

**PROSTHETIC VEIN VALVE:
DELIVERY AND *IN VITRO* EVALUATION**

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Presented to
The Academic Faculty

by

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DELIVERY AND *IN VITRO* EVALUATION**

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LIST OF SYMBOLS AND ABBREVIATIONS

CVI	Chronic Venous Insufficiency
CW Doppler	Continuous-wave Doppler
DVT	Deep Venous Thrombosis
°	degree
Fr	French catheter scale
GT	Georgia Institute of Technology
H&E	Haematoxylin and Eosin stain
hrs	hours
MHz	mega hertz
μL	microliter
mL	milliliters
mL/min	milliliters per minute
mm Hg	millimeters of mercury
PVVB	Percutaneously delivered venous valve bioprosthesis
PVA	Poly(Vinyl) Alcohol
RBC	Red Blood Cells
SG-BVV	Second generation SIS bioprosthesis
SIS	Porcine small intestine submucosa
SFV	superficial femoral vein

SUMMARY

Venous disease will affect 1-3% of the western world at some point in their lives, yet there are few effective treatments for the venous system [1]. One such disease is chronic venous insufficiency (CVI), a painful and debilitating illness that affects the superficial and deep vein valves of the legs. When the valves become incompetent they allow reflux and subsequent pooling of blood. Current clinical therapies are only moderately; and therefore, the need for a better solution remains.

Prosthetic venous valves were constructed from a novel hydrogel biomaterial patented by Georgia Tech. The valves had flexible cusps similar to normal, anatomic venous valves. The purpose of this work was to evaluate the thrombotic potential of the GT venous valve in an *in vitro* study and to design a percutaneous delivery system. *In vitro* thrombosis model provides an appropriate intermediate step between valve development and *in vivo* analysis, which is necessary to determine the biocompatibility of the prosthetic device.

The flow system was modified from a one-pass, flow-through thrombosis assay using whole blood [2] to mimic pulsatile physiologic conditions. Cessation of flow indicated thrombotic obstruction. Histological analysis was performed using H&E staining and Carstairs's stain (specific for platelets). A group of valves were lined with Dacron to confirm the thrombotic potential of the system. All Dacron valves were occluded by thrombus connecting the polymer fibers with adherent platelets.

Whole blood perfused through the GT prosthetic valves exhibited no thrombosis or platelet adherence. All GT valves were patent and competent after blood perfusion. H&E staining revealed no thrombus deposition on the GT vein valves.

A percutaneous delivery system was designed after evaluating the GT valves for their compressibility and plastic deformation over time. Appropriate stents, catheters and sheaths were selected. As designed, this system will be utilized in an ovine trial of the valve. Due to the low *in vitro* thrombotic potential and strong history of PVA as a medical implant material, positive trial results are expected. With successful animal and human trials this valve can provide a potential intervention for the 7 million people suffering from CVI.

CHAPTER 1

INTRODUCTION

Historical Background

The venous system was identified in 335BC when Praxagoras of Cos differentiated arteries from veins [3]. Andrea Visalium accurately depicted the venous anatomy in 1543 [3]; Fabricius elaborated on these findings in 1603 (Figure 1), followed by Harvey in 1628 [4] who identified the direction of blood flow and the concept of circulation with experimental reasoning. Carrel and Guthrie reported the first autotransplantation of animal valve-bearing vein segments in 1906 [5]. Twenty-three years later, Franklin described the anatomy of venous valves in 1929 [6, 7]. Surgical techniques were then largely neglected until 1947 when Johns then studied venous anastomosis [8]. Shortly after in 1953, Eiseman and Malette reported the first technique of valve reconstruction in the canine inferior vena cava by constructing a valve out of the vessel wall [9]. Venous valve autotransplantation was performed in the dog by De Weese and Niguidula in 1960 [10]. The first venous valve repair in man was pioneered by Kistner in 1968 [11]. The first vein valve autotransplant was reported by Taheri et al. in 1982 [12]. The venous system was first identified in 335BC, yet to this day vascular surgeons rarely operate in the venous system, and life threatening complications continue.

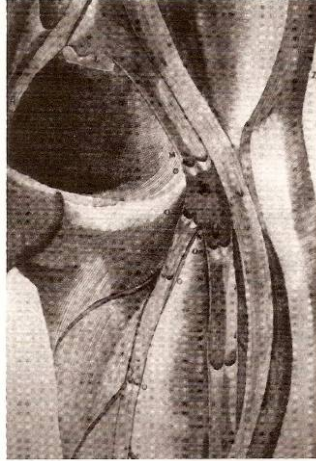


Figure 1: Illustration of Vein Valves from the Fabricus Atlas, 1603

Vein Anatomy and Physiology

Vein Anatomy

The leg venous system has three venous networks, deep veins, superficial veins, and perforating veins (Figure 2). The deep veins are of large diameter and lie deep beneath the muscles of the leg and close to the bones. The deep venous system starts as the tibial vein near the calf, turns into the popliteal vein near the knee, progresses into the femoral vein located in the thigh, and finally becomes the iliac vein prior to emptying into the inferior vena cava. The superficial venous system consists of small diameter veins that are found just below the surface of the skin, and perforating veins connect the superficial veins to the deep veins.

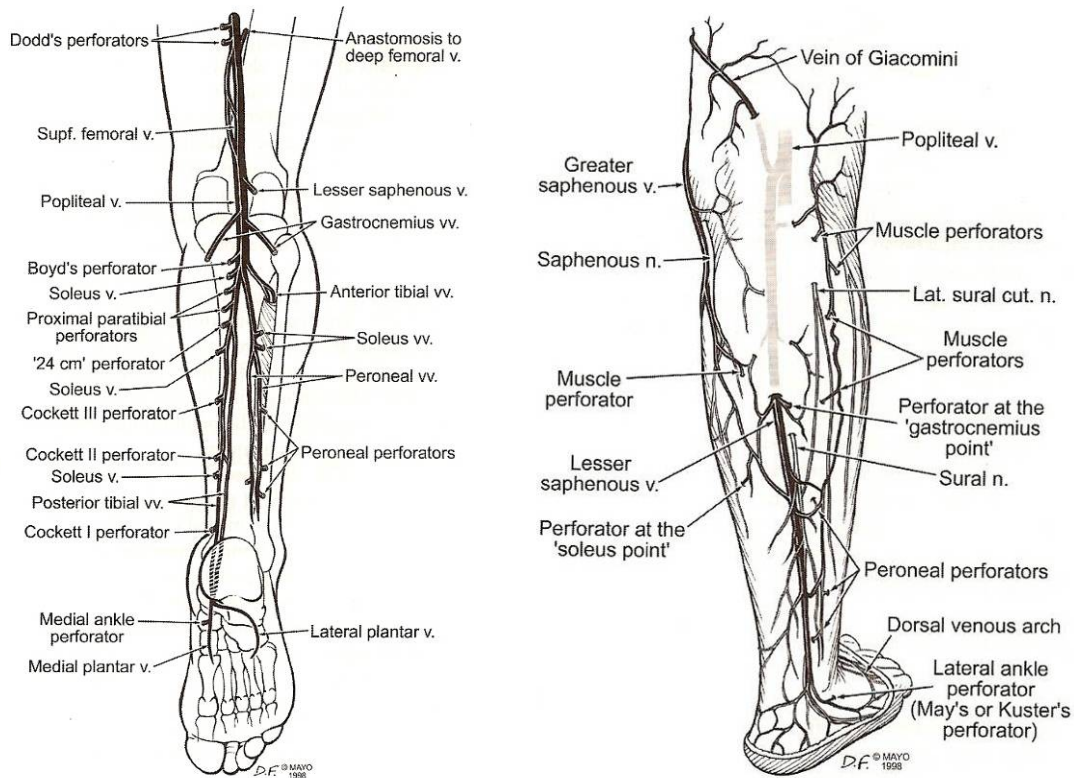


Figure 2: (Left) Deep Veins of the Leg, (Right) Posterior Superficial and Perforation veins of the Leg [13]

Each venous network contain one-way vein valves; though the anatomic structure, location, and number of valves varies among individuals. The most common venous sites are the common femoral vein, just above the entrance of the long saphenous, and in the superficial femoral just below the profunda femoris. The typical location and number of valves in the leg is found in Table 1. The distal vessels contain the greatest number of valves to decrease the pressure exerted by each valve. The anatomic structure varies from one to five leaflets; however, the valves generally are bi-leaflet. The thickness of leaflets varies from 20 to 50 microns. When blood is flowing the leaflets distend to provide an opening area of about 35% of the full luminal area [14]. The muscle pressure on the vessels distort the veins to have an elliptical cross-section. The leaflets are composed of collagenous fibers covered with two unicellular endothelialized layers [15].

Table 1: Average occurrence of valves in deep veins [15]

Deep Vein	# of Valves
Common Femoral Vein (above sapheno-femoral junction)	1
Femoral Vein (below sapheno-femoral junction)	3
Popliteal Vein	1
Posterior Tibial Vein	19
Anterior Tibial Vein	11
Peroneal Vein	10

Vein valves have been classified as either ostial valves or parietal valves. Ostial valves are located where a smaller vein branch meets a larger vein, they aid in distribution of blood throughout the leg during leg muscle contractions. Parietal valves are generally positioned in the larger vessel proximal to a venous junction. Parietal valves are the main pressure-bearing valves and are therefore more critical to CVI.

Vein walls are comprised of 3 layers, intima, tunica media, and adventitia. The intima is the inner most layer and is in contact with the blood, it is composed of elastic media. The tunica media is the middle layer and is a more complex structure consisting of its own three layers. Starting from the inside the first layer is composed of longitudinal muscle fibers, elastin fibrils and connective tissue. The middle tunica media contains bundles of smooth muscle cells separated by elastic fibrils. The outer tunica media comprises longitudinally oriented muscle bundles and fibrous tissue. The vein outer layer is the adventitia, which contains connective tissue, nerve fibers, and vasa vasorum.

Vein Valve Physiology

Vein valve activity is random and sporadic. During any static activity, such as standing or sitting, the valve cusps are open and the pressure in the foot is equal to the haemostatic pressure exerted by the column of blood from the foot to the right atrium. When a leg muscle contracts the intramuscular vein pressure drops to zero, the venous valves will then close preventing reflux. Venous valves are most active during rhythmic activity, such as walking or exercising.

Venous Pumps

The leg contains 3 venous pumps, calf, thigh and foot, which play a vital role in returning venous blood to the heart. The calf pump is the most important because it generates the highest pressure (200 mm Hg) during muscular contraction, the resting venous pressure is approximately 100 mm Hg and reduces to 30 mm Hg after 10 or more repetitive calf contractions. Abnormalities in the valves can impede or negate the function of the calf pump; this results in ambulatory venous hypertension, which is an inability to reduce the superficial venous pressure.

Vein Mechanical Properties

Veins are highly distensible at low pressures, and distention ceases when intramural pressure reaches approximately 50 mm Hg, with diameter expansion limited to 1.5 to 1.6 times the original vein diameter [16, 17]. The distentions of the veins help minimize trauma to the vein, improve comfort for the patient, and reduces the risk of inflammation or thrombus formation in the immediate valve area.

Chronic Venous Insufficiency

Chronic Venous Insufficiency (CVI) is a disease that causes symptoms in the leg including swelling, edema, pain, itching, varicose veins, skin discoloration, ulceration and limb loss [18]. Post-thrombotic damage within the deep veins is the most significant cause of CVI. Thrombus formation permanently damages veins by adhering to the vein wall and becoming permanent when the thrombus organizes, becoming replaced by fibrous tissue and covered by a neoendothelium. Thrombus arising in the valve pockets or coming in direct contact with valve cusps irreparably damage their function. Permanent endothelialized strands of residual organized thrombus (synechiae), may develop across the vein wall, obstructing outflow or binding the valve leaflets to the vein wall [13]. An illustration of competent and incompetent vein valves is shown in Figure 3. Another possible cause of valve leaflet deterioration is fibrinolysis. Fibrinolysis is typically referred to when plasmin is degrading the fibrin and thrombus; however, in the case of CVI, plasmin may also be degrading the delicate valve leaflets [13]. Even though DVT is the most common precursor to CVI, approximately two-thirds of patients with venous insufficiency give no history of DVT [19-21].

Reflux is also not always indicative of CVI, as about 15% of normal limbs have reflux in the common femoral vein. Normal limbs often show incompetence, which is limited to the superficial femoral valves. A strong indicator of CVI is incompetence in the popliteal vein and the deep calf veins. Strong indicators of ulcer development are incompetent distal superficial veins [19, 21]. Patients with persistent valvular reflux develop chronic venous hypertension (venous pressure above normal range) and ambulatory venous pressure (the blood pressure inside veins while walking above normal range). Venous hypertension causes leakage of red blood cells and macromolecules (i.e. fibrinogen and α_2 -macroglobulin) into the dermal interstitium. The initial chronic inflammatory signal

responsible for leukocyte recruitment is the RBC degradation products and interstitial protein extravasation which act as chemoattractants to the leukocytes. A cascade of pathologic events follows, resulting in dermal fibrosis.

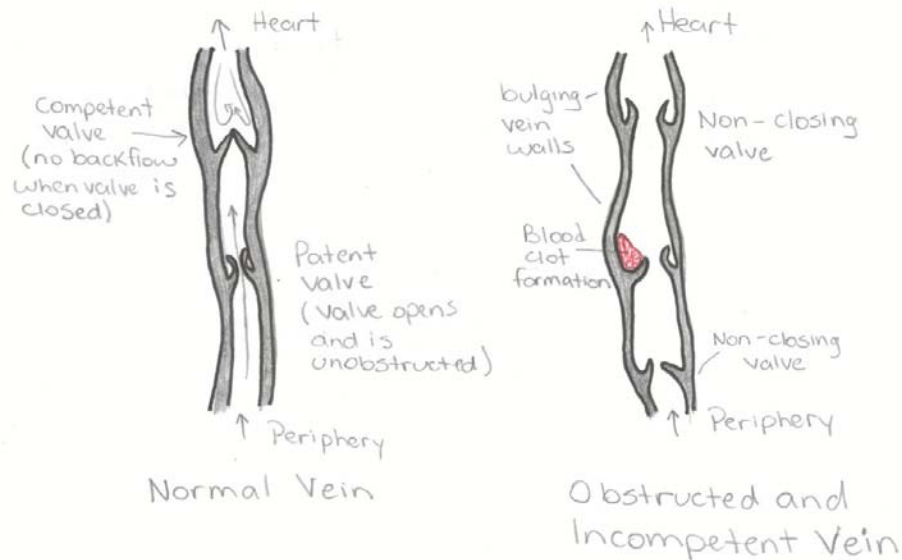


Figure 3: (Left) Competent and patent valves, and (Right) obstructed and incompetent valves

Chronic venous insufficiency can typically be diagnosed as either primary or secondary incompetence. Other causes of chronic venous insufficiency exist and are mentioned below. Primary incompetence is the degradation of a valve due to aging, whereas secondary incompetence is caused by a damaging obstruction in the vein. Primary incompetence is more common, though may progress into secondary incompetence. Patients with secondary incompetence are the primary candidates to receive surgical interventions.

Primary incompetence develops as the structural composition of veins change. As humans age their vein walls naturally distend as the elastic fibers and muscle layers become disorganized. Another symptom of aging includes the elastic lamina of the intima layer becoming fragmented, atrophic, thin, and irregular [22].

As vein walls progressively distend due to primary incompetence the veins become vulnerable to high-pressure distention and thrombus formation. Valve damage can be caused by distention to the point where the valve leaflets no longer meet in the lumen, and/or thrombus formation in the leaflet pockets caused by unnatural turbulence and stagnation areas in the vein [23]. These incompetent leaflets over time will fold over and be absorbed into the vein wall. Secondary incompetence is when the vein valves are lost [24]. The incidence of secondary incompetence in patients with ulcerations and deep venous reflux has been reported as high as 95% [25].

The physiological consequences of venous incompetence depend on the location of the incompetent valve(s). The combination of competent perforating veins, a powerful calf pump and the absence of obstruction can compensate for deep venous valvular incompetence. Perforating veins inevitably become incompetent as they act as safety valves for incompetent deep veins, allowing blood under high pressure in the calf pump to escape. Intervention is necessary when the perforating veins become incompetent and the calf pump no longer compensates.

The absence of one vein valve results in increased pressure to the distal valve. Similar to the initial incompetent valve the distal valve will experience distention and abnormal blood flow regions. This distal valve will likely become incompetent and the disease will cascade down the leg.

Other processes that lead to valvular incompetence and CVI involve vascular trauma, tumor growth, and deep vein thrombosis (DVT). Patients with a history of DVT have over a 25-fold increase chance for developing CVI compared to patients without a history of DVT [26].

Risk factors of CVI include heredity, obesity, pregnancy, a sedentary lifestyle, smoking, or being female over 50 years of age. A patient suffering from CVI may have a rash on the skin of the calves or ankles (indicative of lipodermatosclerosis), reddened or discolored skin on the leg, edema or swelling, ulceration or skin breakdown, or varicose veins. An extreme clinical case of venous incompetence is depicted in Figure 4; this patient has swelling, brownish discoloration, a tough hardened area at the ankle, and ulceration.



Figure 4: Patient with an extreme case of venous incompetence

Diagnosis of CVI is typically accomplished by identifying the signs and symptoms. Anatomic studies are intended to identify the location of incompetent valves for severe cases which will involve invasive surgery, like venous venotomy or valve transplantation. CVI imaging techniques are explored further below. Valves that are typically addressed surgically are the upper superficial femoral vein or popliteal vein. Surgical options are further discussed later.

Imaging

Phlebography

Phlebography (also called venography, ascending contrast phlebography, or contrast phlebography) is an invasive diagnostic test that provides an image of leg veins on a fluoroscope screen [27]. It is the standard for identifying and quantifying reflux in individual veins. The fluoroscope images allow for identification of the location and condition of vein valves and blood clots, and the overall condition of the veins.

The widespread availability and noninvasiveness of duplex Doppler, color Doppler and compression ultrasound has reduced the need for contrast phlebography in venous disease diagnosis; but phlebography is the "gold standard," and the best test by which other imaging methods are judged [28]. Venography is essential for patients who are considered for valvuloplasty or valve substitution, as it is the most accurate test for detecting vein valve incompetence and vein thrombosis. It is nearly 100% sensitive (probability of a positive test among patients with disease) and specific (probability of a negative test among patients without disease) in making this diagnosis. Accuracy is crucial since deep vein thrombosis and CVI are potentially fatal conditions.

Descending phlebography, first established by Bauer in 1948, provides a method to identify vein valve sites and to study their degree of competence [29]. The patient is positioned on a fluoroscopic table inclined to a minimum of 60°. The contralateral common femoral vein is typically chosen as the site of venipuncture, although a brachial or antecubital approach are alternatives. Contrast is injected during a forced Valsalva maneuver. A Valsalva maneuver is an effort to exhale without letting air escape through the nose or mouth. Heparin (5000 units) is intravenously administered prior to catheterization. The examined leg is non-weight bearing. Reflux is observed under

fluoroscopy and graded according to the classification of Kistner and associates [30]. Videotaping for post-procedure evaluation is a common practice, a competent vein valve imaged with descending venography is presented in Figure 5.

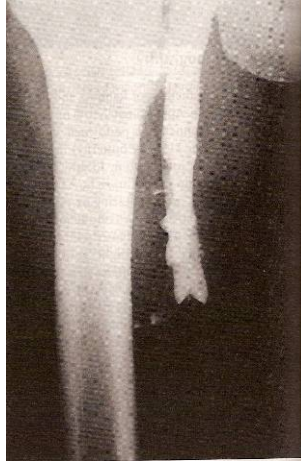


Figure 5: Descending venogram of a competent vein valve [13]

The risk associated with phlebography is a reaction to iodinated contrast. The contrast is an irritant to the intima which causes thrombosis in 1-2% of cases [31]. False-positive interpretations are possible, owing to the relatively high density of the contrast medium, which may trickle down the relaxed leg through normal but partially open valves.

Ambulatory Venous Pressure

Ambulatory venous pressure provides a general measurement for venous insufficiency. Pressure measurements are obtained by placing a needle in the foot and having the patient perform maneuvers. A normal patient will have pressures between 0-20 mmHg, someone with incompetence will have elevated pressures. Venous obstruction, valvular reflux and calf muscle pump failure cannot be separately identified with this method. This test has been replaced by non-invasive alternatives due to its invasive procedure.

Duplex Scan

Duplex scanning is a noninvasive test that is the most clinically useful test for detecting the location of venous valves, and evaluating venous valvular incompetence and chronic venous obstruction [13]. Duplex scanning incorporates a pulsed-Doppler flow-velocity detector; which confirms the presence or absence of flow indicates the direction of flow, and records flow patterns. The addition of a color-coded flow map allows immediate identification of arteries and veins. Blood flowing towards the heart is depicted as blue (indication of veins), and red for blood flowing towards the peripheries (indicating arteries). Encroachment or absence of flow is easily detected. Color is helpful to immediately identify reflux. Duplex scanning has a sensitivity between 82-87% and a specificity between 92-99% [32, 33].

Duplex scanning depicts the vein over a relatively long region and will show multiple vessels simultaneously; these benefits eliminate the need to interrogate the leg every centimeter (which is necessary with CW Doppler). In contrast, venograms provide an even larger image region.

Veins are identified by their characteristic audible Doppler signal and velocity spectrum (or color-flow map). The probe is adjusted to provide a long-axis view of the segment of interest. The patient is then asked to perform a Valsava maneuver to reverse the normal pressure gradient. Competent valves will prevent retrograde flow by valve closure, where incompetent valves will permit retrograde flow. Retrograde flow is indicated by an inverted spectrum, or by a change in the flow-map color from blue to red. A reflux flow longer than 1.0 seconds is considered abnormal.

An alternative method to quantify valvular incompetence involves the patient in a standing position, a pneumatic cuff is positioned around the leg about 5 cm below the transducer site, and the cuff is inflated to 80-120 mm Hg (depending on cuff location) for about 3 seconds [34]. Normal valves close rapidly when the cuff is deflated in response to temporary flow reversal. In 95% of competent veins, reflux flow is less than 0.5 seconds; therefore, retrograde flow greater than 0.5 seconds indicates valvular incompetence; in most cases incompetence reflux is about 3-4 seconds. This technique has proven to be more sensitive than the supine-manual compression or Valsalva methods [35].

Valves can be identified on duplex scanning by their geometry, as healthy sinuses have an elliptical configuration and the leaflets are thin and mobile, Figure 6 illustrates venous valve geometry. Diseased valve sinuses are distorted, may contain echogenic material, and the thick and short leaflets are rarely visible. Many duplex instruments permit the diameter of the vein to be measured, the machine can then determine the mean flow rate by multiplying the reflux flow velocity by the cross-sectional area. Locating the valves is clinically desirable, though is not always possible, due to their degenerated state and the echogenicity of the blood.

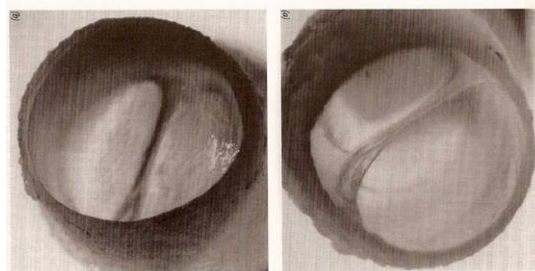


Figure 6: Stereo Microscope Image of a Venous Valve (Magnification of x 11.2), the left image is a distal view, the right image is a proximal view [13].

Continuous-Wave Doppler

Continuous-wave Doppler (CW Doppler) is an older technology than Duplex scanning, though it may provide sufficient information if Duplex scanning is not available. CW Doppler inversely relates the frequency of the transmitted ultrasound to the sonic penetration. To evaluate deep veins a low frequency around 5 MHz is used, and for superficial veins a higher frequency of 10 MHz is used. Examinations are performed with the patient relaxed and comfortable in a slightly inclined supine position of 10-15° (Trendelenburg position). The initial step is to locate the corresponding artery (easily identified by direction of flow) and to shift the probe towards the vein. The common femoral, superficial femoral, popliteal and posterior tibial veins can all be identified. Care must be taken to apply minimal external probe pressure to prevent the veins from collapsing. Compressing the leg below the probe displaces blood up the leg; thereby, increasing the Doppler audible signal. Compressing the leg above the probe restricts venous outflow causing blood to back up in the veins below the site of compression, upon relief of compression the blood rushes up the leg. If the probe is shifted more than a centimeter from the corresponding artery then the vein is likely not to be patent.

Valvular incompetence can be demonstrated by reversal flow in the vein. The patient performs a Valsava maneuver where the intra-abdominal pressure is increased; thereby, forcing blood out of the inferior vena cava and iliac veins. Competent valves will prevent retrograde flow by valve closure, where incompetent valves will permit retrograde flow. It is important to note that one competent valve proximal to the probe will prevent reflux and may lead to a false-negative result. To minimize a false negative the common femoral or superficial femoral vein are examined to detect reflux in the common femoral level.

CW Doppler demonstrates a sensitivity to identify incompetence of 73% in the saphenous vein, 33% in the lesser saphenous, and 48% in the deep veins [36]. CW Doppler has specificity of 90%. More accurate tests, like venography and duplex scanning, are more commonly used.

Plethysmography

Plethysmography is a noninvasive test that measures volume displacement in a limb segment. An air filled plastic sleeve is typically placed around the calf, the patient is then asked to perform a series of maneuvers, and the pressure in the pneumatic sleeve monitors the change in limb volume. The volume is recorded by a pulse volume recorder (plethysmograph) that measures the pulse waves from each cuff. Plethysmography may be performed as a basic diagnostic test, prior to more in depth alternatives.

Plethysmography provides quantitative information for venous outflow obstruction, valvular incompetence, and calf muscle pump function [13]. For obstructions the rate of volume change in an elevated limb is measured. The volume-measuring device is positioned around the calf and the pneumatic cuff is placed around the thigh. The leg is elevated above the heart and the venous flow is occluded by inflating the cuff to 60-80 mmHg. Blood volume accumulates, stabilizes and then the cuff is rapidly vented. The output trace compared against normal values is used to determine obstruction. Valvular incompetence using plethysmography involves placing the transducer distally on the leg. Measurements are conducted by having the patient perform a calf exercise followed by a relaxation period; refill times are recorded and compared against normal values to determine incompetence. Slow refill times indicate incompetent valves. Calf muscle pump function is evaluated with air plethysmography by comparing the percentage of venous volume ejected by the calf against the percent venous volume which cannot be

expelled. Plethysmography accurately quantifies CVI and is therefore a common test used to support venography.

Surgical Repair of Incompetent Venous Valves

Historically, surgical treatment was avoided due to lack of accurate surgical technology and the extreme risk of post-operative thrombosis. Through the advancement of diagnostic imaging, surgical technology, and anticoagulation, the clinician's options for addressing venous disease has greatly increased over the past 40 years; however all procedures are extensively time consuming and extreme cases are only pursued surgically. Invasive venous valve repair or replacement techniques are reserved for patients with a congenital absence of functional valves and severe cases of DVI. An evaluation is conducted, including a complete history and physical examination, assessment of the clinical status [37], and imaging with duplex ultrasound and plethysmography.

The first venous surgical technique was ligation of grossly incompetent veins by Linton in 1938 [38]. Ligation is the stripping of superficial vessels and does not address the problem of deep venous incompetence. Ligation was the only surgical treatment until Kistner, in 1968, developed the technique of internal valvuloplasty [11], as illustrated in Figure 7. The current surgical procedures for deep venous incompetence include valvuloplasty, vein segment transposition, and vein valve transplantation [39].

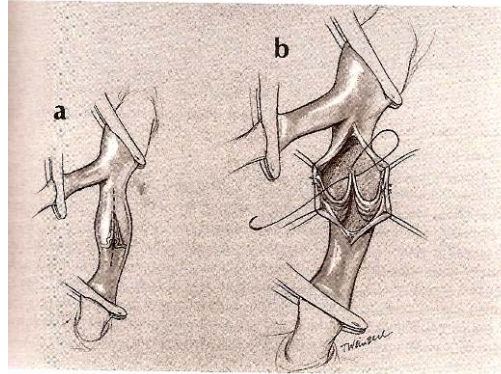


Figure 7: Longitudinal Venotomy and Valve Repair [13]

Valvuloplasty

The standard of care in the surgical correction of primary reflux is a valvuloplasty. This surgical procedure involves a venotomy, where the valve cusps are plicated 20-25%; a PTFE sleeve is often placed around the operation site to maintain valve integrity, as illustrated in Figure 8. Kister who pioneered this technique, omitting the PTFE sleeve, concluded that one competent vein valve in the deep-venous system of the leg at the femoral level would reverse abnormal hemodynamics [40]. Further reports have indicated that reversing abnormal hemodynamics is more complicated and that repair of the most proximal valve in the superficial femoral vein is only sufficient when the profunda femoris vein is competent [41, 42]. In these rare cases a valvuloplasty has a 60% success rate over 5 years [43]. A singular valvuloplasty has shown not to be valid for systems that include occlusion of the femoral or popliteal vein, or absence/incompetence of the communicating leg veins. These conclusions are supported by the following clinical studies.

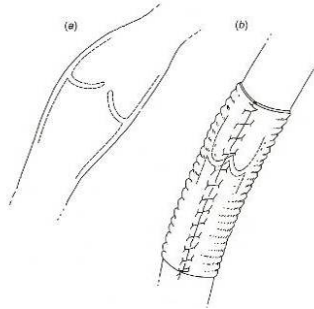


Figure 8: Placement of PTFE sleeve as a support following a venotomy [13]

Johnson demonstrated in 10 patients that venous valve refilling time can be temporarily improved by repair of the femoral valve [44]. Unfortunately this valve repair failed to demonstrate improvement in pressure or venous refilling times in 12 to 18 months post operation. They concluded that repair of the femoral valve alone does not cure chronic venous inefficiency, and the ineffective calf communicating veins contribute to this inefficiency.

In another study, 125 extremities with reflux in the greater saphenous and superficial femoral veins were followed for 7 to 8 years [45]. Surgical procedures included phlebectomy and Kistner's Technique [40]. Phlebectomy is a surgical technique involving stripping of the greater saphenous vein with stub avulsion of varicose tributaries. Their results indicated that disease was aggravated in 24% of extremities, though 92% of the corrected valves demonstrated clinical improvement. They concluded that surgical correction of the incompetent superficial femoral vein valve would change the course of primary chronic venous insufficiency.

A large study of 582 venous valve reconstructions in 347 limbs was performed from 1979 to 1994, by Raju, S. and Hardy, J.[43]. This study provided a collection of venous operational techniques and evaluation parameters for determining venous valve surgery success. Patients were selected for surgery once conservative therapy failed or recurrent

complications developed. Those selected for surgery experienced primary reflux (55%), post-thrombotic reflux (39%), and congenital dysplasia (6%). The valves were evaluated based on 4 measurable parameters. These parameters included restoration of valve competence, maintenance of patency without hemodynamic stenosis, healing without damage to the repaired valve due to hematoma or infection, and rapid resolution of stasis ulceration after surgery. Postoperative duplex examination showed competence in 78% of valves, with 16% partially competent and 6% reflexive. The femoral valve repair for primary valve reflux was successful in 60% of cases over 5 years. Deep venous thrombus developed in 3% of valves. Stasis ulcers were common, occurring in 267 limbs, though rapid healing occurred in 93% of these ulcers. This study concluded that an internal valvuloplasty is ideal for repair of a single valve in the leg, though this procedure is time consuming and is therefore impractical for multiple valve reconstructions. A popular alternative to valvuloplasty is the transcommissural technique (described below), which combines the benefits of both open and closed techniques, and it is rapidly becoming the first choice for valve repair.

The transcommissural valve repair is performed by placing transluminal sutures along the valve attachment lines, which simultaneously closes the valve attachment angle and tightens the valve cusps [46]. Raju, Berry, and Neglan reported in 2000 the repair of 141 limbs after 30 months of follow up with duplex Doppler ultrasound. Complications arose in 9% of limbs, including: infection, hematoma, seroma, thrombosis, and embolus. Post-operatively the valves were competent in 78% of limbs, and at 30 months 59% were competent. They concluded that transcommissural valvuloplasty is safe with a low morbidity. Compared to internal valvuloplasty, it is a fast and simple procedure, small veins can be repaired, and multiple valves can be addressed in a single stage.

Advances in venotomy include external valvuloplasty, and angioscopic-assisted valvuloplasty [47]. The external valvuloplasty is performed without the use of a venotomy; it places the placating sutures from outside the lumen to tighten the commissural angle. The angioscopic-assisted valvuloplasty uses an angioscope that is introduced through a side branch and advanced into the proximal SFV, it is then positioned directly above the valve. Blood is cleared from the field by irrigation. Through use of a video-enhanced, magnified angioscopic visualization, sutures are passed from outside to inside the lumen. These techniques lessen vessel trauma and reduce the dependence of anticoagulants. Unfortunately, when the valve apparatus is completely destroyed, limited options are available; they include axillary vein transfer and *de novo* valve reconstruction.

Vein Segment Transposition

When a normal functioning vein valve is in close proximity to the incompetent vein valve, a vein segment transposition procedure may be performed, as illustrated in Figure 9. The incompetent vein is dissected, mobilized, and transposed on to the normal vein distal to a functional valve. As CVI cascades down the leg the distal vessel is often also afflicted. The long term patency rate for transposed valves is 17-66% [48].

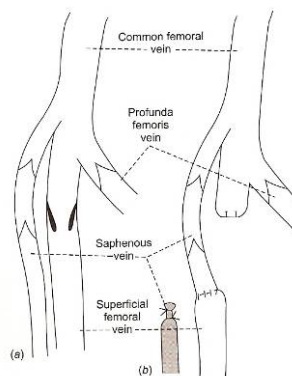


Figure 9: Vein Segment Transfer [13]

Vein Valve Transplantation

A vein valve transplantation is performed by mobilizing a valve containing vein segment from either the axillary or brachial veins and transplanting it into either the popliteal or femoral vein systems, as illustrated in Figure 10. Allograft and cadaveric vein transplantations are not common practices due to the low quantity of suitable donor valves, and the negative immunogenic response.

Valve transplant can cause unnecessary trauma to the patient's leg, and most procedures require indefinite post-operative anti-coagulation. Therefore, transplantation is used only when alternative methods have failed, such as medication, physical rest, therapy, and other less invasive surgical procedures. Problems arise even prior to surgery because it is difficult to find a suitable donor valve. This is evidenced by the fact that 30 to 40% of axillary vein valves, which are often used for superficial femoral venous valve replacement, are found incompetent prior to harvesting [49]. The long term patency rate for transplanted valves is 17-66% [48].

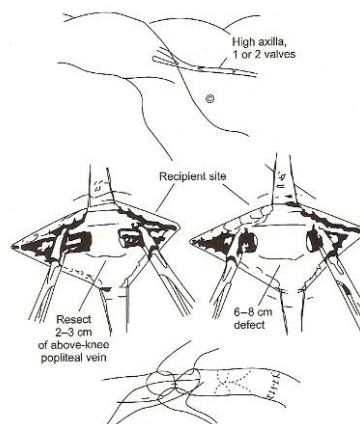


Figure 10: Vein Valve Transplantation [13]

Cryopreserved Human Vein Valves

Cryopreserved human vein valves result in high morbidity (48%), poor patency (41% patent at 24 months), poor competency (27% competent at 24 months) and poor clinical results [50]. Furthermore, ulcers reoccurred in 50% of patients, pain relief was dissatisfactory, and swelling was not improved. It was found that nearly three-fourths of the cryopreserved valves required some transcommissural repair prior to implantation. The poor performance of cryopreserved valves was attributed to the effects of cryopreservation on structural integrity and the immunogenic response elicited after implantation [50].

Prosthetic Vein Valves: Animal Studies

Several animal trials to test prosthetic venous valves have been performed since 1985. Canine (dog) was the most common animal used prior to 1995, and since the animal models were swine (pig) and ovine (sheep). It is appropriate to assume that the canine model was progressively phased out due to the hyperthrombogenic potential of canine blood. The lengths of the animal trials were for 1 week up to 8 months.

In 1985, Hill *et al.* designed implantable venous valves, one set fabricated of Pellethane®, a polyurethane elastomer, and others were fabricated from glutaraldehyde-fixed umbilical cord segments. They implanted the valves in the external jugular vein and contralateral jugular vein of canine for up to eight days. All umbilical vein valves were occluded within 48 hours. Two of the eight Pellethane valves were patent at 5 days, though all were occluded by 8 days [51]. These valves demonstrated poor patency results.

Gerlock *et al.* implanted Bioprosthetic cardiac valves in the vein valve position, in 1985. These valves were trileaflet and constructed from gluteraldehyde-fixed pericardium and a Dacron graft. They implanted these valves into the inferior vena cava of four dogs. Two dogs were assessed at six months and two at eight months, all valves were patent at both time points [52].

In 1988, Taheri *et al.* developed a bi-leaflet mechanical vein valve [53, 54]. They performed both *in vitro* and *in vivo* experiments on platinum and pyrolytic carbon-covered titanium valves. They implanted the valves in the femoral vein and inferior vena cava of 10 canine by transverse venotomy. At 3 months, seven of the ten valves remained patent and competent. Those valves that failed resulted in severe thrombotic occlusion. Taheri *et al* further reported in 1995 that after two years, these valves developed dense ingrowth of intimal hyperplasia which rendered the valves functionless [55].

Rosenbloom *et al.*, in 1988, created an autogenous venous valve constructed from canine external jugular veins [56]. Fifteen valves were implanted into fifteen dogs. Six canine were euthanized immediately after valve placement, six canine were euthanized at 1 week, and three canine received heparin and were sacrificed within 7 to 13 days. All acute animals demonstrated patent and competent valves. Two of six dogs at the 1 week time point were both patent and competent, the remaining 4 dogs had complications arising to thrombosis. All three valves from the heparin group were patent and competent.

Stent based polyetherurethane membrane vein valves were studied in 1993 by Uflacker [57]. They implanted the prosthetic valve in the superior and inferior vena cavae of swine. The valves exhibited good function at 1 week follow-up on supine and upright

cavograms (phlebography on the vena cava). However, partial thrombosis appeared inside the valve cusps.

Gomez-Jorge *et al.* percutaneously delivered eleven glutaraldehyde-fixed bovine external jugular vein bioprosthesis mounted on a Nitinol stent into the inferior vena cavae or external iliac vein of eleven swine [58]. An acute experiment was performed on seven of the eleven animals; the four remaining animals were evaluated for 2 weeks. Deployments of the bioprostheses were successful in nine of 11 swine. In the survival group, 2/4 valves were patent without evidence of thrombus formation by ascending and descending venography. Complications included hemarthrosis, death, and bioprosthesis thrombosis immediately after deployment. Histopathologic analysis showed endothelial cells covering the luminal surfaces. The wall of the bioprostheses had granulomatous response and foreign body reaction. Bacterial contamination was noted in one bioprosthesis. Prior to a long-term study, Gomez-Jorge *et al.* are evaluating the bioprosthesis durability, immunogenicity and leaflet function of their bioprosthetic vein valves.

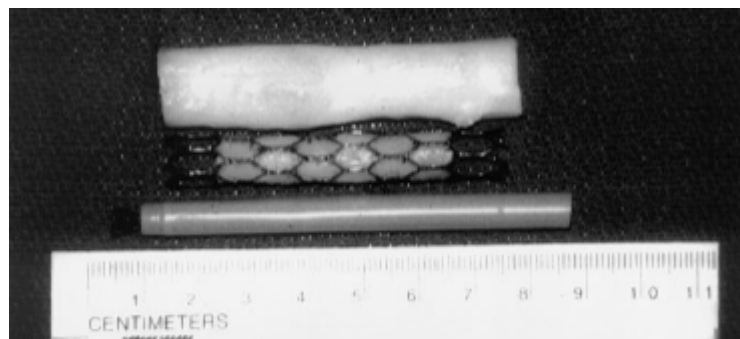


Figure 11: Bioprosthetic, glutaraldehyde-fixed bovine external jugular vein, attached to a Nitinol stent [58]

The percutaneously delivered venous valve bioprosthesis (PVVB) valve, currently in clinical trials, was evaluated in a porcine study during 2003 [59]. The PVVB is a glutaraldehyde-preserved bovine jugular valve-bearing venous xenograft sutured inside a Nitinol frame, as shown in Figure 12. Nine pigs underwent catheter delivery. One PVVB

was inserted and deployed in each iliac vein under fluoroscopic control. PVVBs were evaluated with phlebography at either 2 or 4 weeks to assess patency and competence; all PVVBs were explanted and processed for histological analysis. In 8 animals, the PVVB was successfully deployed in both the left and right iliac veins. Four animals were sacrificed at 2 weeks, 7 of 8 valves were patent and 3 of 8 were competent. Five animals were sacrificed at 4 weeks, 5/10 were patent and 2/10 were competent. Histology showed thrombosis as the cause for all non-patent valves. Clinical trials are currently underway; they are further discussed under the Clinical Trials heading.



Figure 12: First Generation PVVB [59].

Bonhoeffer *et al.* evaluated a fresh bovine vein valve as an option for pulmonary valve replacement [60]. Despite the fact that the valve position is different to the vein valves, the Bonhoeffer *et al.* results are discussed to demonstrate a simple *in vitro* patency and competency test that may be performed post explantation. A vein segment containing a native biological valve was harvested from a bovine jugular vein and sutured into a platinum balloon expandable stent, as seen in Figure 13. Eleven lambs underwent catheterization for transcatheter pulmonary valve replacement. The successfully placed valves were explanted 2 months after implantation. Competent and patent valves were evaluated by restoring function *in vitro* after removal of the pannus, as seen in Figure 14. For a competent valve, water was flown in the retrograde direction, as seen in Figure 14 the valve demonstrates effective closure of the valve. To demonstrate patency, water is

flown from distal to proximal, again as seen in Figure 14 the valve demonstrates effective opening of the leaflets.



Figure 13: Fresh bovine jugular vein attached to a platinum stent [60].

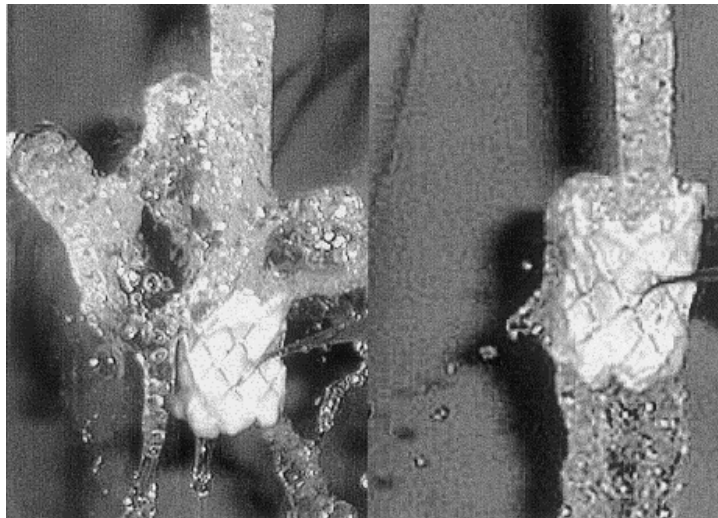


Figure 14: *In vitro* competency and patency test of bioprosthetic pulmonary valves after 2 months *in vivo*. Left, competency test; and right, patency test [60].

Between 2000 and 2005, Pavcnik *et al* reported on percutaneously delivered vein valve prosthesis [61-69]. The valve was constructed from Nitinol or stainless steel wires and a sheath of porcine small intestine submucosa (SIS). SIS is an acellular material derived from small intestines [62]. Unlike other xenograft tissues which promoted host fibrotic tissue growth, SIS tissue promotes host tissue generation specific to the site of implantation. The first generation bioprosthesis is shown in Figure 15, and collapsed for transcatheter delivery in Figure 16. In 2002, 25 valves were implanted into the external jugular veins of 12 sheep. The valves were assessed over a period of 6 months, where

88% of the valves demonstrated good long term patency and competency [70]. They also reported that host cells integrate into the SIS collagen matrix [68]. All 3 failed valves were the result of prosthesis tilting, where one valve thrombosed at one month and the other two were patent with moderate reflux at three months. Tilting refers to the valve being misplaced and not positioned perpendicular to flow. With these vein valves, a clinical trial was performed, the results from this trial are further discussed under the Clinical Trials section [64].



Figure 15: SIS vein valve bioprosthesis [67]

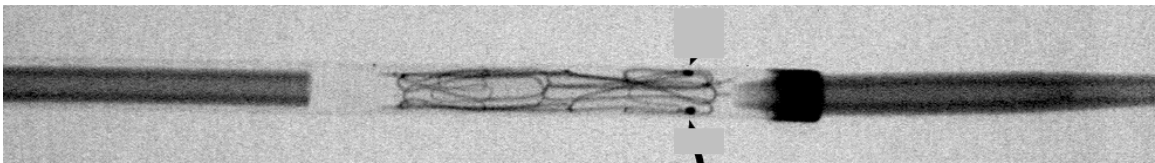


Figure 16: SIS vein valve bioprosthesis, collapsed for catheter delivery [67]

A second generation SIS bioprosthesis (SG-BVV), by Pavcnik *et al.*, was evaluated in 2004 and 2005 [66, 67]. The revised bioprosthesis included a modified stent from the original design to prevent tilting. The second generation SIS vein valves are shown in Figure 17, stainless steel and Nitinol versions were designed. Forty-eight prostheses were implanted, via catheter delivery, into the jugular veins of 24 sheep. The animals were

ethanized at 6 weeks. The prosthesis prevented reflux in 44/48 valves (91.6%) The failed valves were the result of stent over expansion and prosthesis over sizing. Pavcnik *et al* indicated that matching valve size with vein diameter is essential for good valve function. They suggested that an optimal prosthesis is 10% to 15% undersized. Clinical trials have yet to be reported on the second-generation bioprosthetic venous valve.

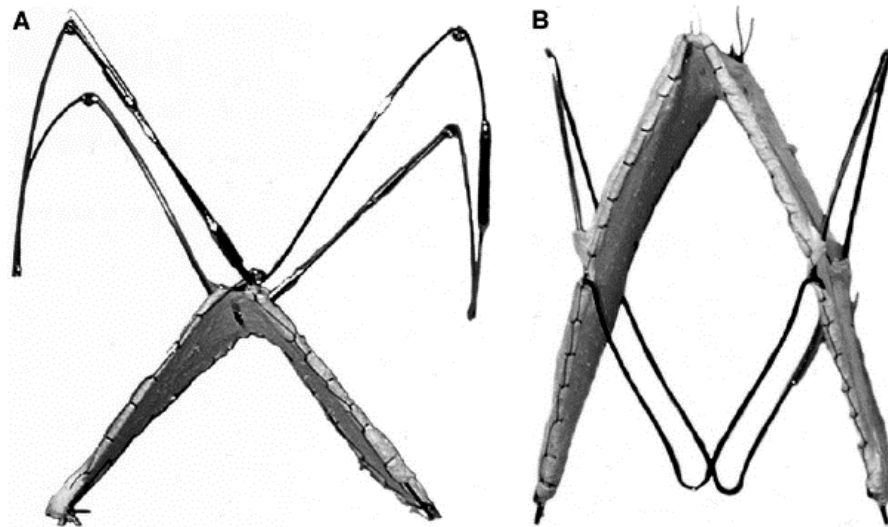


Figure 17: Second generation SIS bioprosthesis venous valve. (A) Stainless steel, and (B) Nitinol versions [66].

There are currently no commercially available prosthetic vein valves. However, the success of the animal trials by Pavcnik *et al.* and the PVVB study indicate positive progress in the prosthetic venous valve industry [59, 67]. Several major achievements have developed since the infancy of prosthetic vein valve animal trials; such as, standardization of imaging techniques, the choice of more appropriate animal models, and the advancement in surgical procedures. Initially venograms were not consistently used in animal trials; however, this imaging technique eventually became the standard to test for patency at implant and explant. An attractive evaluation technique was performed by Bonhoeffer *et al.* who provided a simple test for *in vitro* patency and competency. It is appropriate to assume that the canine model may be progressively phased out due to the

hyperthrombogenic potential of canine blood. Ovine and porcine models have similar thrombosis potentials to humans, though they are lower than the canine thrombotic potential [71]. The surgical procedure has improved from transverse venotomy to percutaneous delivery. Dotter first suggested percutaneous delivery of venous valves [72] in 1981, though it was not successfully executed and reported until 2000 [58, 61].

The preceding list provides a relatively thorough literature review, yet developing a historical timeline of medical devices is limited due to the proprietary nature of the industry. For instance, in 1991, Baxter reported the development of a hydraulic mock circuit that accurately mimicked natural venous flow; however, the paper does not describe the venous valve [73]. Also, other surgeons have implanted venous valves provided by companies into the iliac and jugular veins of sheep, yet the venous valves are proprietary; and therefore the study results are not available.

The preceding studies failed due to either *in vivo* thrombosis or biocompatibility problems. For instance, the umbilical vein valves and pellethane valves by Hill *et al.* all failed due to occlusion [51]. The three-month non-patent mechanical valves by Taheri *et al.* failed due to severe thrombotic occlusion. Two-thirds of the autogenous venous valves by Rosenbloom *et al.* failed from complications arising to thrombosis [56]. Partial thrombosis appeared in the valve cusps of the polyetherurethane valves by Uflacker [57]. Thrombosis occurred immediately after deployment of the gluteraldehyde-fixed bovine external jugular by Gomez-Jorge *et al.* [58]. Histology revealed that thrombosis was the cause for all non-patent valves in the PVVB study by de Borst *et al.* [59].

Biocompatibility was the primary concern for Taheri *et al.* and Gomez-Jorge *et al.*

The two-year non-patent mechanical valves by Taheri *et al.* failed due to dense in-growth of intimal hyperplasia which rendered the valves functionless. The gluteraldehyde-fixed bovine external jugular by Gomez-Jorge *et al.* produced a granulomatous response and

foreign body reaction [58]. Biocompatibility and thrombus formation were the two failure causes in the animal studies.

Prosthetic Vein Valves: Clinical Trials

Prosthetic vein valves have been presented in two case studies; including, the second generation bioprosthetic venous valve (SG-BVV) trials [64], and the percutaneously delivered venous valve bioprosthesis (PVVB) trials [74].

A first generation BVV was evaluated in three patients [64]. The first generation BVV is shown in Figure 15. The first patient received the implant in the femoral vein, at twelve months the leg ulcers had healed and edema was improved; though, the valve leaked when Valsalva was applied. The second patient received an oversized BVV in their femoral vein. This valve was patent and competent at 12 months, though clinical symptoms and disability scores did not improve. The valve tilted in the femoral vein of the third patient, though minimal leakage was present at 12 months, leg pain decreased and the valve continued to function with minimal leakage. The SG-SVV bioprosthesis was revised to include a modified stent to prevent tilting, as shown in Figure 17. SG-BVV clinical trials are planned.

Patent rights of the PVVB belong to Venpro Inc. (Irvine, California). The phase one PVVB valves were implanted into fifteen patients between 2001 and 2002 [75], the clinical results of two patients are reported by Gale *et al.*[74]. The valves were inserted into the mid-thigh femoral vein, via a right internal jugular vein puncture, as illustrated in Figure 18. Percutaneous deployment was successful in both patients. The first patient's prosthetic was both patent and competent. However, complications with the prosthetic valve developed and the following treatments were necessary to prevent undo pain for the

patient. The treatments included ligation and stripping of the left saphenous vein after 12 months, a 2 month course of nanocrystalline silver, firm compression dressings, and skin grafts. The patient was free of discomfort, though this successful outcome can not be attributed to the valve alone, as other medical interventions were used. The second patient suffered from post-insertion thrombosis, and multiple secondary procedures were performed to restore valve patency and competent. This clinical trial does not demonstrate the effectiveness of the PVVB, as both patients required multiple post-operative procedures. The PVVB was redesigned by replacing the bovine jugular venous valve with pericardial tissue, increasing the radial stiffness of the Nitinol stent, and incorporating a heparin coating. The second generation PVVB is displayed in Figure 19; phase II clinical trials are planned in Europe and the U.S.A. For the reader's information, phase I is an initial safety evaluation; whereas, Phase 2 Clinical trials determine the effectiveness of the product, and associated side effects and risks.

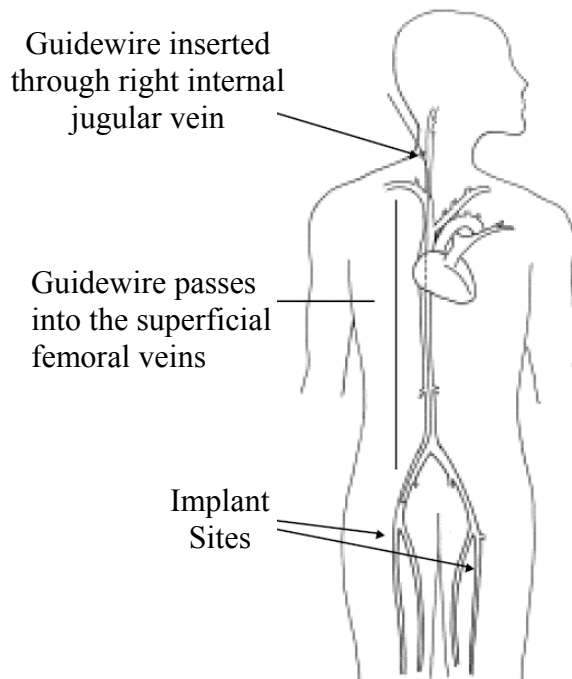


Figure 18: Percutaneous delivery technique for the PVVB [74].

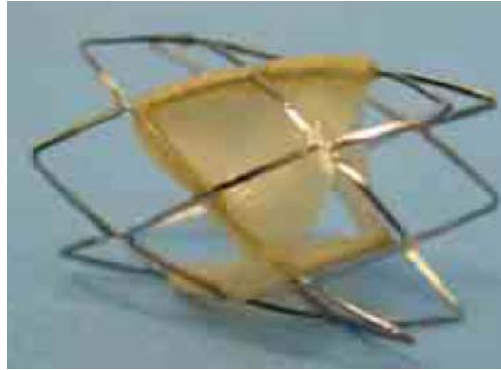


Figure 19: Second Generation PVVB [75].

Blood

Blood Composition

Blood is a fluid connective tissue with plasma as its matrix [76]. Plasma makes up 46-63% of the blood volume. Plasma has a higher density than water since the plasma proteins are in solution rather than forming insoluble fibers like those in other connective tissues. Three formed elements (37-54% of blood volume) are suspended in the plasma, they include red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes). The distribution by volume of formed elements is 99.9% erythrocytes, and a combined 0.1% for leukocytes and platelets.

Platelets may be considered the smallest blood cells, though like RBCs they do not have a nucleus. They play a major role in blood clotting. The role of platelets is to stop bleeding at an injury site; they accomplish this by swelling, clumping together, and forming a sticky plug. Platelets will activate in high shear environments [77], sites of vessel injury, and when exposed to non-biocompatible medical implants. Vessel injury and non-biocompatible medical devices trigger an inflammatory response, where the platelets adhere to the exposed collagen and localized fibulin and fibrinogen [78].

Blood by Species

Porcine whole blood may act like human blood for intravascular thrombosis. The platelet count in the porcine model is higher than the platelet count in humans; therefore, porcine blood may be more likely to coagulate under flow conditions. If the model can be proven in the porcine model, then the vein valve may be successful in a human. The composition of blood by species is listed in Table 2. The blood composition of ovine is also listed, because an ovine animal trial is planned. Ovine blood also has a higher platelet count than humans; and will therefore provide strong thrombotic potential results when considering a clinical trial.

Table 2: Composition of blood by species and cell type [71, 76]

Species	Erythrocytes	Leukocytes	Platelets
Human Male	4,600,000 - 6,200,000 / μ L	4,500 - 11,000 / μ L	140,000 - 450,000 / μ L
Female	4,200,000 - 5,400,000 / μ L		
Porcine	5,000,000 - 8,000,000 / μ L	11,000 - 22,000 / μ L	250,000 - 850,000 / μ L
Ovine	9,000,000 - 15,000,000 / μ L	4,000 - 12,000 / μ L	250,000 - 750,000 / μ L

Blood Viscosity

Viscosity is a measure of the resistance of a fluid to deform under shear stress. Whole blood has a viscosity of 3 to 4 centipoises depending upon hematocrit, temperature, and flow rate [79]. The viscosity of plasma is about 1.8 times the viscosity of water at 37°C, and is related to the protein composition of the plasma. As hematocrit increases, there is a disproportionate increase in viscosity. As temperature decreases, viscosity increases. Viscosity increases approximately 2% for each degree Celsius decrease in temperature. The flow rate of blood also affects viscosity. At very low flow states the blood viscosity increases, due to cell-to-cell and protein-to-cell adhesive interactions that can cause erythrocytes to adhere to one another and

increase the blood viscosity. An acceptable average measure of viscosity for blood is 3.5 centipoises.

Biocompatibility of Poly(Vinyl) Alcohol (PVA)

PVA hydrogels have been used for numerous biomedical and pharmaceutical applications; such as, contact lenses, linings for artificial organs, development of artificial cartilage, vocal cords, drug delivery, soft tissue replacements, catheters, artificial skin, and hemodialysis membranes [80]. The biomedical advantages of PVA hydrogels include non-toxicity, non-carcinogenicity, bioadhesive characteristics, ease of processing, and shape-memory properties.

Peppas and Merrill conducted some of the earliest work on PVA hydrogels as a biomaterial, in 1977 [81, 82]. The gels were examined for their blood compatibility and elastic behaviors. These researchers further investigated the possible reconstruction of vocal cords and bio-membranes in artificial kidney applications.

Biocompatibility of PVA was investigated by Tamura *et al.* in 1986 [83]. The characteristics of the gel resembled those of natural tissue, and these characteristics were not affected after long term implantation. Subcutaneous or intramuscular implantations in rabbits showed good bioinertness. Despite initial presence of inflammatory cells, they disappeared within two weeks of implantation and showed no adhesion to surrounding tissues. They concluded that the material would be useful be clinically useful.

One cause of vessel trauma is the implantation of percutaneous devices; therefore, an inflammation reducing drug could be incorporated into the PVA matrix to reduce or prevent inflammation. PVA gels have been extensively investigated in terms of their

diffusive characteristics. Peppas and collaborators analyzed drug and protein diffusion [84]; and in another study investigated the transport of oxygen across a PVA membrane [85].

Relevant Previous PVA Vein Valve Results

The PVA vein valve was previously evaluated for patency, competency and cyclic life [86]. The valve withstood 300 mm Hg of backpressure with less than 0.3 mL leakage per minute, demonstrated a burst pressure of 530 ± 10 mm Hg , opened with a pressure gradient as low as 2.0 ± 0.5 mm Hg, and met criteria 1 and 2 after 500,000 cycles of cyclic testing.

CHAPTER 2

METHODS: THROMBOTIC POTENTIAL

Experimental Design

The purpose of this work was to evaluate the thrombotic potential of the PVA vein valve in a bench-top analysis. A flow system with venous flow conditions was designed and the PVA valves were attached to this system. Two flow systems were utilized, the initial flow system design was modified from a system previously designed in our lab [2], the second flow system utilized a pulsatile pump to imitate the venous pulsatile environment.

Valve Manufacture

The PVA valves were manufactured according to the procedure outlined in “Design and Development of a Novel Implantable Prosthetic Vein Valve”, by Rahul Sathe. The valve material was a 15% poly(vinyl-alcohol) hydrogel, manufactured per U.S. Patent 5,981,826. The valves were fabricated by injecting PVA into a two-part silicone cavity mold, and subjecting the valves to a freeze-thaw curing process. The curing process consisted four 12-hour freeze periods intermittent with three 6-hour thaw periods. The valve orifice was then cut open with a two-sided surgical blade (no. 11, Miltex, York, PA) by placing the blade tip at the center of the orifice and applying pressure down through the orifice to separate the leaflets with one slice, thereby reducing the jagged edges associated with cutting the orifice with scissors.

Blood Collection

Fresh blood was collected from an abattoir (Holifield Farms, Covington, GA). Whole blood samples of approximately 1000 mL were harvested from pigs and quickly anti-

coagulated with 1000 USP units/mL porcine heparin (Elkins-Sinn Inc. Cherry Hill, NJ) at 6.0 ± 0.2 U/mL. The samples were transported to the laboratory in an insulated container, to limit temperature fluctuations during transport. The blood was able to drop in temperature from 37 °C (body temperature) to 20 °C (room temperature). In the laboratory, the collection time, volume, heparin condition, and presence of aggregates was recorded. Samples free of bubbles and aggregates were selected for experimental processing. The selected sample was mixed with a nutating rocker (Shelton Scientific) at approximately 42 rpm for 15 minutes prior to the experiment. Experiments were completed within eight hours of harvesting the blood and conducted at room temperature.

The heparin dosage was intentionally low compared to clinical heparin dosages, as this increases the likelihood of *in vitro* thrombosis. *In vitro* human donated blood experiments testing the deposition of platelets on collagen or under stasis conditions have used heparin dosages of 10 U/mL and 20 U/mL (LMWH) [87-89]. In an *ex vivo* porcine study, low levels of heparin (1.37 ± 0.036 U/mL) did not significantly affect platelet deposition [90]; therefore, the low heparin dosages used in the blood collection for this thesis should minimally affect platelet deposition. A heparin concentration of 6.0 ± 0.2 U/mL was selected based on previous *in vitro* analysis [2].

Non-pulsatile Model

The flow system was modified from a one-pass, flow-through thrombosis assay using whole blood [2]. 250 mL of fresh, whole, porcine blood with heparin (6.0 ± 0.2 U/mL) was transferred into a blood donor collection bag (Pall Corporation, East Hills, NY). The collection bag was raised 30 cm above the test section, rotated on an orbital mixer (Labnet Int., Woodbridge, NJ), and attached to 90 cm of vinyl tubing (3mm inner diameter), followed by a 3-way valve, a pressure tap and the test section. Pressure

upstream of the vein valve was recorded with a pressure transducer (Harvard Apparatus, South Natick, Massachusetts) and the flow rate was calculated from measurements with a graduated cylinder and a stopwatch. The experiment proceeded until flow cessation or the contents of the fluid reservoir were emptied. The flow set-up is illustrated in Figure 20 and Figure 21.

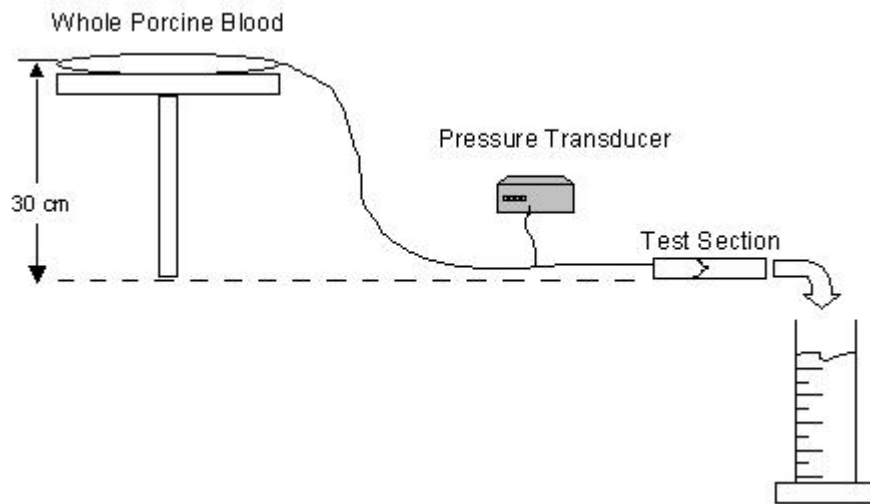


Figure 20: Diagram of non-pulsatile flow set-up

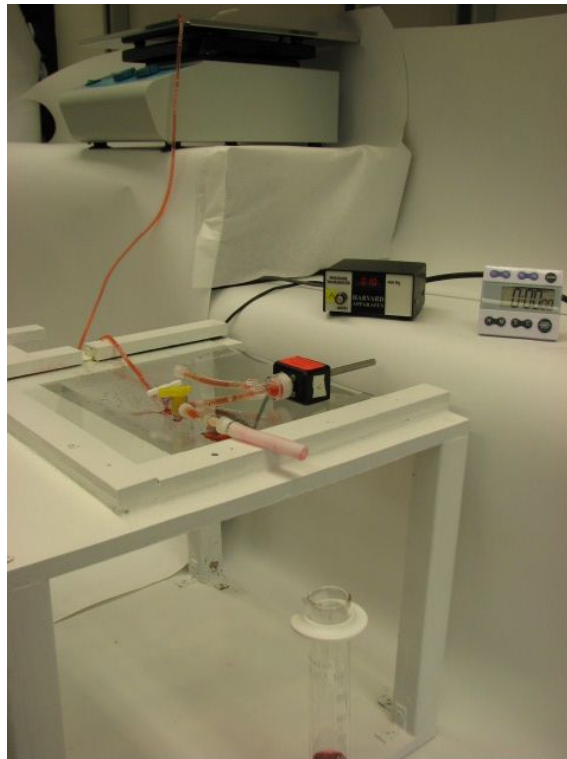


Figure 21: Non-pulsatile flow set-up

Pulsatile Model

The flow system was modified from a one-pass, flow-through thrombosis assay using whole blood [2]. This pulsatile system was designed to mimic the physiologic flow conditions present in the lower extremity venous system. During normal walking, calf compression propels 10 to 20 mL of blood through the veins, and compression occurs about 40 times a minute (0.67 Hz) [23, 25]. In accordance to previous *in vitro* studies performed, a blood collection time of at least 20 minutes was selected; as this provides enough time for thrombus to form [2]. Taking the frequency and time into consideration, the pulsatile pump was set to 0.75 Hz. Since there are three journal bearings across the circumference of the pump head, each revolution of the pump head accounts for three expansions and contractions of the tube. For instance, one revolution of the pump head took 4 seconds, so the frequency of the pump was 3 cycles / 4 seconds = 0.75 Hz. Fresh, whole, porcine blood with heparin (6.0 ± 0.2 U/mL) was transferred into a blood donor collection bag (Pall Corporation, East Hills, NY). The collection bag was raised 30 cm above the test section, rotated on an orbital mixer (Labnet Int., Woodbridge, NJ), and attached to 90 cm of vinyl tubing (3 mm inner diameter), followed by a 3-way valve, a pressure tap and the test section. Downstream of the test section, a 50 cm segment of tubing (3.5 mm inner diameter) was passed through a Masterflex pulsatile pump (Model 7520-25, Cole Parmer. Co, Chicago, IL) rotating at a frequency of 0.75 Hz. Pressure upstream of the vein valve was recorded with a pressure transducer (Harvard Apparatus, South Natick, MA) and the flow rate was calculated from measurements with a graduated cylinder and a stopwatch. The experiment proceeded until flow cessation or the contents of the fluid reservoir were emptied. The flow set-up is illustrated in Figure 22 and Figure 23.

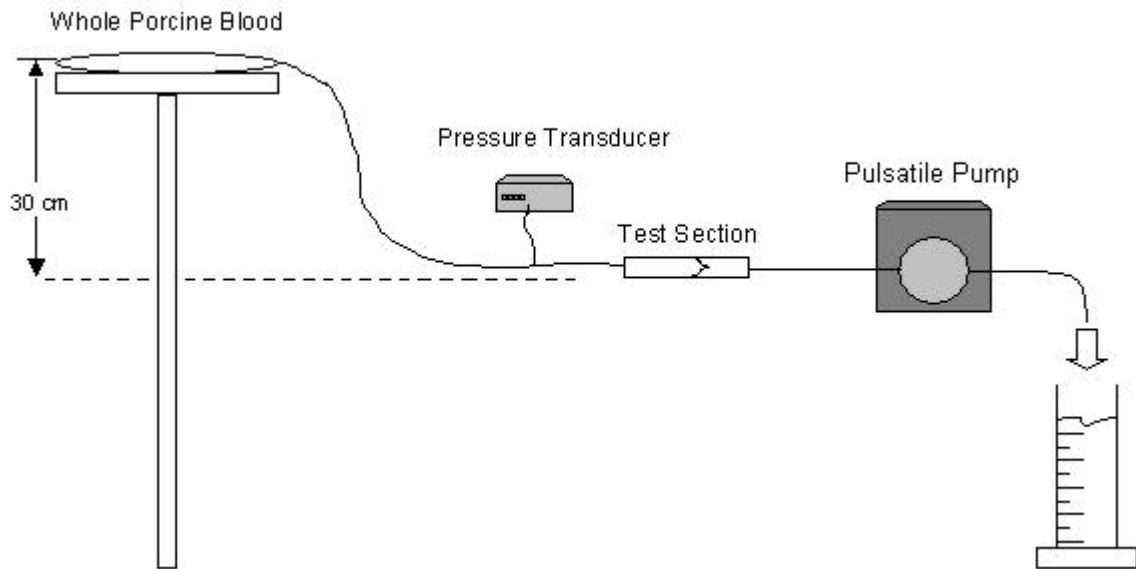


Figure 22: Diagram of pulsatile flow set-up

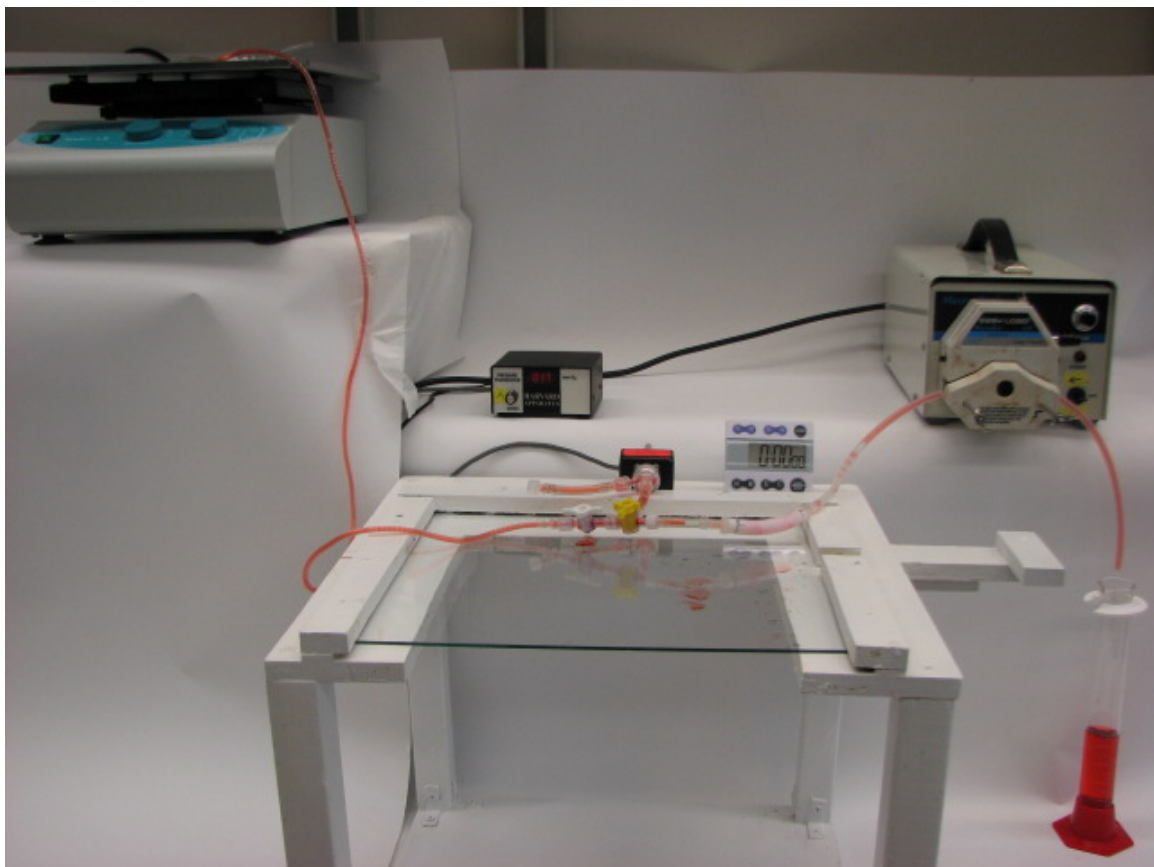


Figure 23: Pulsatile flow Set-up

Test Section

The test section included a vein valve, a flexible venous-like tube, and suture material.

The vein like tube was manufactured from the PVA hydrogel, described above. The valve was inserted into the flexible tube and tightly tied in place to prevent blood from passing between the valve and the vessel wall. The flexible tube was further attached to the vinyl tubing by securing it with suture.

Dacron Positive Controls

A different positive control was used for each the non-pulsatile and pulsatile experiments. A positive control reveals that a procedure works according to expectations; in the blood flow experiments, this refers to a system that can form thrombus. A Dacron sleeve was constructed for the non-pulsatile experiments. The Dacron sleeve did not effectively mimic the PVA valve as a positive control, as the sleeve manufacturing was dependent on sewing techniques and the downstream opening was not a consistent size. Therefore a Dacron lined valve was constructed for the pulsatile experiments, where the geometry directly imitated the PVA valves.

Dacron Sleeve

A Dacron sleeve was constructed out of a polyester blend material, suture and a steel wire. The wire was bent into a 10-12 mm loop. The polyester was wrapped around the wire so that the material edges were positioned on the outside, it was then sewn to create a cone shaped sleeve, with a 10 mm opening upstream and 1 mm opening downstream, as seen in Figure 24. The Dacron sleeve was used as a positive control in the system.

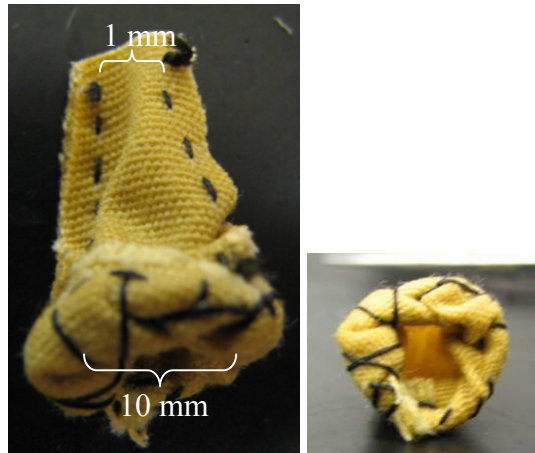


Figure 24: Dacron Sleeve

Dacron Lined Valve

A Dacron lined valve was constructed from a cardiovascular Dacron patch (Hemashield Finesse, Boston Scientific Co., Oakland, NJ), suture and a PVA valve. Two same size pieces of Dacron were sized to fit from the top of the upstream side, through the orifice, to just beyond the edge of the leaflets downstream. The approximate Dacron size was $14 \text{ mm} \pm 1 \text{ mm}$ by $9 \text{ mm} \pm 0.5 \text{ mm}$. One stitch was placed on each Dacron piece on the upstream side, these sutures held the Dacron against the PVA valve. A Dacron lined valve is illustrated in Figure 25.



Figure 25: Dacron lined Valve

Opening Pressure Test

Opening pressure was measured by applying distal pressure on the valve and reading the pressure when water was visible downstream of the leaflets. A syringe was attached to a three-way valve with the test section and the pressure transducer (Harvard Apparatus, South Natick, MA); downstream the test section was open to atmosphere, as seen in Figure 26. The vein valve was orientated such that the distal end of the valve was closest to the syringe, and the proximal end was facing the ambient atmosphere. The test section was secured to the system with suture. Pressure was applied with the syringe and the valve was monitored for water collecting on the proximal side of the leaflets. The pressure indicated on the transducer when water collected downstream was recorded as the opening pressure for that valve.

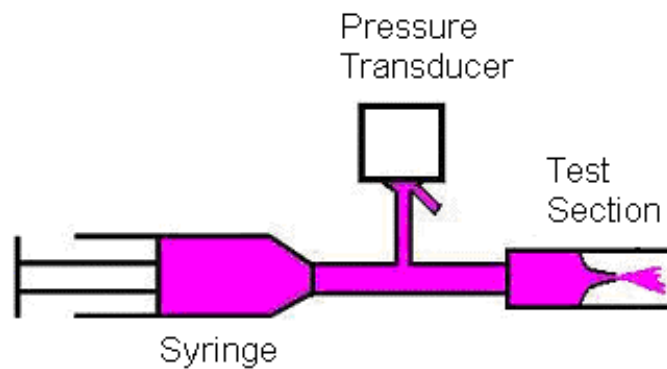


Figure 26: Opening Pressure Test set-up

Backpressure Test

Reflux leakage was measured by applying proximal pressure on the valve and reading the pressure when water was visible downstream of the leaflets. A syringe was attached to a three-way valve with the test section and the pressure transducer (Harvard Apparatus, South Natick, MA); downstream the test section was open to atmosphere, as seen in Figure 27. The vein valve was orientated such that the proximal end of the valve was

closest to the syringe, and the distal end was facing the ambient atmosphere. The test section was secured to the system with suture. Pressure was applied with the syringe and the valve was monitored for water collecting on the distal side of the leaflets. Pressure was measured up to 100 mm Hg, as to prevent valve failure. The pressure indicated on the transducer when water collection was observed was recorded as the backpressure for that valve.

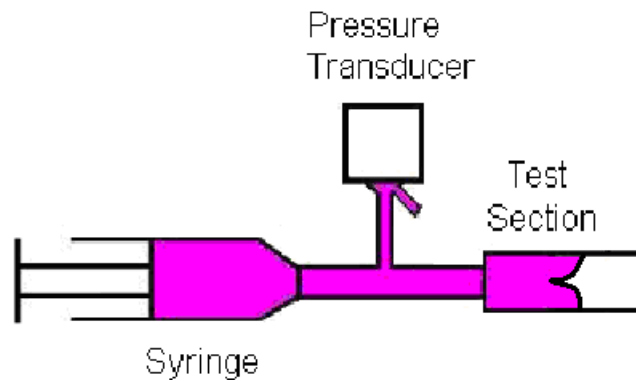


Figure 27: Backpressure test set-up

Histology

Samples were fixed in 10% formalin (VWR International, West Chester, PA) for at least 72 hours. Formalin fixes at 1mm/day; in the thickest area, the sample was approximately 2mm; therefore, 72 hours was sufficient. Samples were processed and embedded in paraffin. Deformation of the samples during processing was expected to be between 30 to 50%. Samples were cut into 5-micron thin circular cross-sections, oriented perpendicular to flow. Eight sections from orifice areas, leaflet contact areas, were collected from each sample. Even numbered samples were stained with Haematoxylin and Eosin stain (H&E), and odd numbered samples were stained with Carstairs' stain. Sections were microscopically analyzed using a Nikon E600 microscope, a digital camera and Q-capture software. Three magnifications were captured: 4x, 10x, and 20x.

H&E Stain

H&E stain is the most widely used stain in medical diagnosis, it stains for basophilic and eosinophilic structures. H&E stain was prepared using a standard program in the auto-stainer (Leica AutoStainer XL, Wetzler, Germany). The procedure included three changes of Xylene Substitute; two changes of 100% alcohol; water washes intermittent with exposure to Hematoxylin, acid alcohol, Scott's, and Eosin; followed by three changes of alcohol; and two changes of Xylene substitute. The following structures may be identified: nuclei (blue); erythrocytes and eosinophilic granules (bright pink to red); and cytoplasm and other tissue elements (various shades of pink).

Carstair's Stain

Carstair's Stain is used to detect fibrin and platelets in clotted blood or thrombus. The Carstair's Stain procedure was obtained from Sheehan's Theory and Practice of Histotechnology [91]. The samples were deparaffinized and rehydrated in distilled water. To mordant, 5% ferric alum was exposed to the sections for 10 minutes and rinsed in tap water. To stain, Harris' hematoxylin was exposed for 5 minutes, rinsed in tap water, and then exposed to picric acid-orange G solution (80 mL saturated picric acid, 320mL isopropanol, 0.8g orange-G) for 20 minutes (up to 1 hour). The sections were then rinsed in distilled water and stained in ponceau fuchsin (2.0g acid fuchsin, 2.0g pnceau 2R, 4mL glacial acetic acid, 396mL distilled water) for 5 minutes. To differentiate, the sections were exposed to 1% phosphotungstic acid for 2 minutes, and then rinsed well in water. The final staining was to expose the sections to aniline blue solution (4.0g aniline blue, 400mL 1% acetic acid) for 10 minutes. Samples were rinsed in distilled water, dehydrated, and mounted. The following structures may be identified: fibrin (red), muscle (red), platelets (gray-blue to navy), collagen (bright blue), and red blood cells (clear yellow).

CHAPTER 3

RESULTS: THROMBOTIC POTENTIAL

Non-pulsatile Blood Flow

The non-pulsatile flow system was designed to impose a constant upstream pressure of blood on the test section, similar to the pressure in the venous system. Blood was perfused through 7 PVA valves; flow was established for all test sections. The flow profile refers to the collected blood volume over time, which was plotted in terms of milliliters per minute. The upstream pressure over time was recorded in millimeters of mercury and plotted with their corresponding blood volume graphs. All 7 PVA valves demonstrated significantly different flow profiles, as illustrated in Figure 28 through Figure 34. The flow profiles presented in Figure 28 through Figure 34 illustrate both the volumes collected and the pressure fluctuations over time. Valves 1, 5, 6, and 7 produced very little flow, none of them ever passing more than 23 mL of blood through the valve orifice, the upstream pressures were relatively constant at 24, 21, 21, and 23 mmHg, respectively. The limited flow could have been attributed to the pooling of blood downstream of the valve, since the valve was naturally in a closed position, it is hypothesized that stagnant blood collected upstream of the leaflets. Valve 2 produced an “S” shaped profile with a gradual cessation of flow; the pressure remained fairly constant around 24 mmHg. Valve 3 had a reasonably constant flow with exhaustion of blood at 32.5 min; the pressure measurements fluctuated between 17 to 20 mmHg. Valve 4 was an interesting case where the flow rate increased and decreased throughout the run. Periodic adjustments of the blood bag would initiate the sudden increase in flow. The pressure would spike just prior to the flow increasing, indicating that an increase in pressure would re-establish flow. Pressure measurements varied between 10 to 19 mmHg throughout the run. All valves were photographed before and after attachment to the

apparatus, as presented in Figure 35. Figure 35 illustrates a valve prior to placement in the apparatus where the orifice is intact and clean; also, a valve after removal from the apparatus where blood has collected on the leaflets and orifice area.

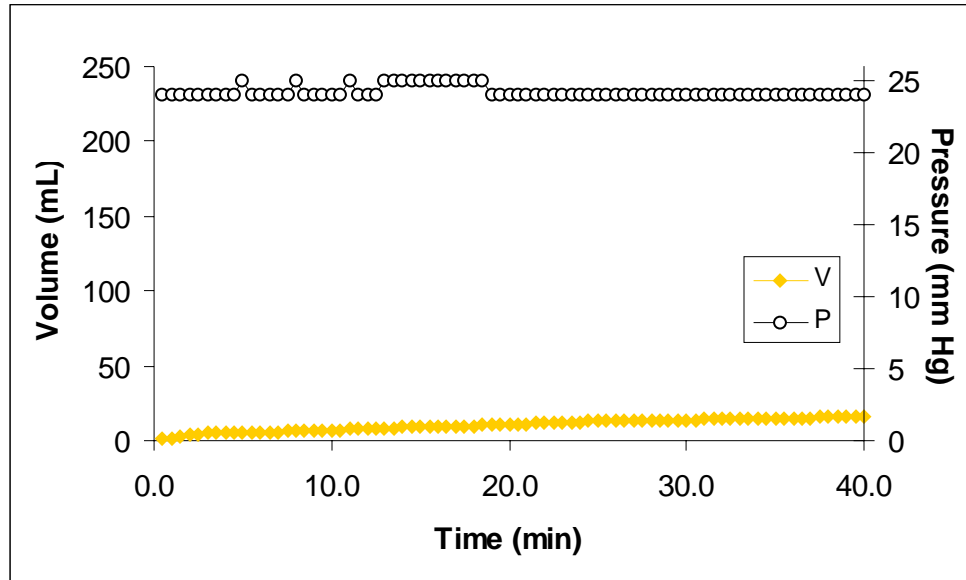


Figure 28: Non-pulsatile flow of porcine blood through PVA valve 1

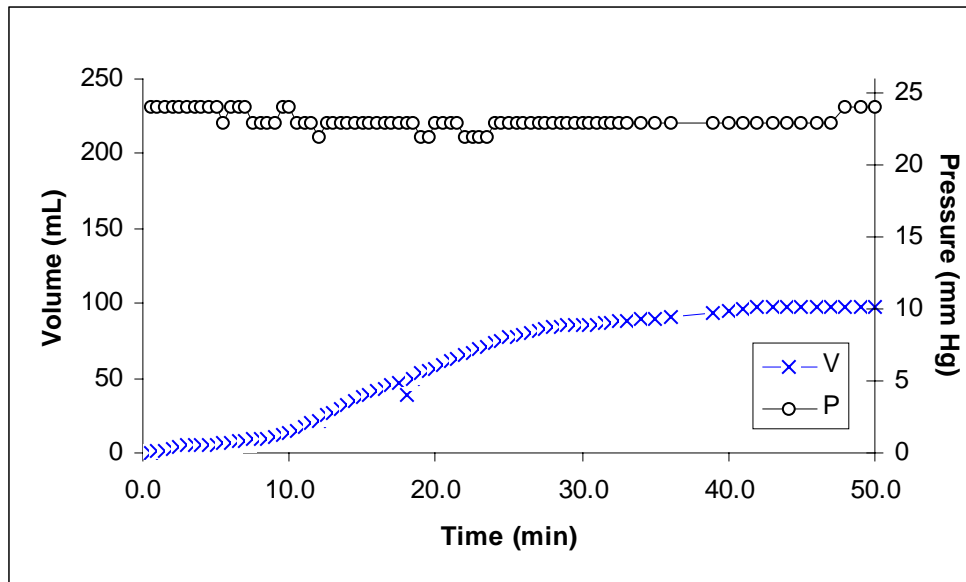


Figure 29: Non-pulsatile flow of porcine blood through PVA valve 2

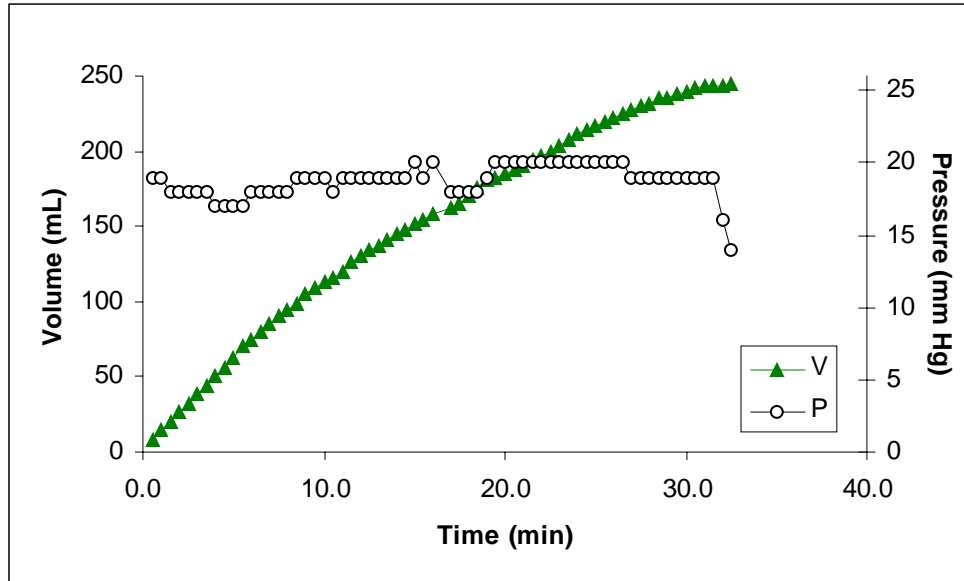


Figure 30: Non-pulsatile flow of porcine blood through PVA valve 3

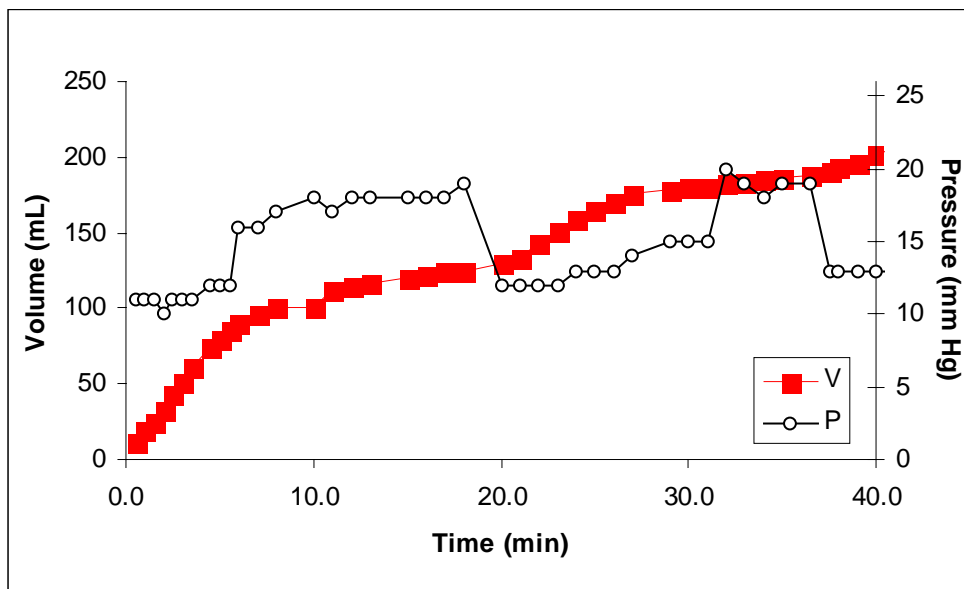


Figure 31: Non-pulsatile flow of porcine blood through PVA valve 4

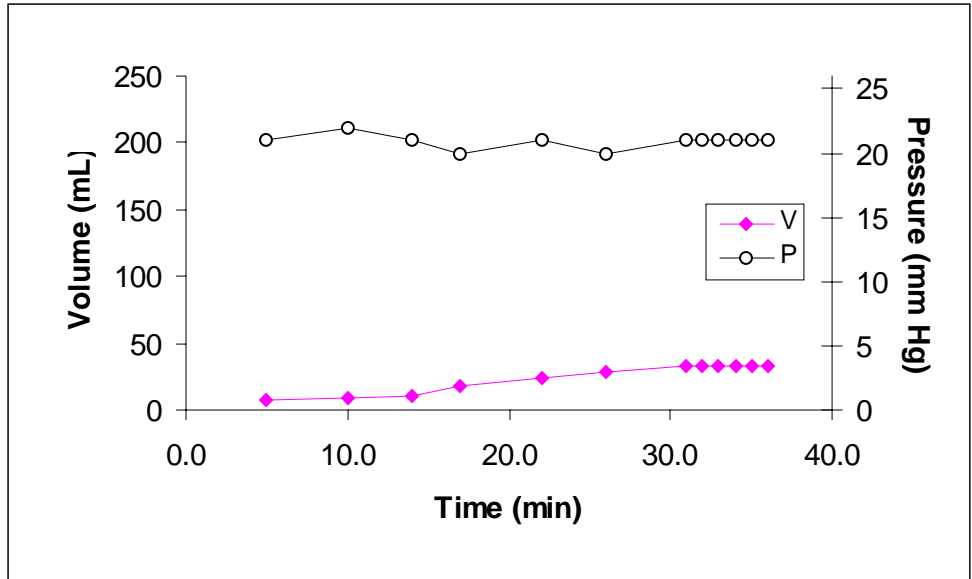


Figure 32: Non-pulsatile flow of porcine blood through PVA valve 5

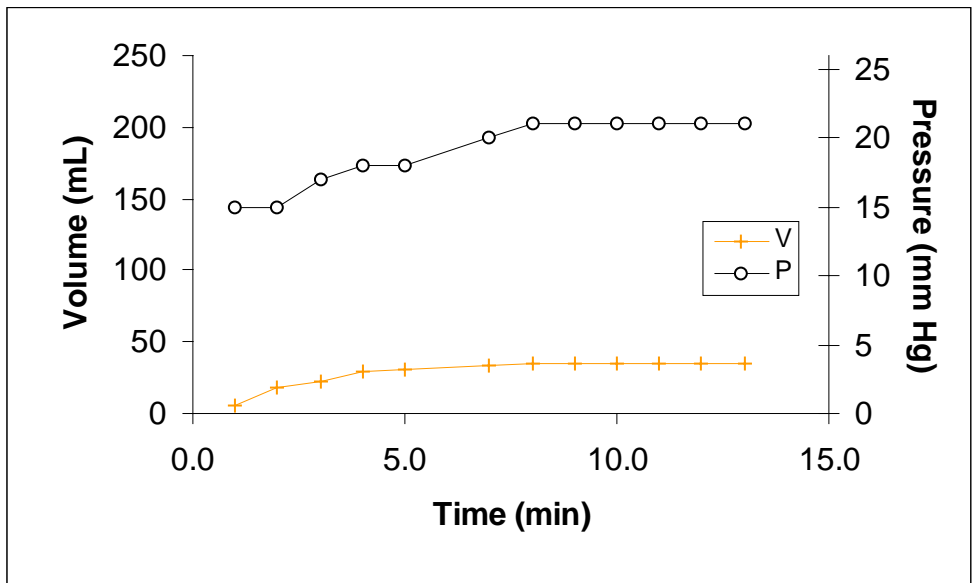


Figure 33: Non-pulsatile flow of porcine blood through PVA valve 6

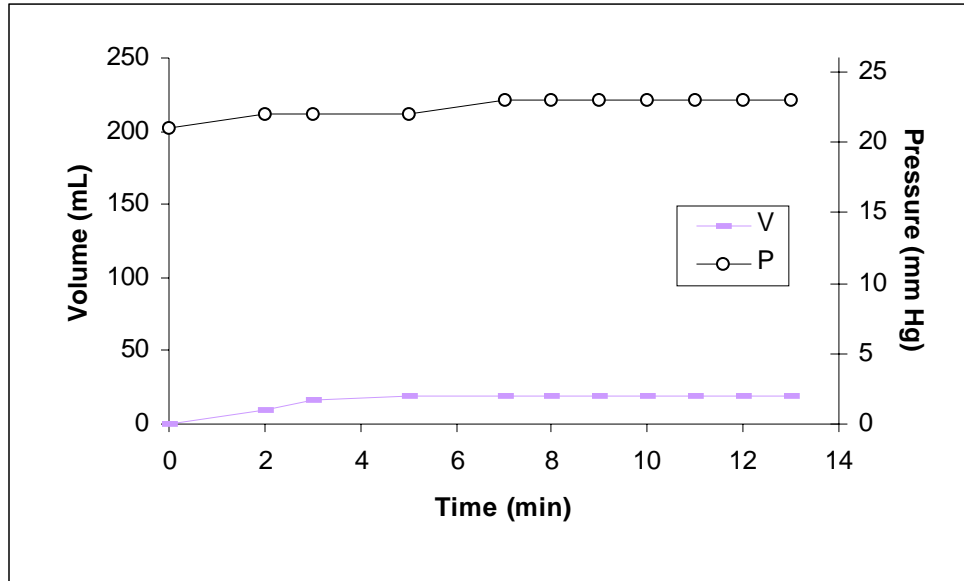


Figure 34: Non-pulsatile flow of porcine blood through PVA valve 7

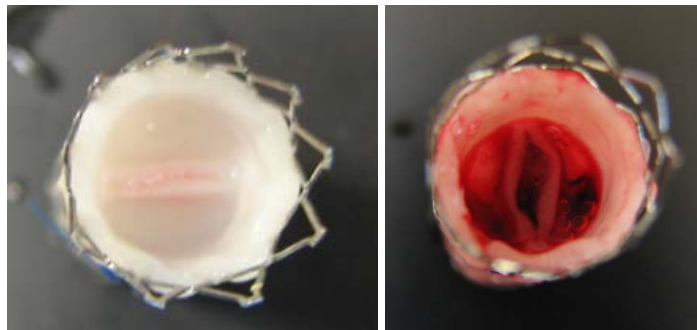


Figure 35: PVA valve (A) prior to placement, and (B) after placement in the non-pulsatile blood system

Visual confirmation that blood was flowing through the valve, as opposed to around the valve, was accomplished by noting no visible blood between the valve and the PVA tube, as seen in Figure 36. Upstream and downstream of the valve the tube appeared red; though, at the valve location the tube appeared white, since at this location the PVA valve was flush with the PVA tube.

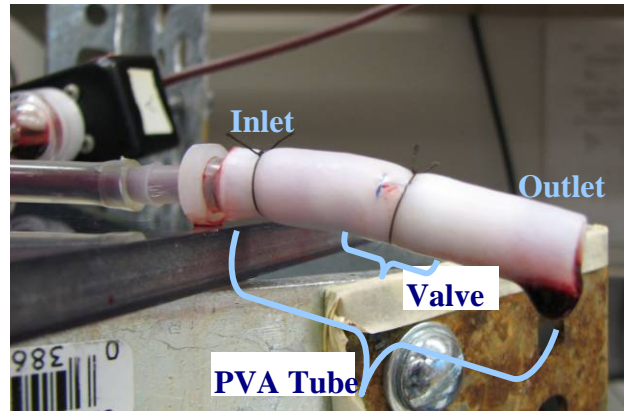


Figure 36: Close-up of test section in non-pulsatile system

A Dacron sleeve was placed in the flow set-up as a positive control. The Dacron sleeve for both before placement and after removal from apparatus is illustrated in Figure 39. As a positive control, the Dacron should have occluded the system faster, compared to the PVA valves. Two Dacron sleeves were evaluated and both demonstrated very different flow profiles, as illustrated in Figure 37 and Figure 38. Dacron sleeve 1 produced very little flow, never passing more than 24 mL through the valve orifice, the pressure upstream was a constant 24 mmHg. Dacron sleeve 1 acted very similar to valves 1, 5, 6, and 7. Dacron sleeve 2 started with a fairly steep flow profile, though there was a gradual cessation of flow at 25 min. The flow of Dacron sleeve 2 behaved similar to valve 3, though the pressure profiles were different. Dacron sleeve 2 had a gradual increase in the pressure as the flow started to slow; whereas the upstream pressure on valve 3 did not modulate in unison with the flow. The Dacron sleeves were not identical, as the construction was variable due to sewing technique. The flow profile variations could possibly have been caused by the construction variability. A Dacron sleeve attached to the flow set-up is illustrated in Figure 41. The polyester material bulked on the exterior of the metal ring, and when placing the Dacron sleeve inside the vein like tube it would distend the tube. Regardless, the upstream inside diameter was $10 \text{ mm} \pm 1 \text{ mm}$, which is consistent with the PVA valves. The material composition of the polyester was not confirmed as 100% polyester, as the material was purchased from a fabric store and this

information was not available. This unknown material content could have attributed to the unexpected flow profile of Dacron sleeve 2. Evident from the non-reproducible results of the PVA and Dacron flow profiles, the Dacron sleeve did not successfully perform as a positive control. A summary of the flow profiles of all PVA valves and Dacron sleeves are illustrated in Figure 40. This system did not provide a consistent flow profile for the PVA valves; therefore, the pulsatile flow set-up was designed.

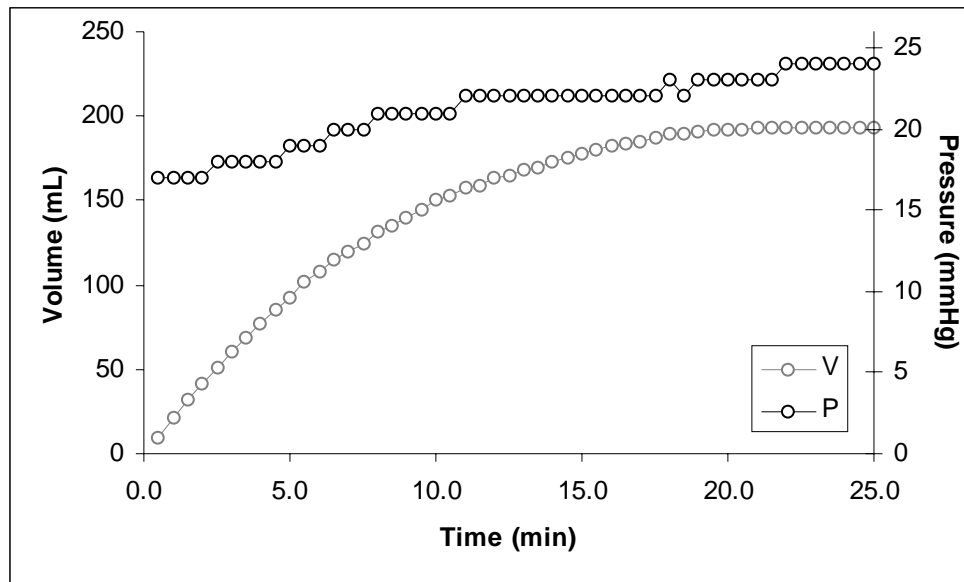


Figure 37: Non-pulsatile flow of porcine blood through Dacron Sleeve 1

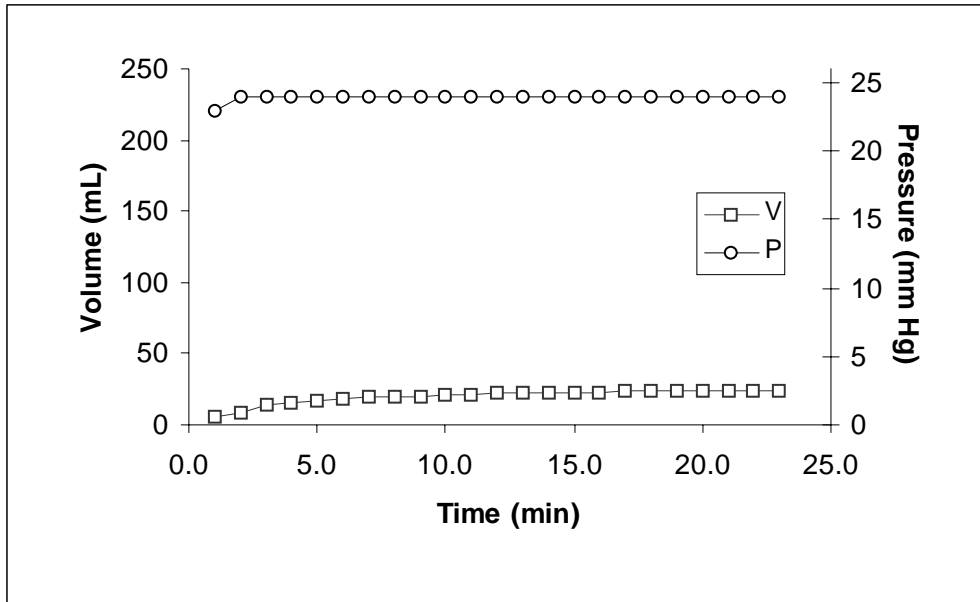


Figure 38: Non-pulsatile flow of porcine blood through Dacron Sleeve 2



Figure 39: Longitudinal View of Dacron Sleeve (A) prior to blood flow exposure, and (B) after blood flow exposure. Cross-sectional View of Dacron Sleeve (A) prior to blood flow exposure, and (B) after blood flow exposure

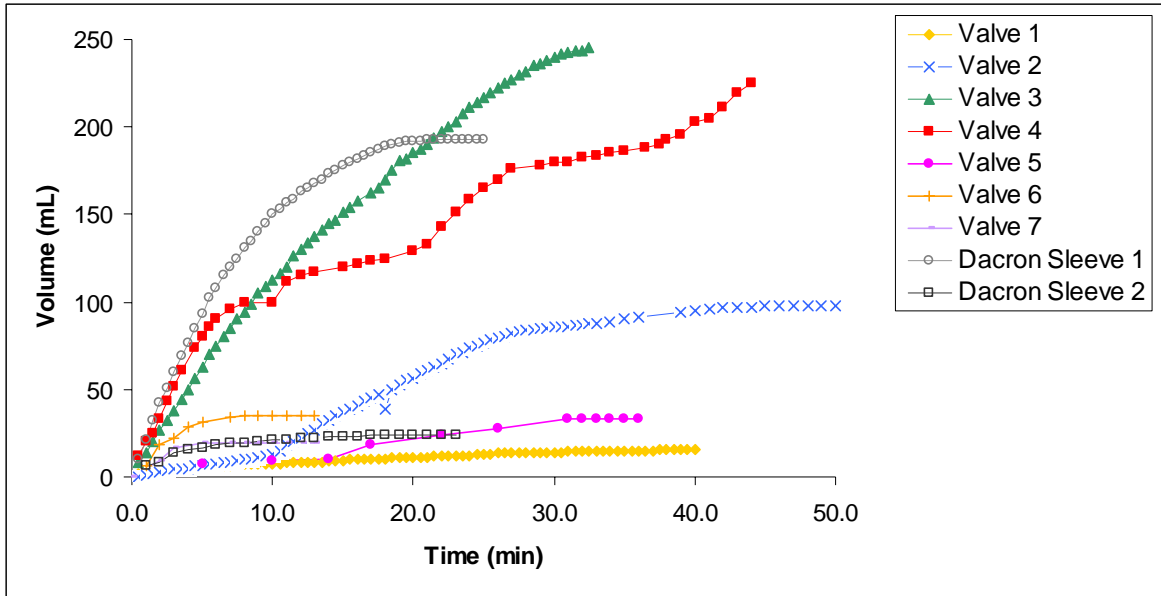


Figure 40: Non-pulsatile flow through PVA valves and Dacron Sleeves

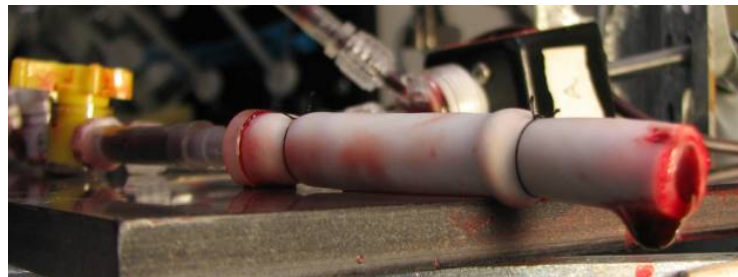


Figure 41: Dacron sleeve attached to non-pulsatile system

Pulsatile Blood Flow

Blood was perfused through five PVA vein valves. All five valves remained patent after 20 minutes of blood flow without significant flow rate deterioration. A patent valve attached to the pulsatile blood set-up is shown in Figure 50. The flow rates and corresponding pressure measurements for each valve and Dacron lined valves are shown in Figure 42 through Figure 49. PVA valve 1 through 5 all produced the same volume flow profiles, though their pressure profiles are slightly different. The range on each pressure measurement point corresponds to the stop and start of flow from the pulsatile

pump. The pressure for PVA valve 1 is between 13 – 15 mmHg, this is low compared to all other PVA valves. The pressure range for valves 2 through 5 is 15 – 21 mmHg. Once the system was exhausted of blood the pressure dropped off to just above 10 mmHg, it did not drop further since the system was not airtight. All Dacron lined valves displayed a gradual cessation of flow. The Dacron lined valves, 1 through 3, passed 35, 83, 104 mL of blood prior to cessation of flow at 2, 7, and 9 minutes, respectively. The pressures fluctuated until flow cessation, where they then produced a relatively constant pressure output of 25, 23 and 24 mmHg.

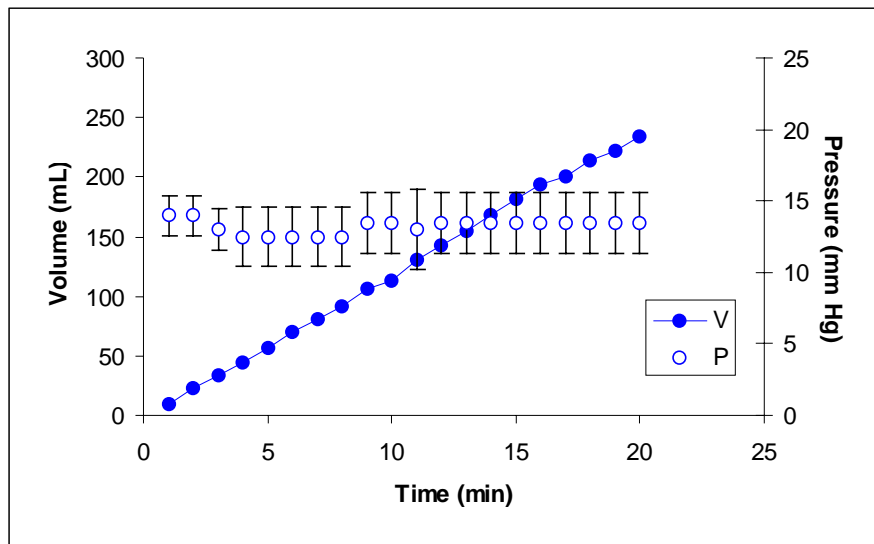


Figure 42: PVA Valve 1, blood volume collection and pressure fluctuation measurements

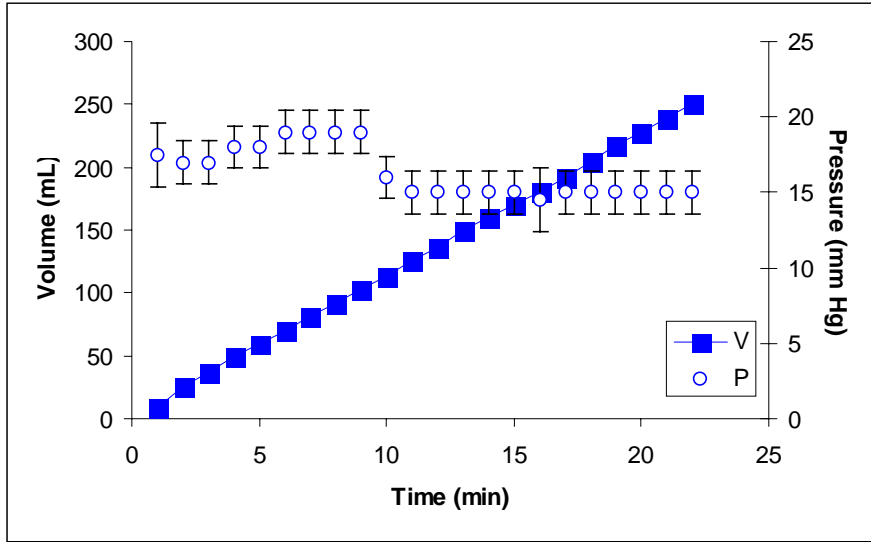


Figure 43: PVA Valve 2, blood volume collection and pressure fluctuation measurements

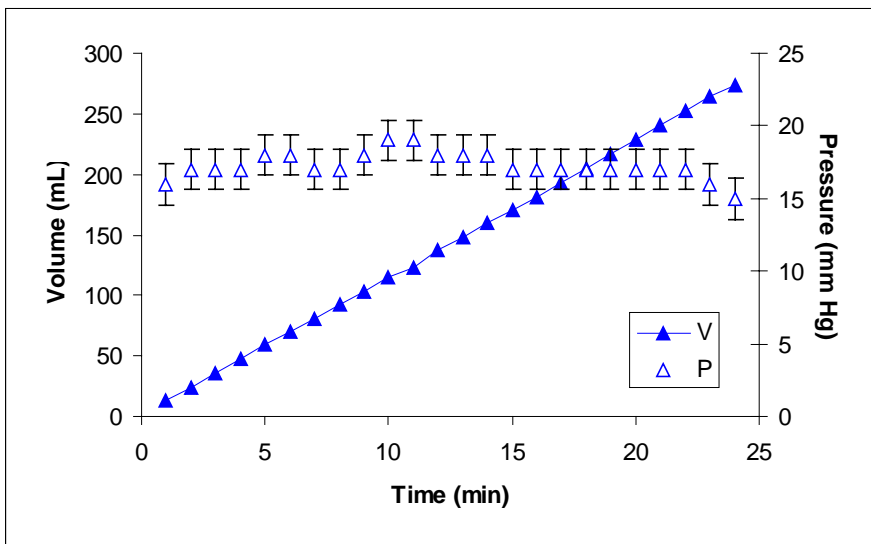


Figure 44: PVA Valve 3, blood volume collection and pressure fluctuation measurements

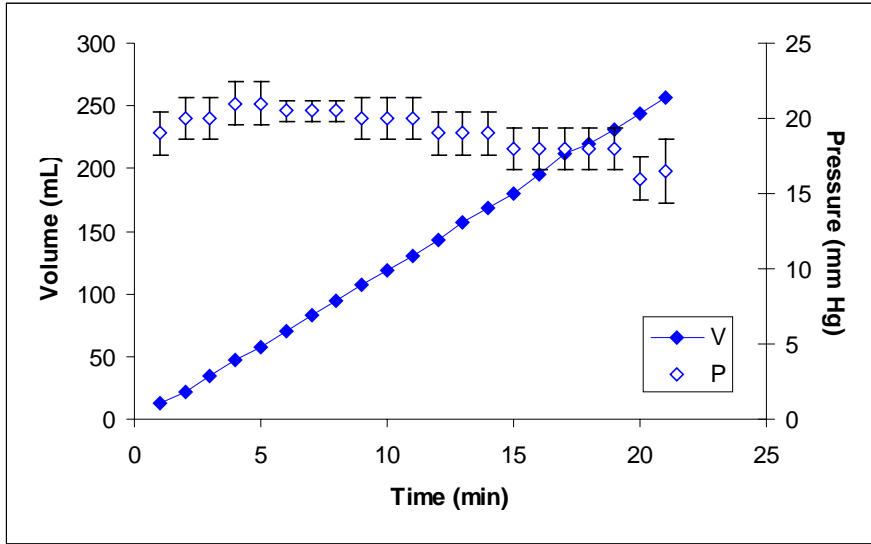


Figure 45: PVA Valve 4, blood volume collection and pressure fluctuation measurements

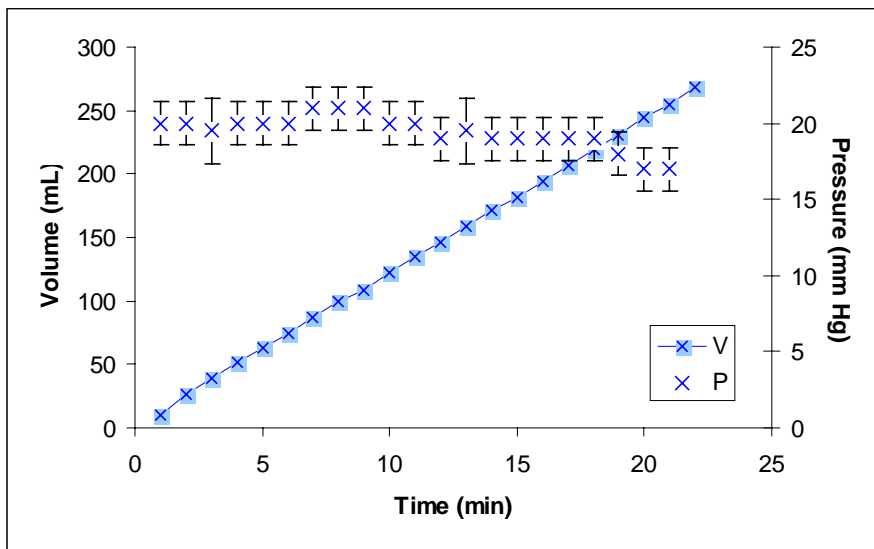


Figure 46: PVA Valve 5, blood volume collection and pressure fluctuation measurements

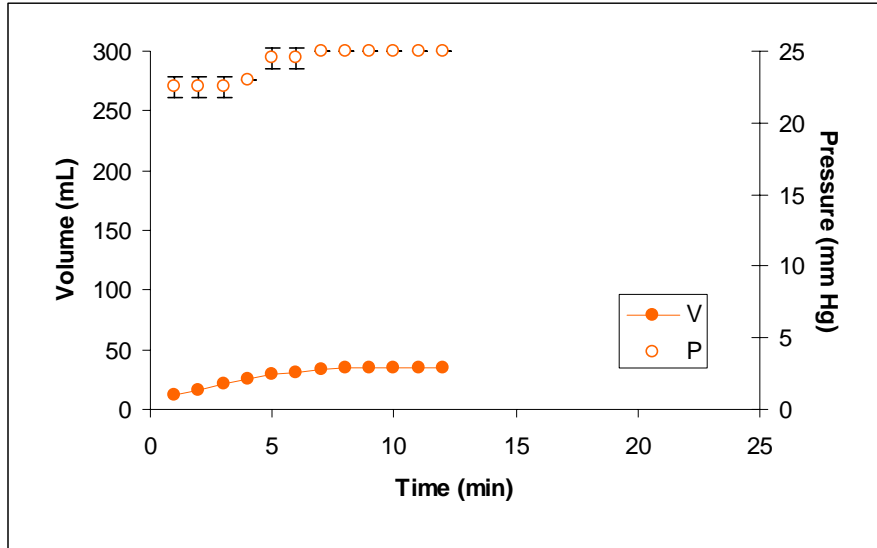


Figure 47: Dacron Lined Valve 1, blood volume collection and pressure fluctuation measurements

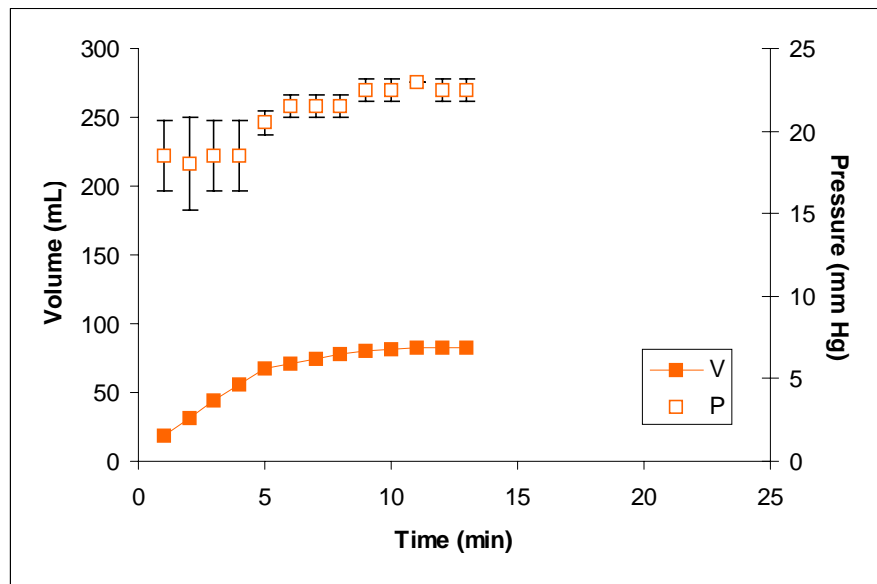


Figure 48: Dacron Lined Valve 2, blood volume collection and pressure fluctuation measurements

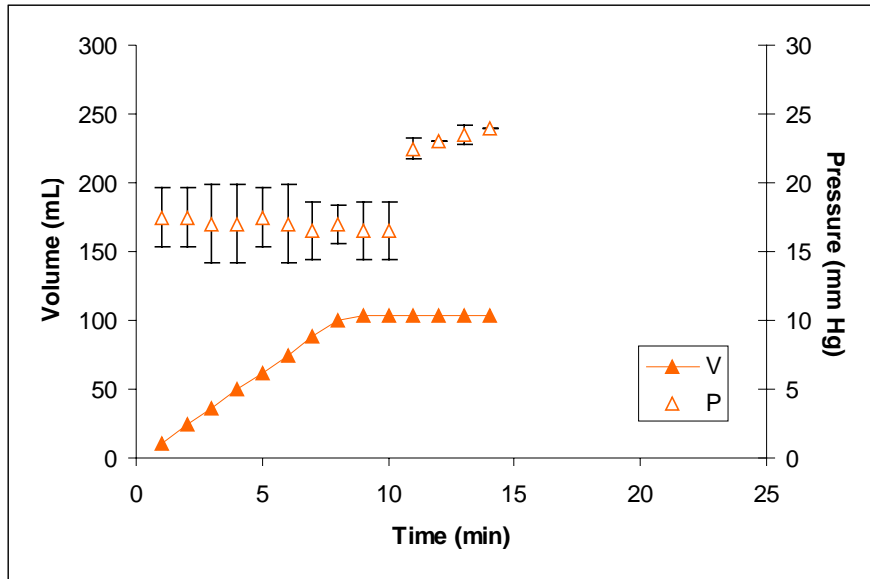


Figure 49: Dacron Lined Valve 3, blood volume collection and pressure fluctuation measurements

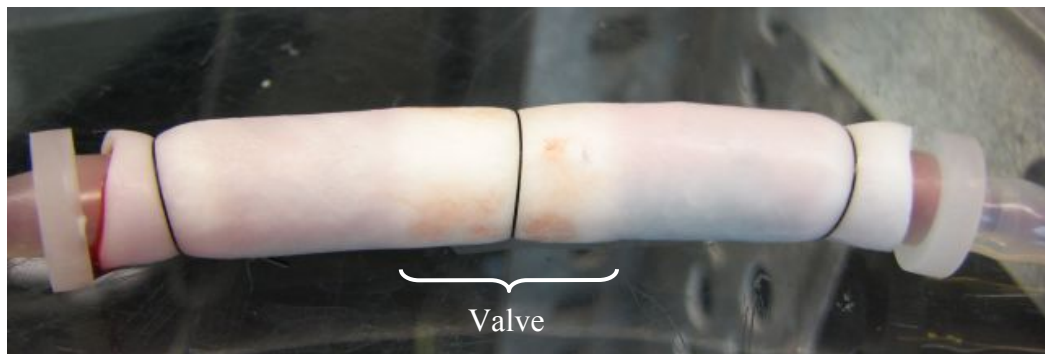


Figure 50: Test section with patent valve.

The average blood flow rate of 11.8 ± 0.4 mL/min through the valves remained constant for greater than 20 minutes, as shown in Figure 51. There was no gross thrombus visible on any of the valve leaflets. The leaflets remained functional and the valves remained competent against backpressure. When the flow system depleted the blood reservoir the roller pump tried to suck blood through the valve, the entire valve would collapse due to the negative pressure, as seen in Figure 52.

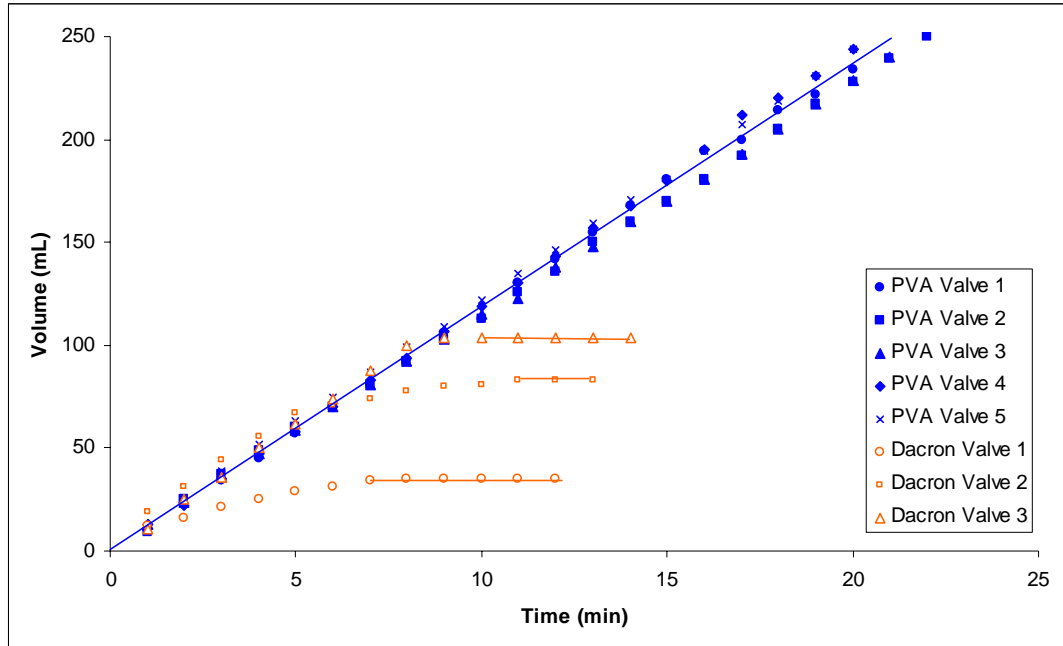


Figure 51: The PVA valves produced a constant flow rate; whereas, the Dacron lined valves produced a gradual cessation of flow.



Figure 52: Test section with a patent valve, though the reservoir of blood was exhausted and the pulsatile pump was still trying to draw blood.

The Dacron lined valves initially produced the same velocity profiles as the PVA valves; though, they did not remain patent for the experiment, but rather occluded completely. The flow rate reduced after two to eight minutes into the perfusion. On average, the polyester valves occluded after 6 ± 3.6 min of perfusion. The frequency of occlusion for the polyester valves in this assay was significant to $p < 0.02$.

The occluded valve created an unmistakable visible image as the roller pump tried to suck blood through the valve. With occlusion, the flexible tube collapsed violently

instead of the valve reopening. Thus, the system was a severe demonstration of the adherent nature of the occluding thrombus. A test section containing a collapsed downstream test section is shown in Figure 53. After removing the Dacron valves from the flow system, the polyester fibers were covered with blood and were visibly matted down, as seen in Figure 54. After occlusion, some red clot remained in the lumen of the tubes. The Dacron lined valve was preserved for histological analysis.



Figure 53: Test section containing a Dacron lined valve, the downstream section is collapsed due to cessation of flow.

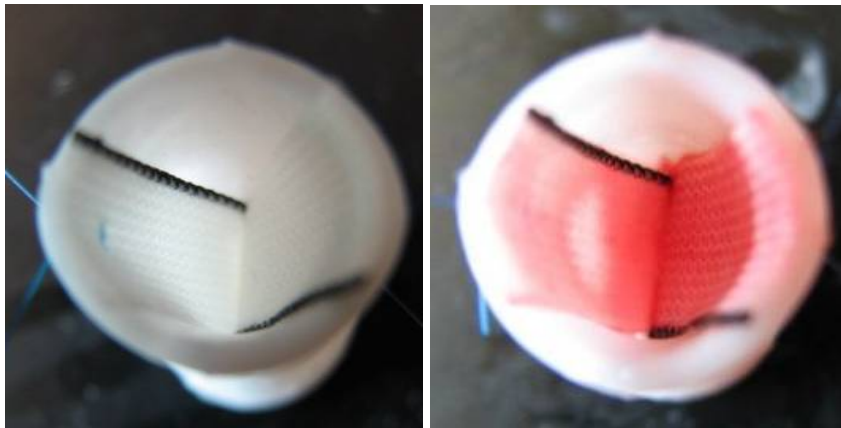


Figure 54: Left, Dacron lined valve prior to blood exposure; right, Dacron lined valve after blood exposure.

The Student's t-test was used to compare the time to occlusion between the PVA valves and the Dacron lined valves were statistically different. The p-value was 0.02. The time to occlusion for the PVA valves was assumed as >20 min, as seen in Table 3. The time to occlusion for the Dacron lined valves was averaged as 6 min, as seen in Table 3. The t-

statistic was 6.73, and the two tailed t-critical was 4.30. With a 98% confidence, the time to occlusion of the PVA valves versus the Dacron lined valves were statistically different.

Table 3: Data points for Student's t-test comparison between means

PVA Valve Time to occlusion (min)	Dacron Lined Valve Time to occlusion (min)
20	9
20	7
20	2
20	
20	

Glycerine

The pulsatile flow system was designed to test the thrombotic potential of the PVA valve, to eliminate the possibility that the Dacron leaflets would stick to each other in a non-blood environment. The Dacron cardiovascular patch is designed to readily attach to host tissues; therefore it is reasonable to assume it might attach to itself in a flow environment. An inert solution with the same viscosity as blood was perfused through the system. A 20% glycerine solution in water created a viscosity similar to blood, of 3.5 centiPoise. This solution was perfused through the pulsatile system with the PVA valve and subsequently the Dacron lined valve. The average flow rate of both the PVA valve and Dacron lined valve was 10.2 mL/min, as seen in Figure 55. Both the PVA valve and the Dacron lined valve developed similar flow profiles; therefore, the cessation of flow in the pulsatile system, perfused with blood, was the result of blood characteristics and not Dacron characteristics.

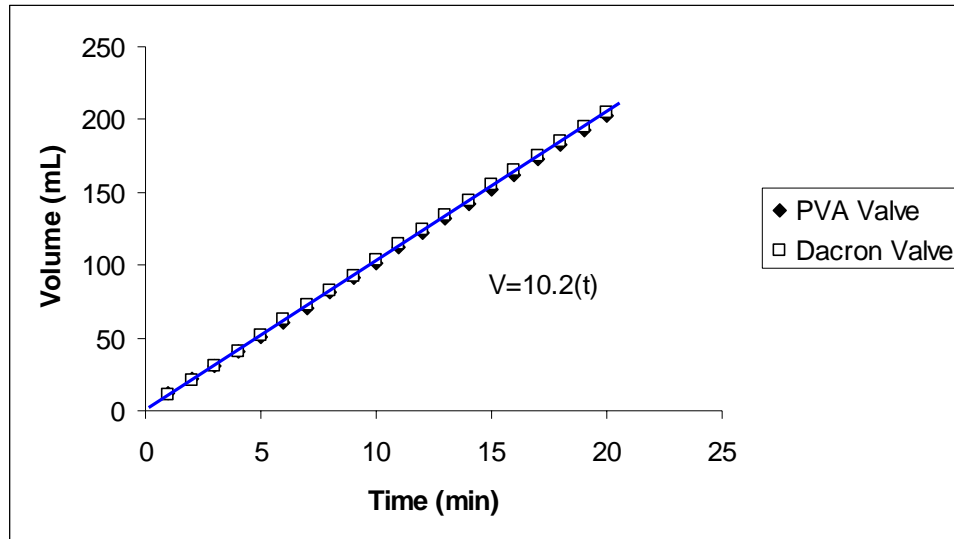


Figure 55: The PVA valve and the Dacron lined valve produce constant flow rates, in 20:80 Glycerine Solution

Histology

Histology was performed on both the PVA and Dacron lined valves to identify cell accumulation and the cause for Dacron cessation of flow. The histology stains utilized were Haematoxylin and Eosin stain (H&E), and Carstairs' stain (specific for platelets). A pictorial representation of how the PVA vein valve appears after histological processing is depicted in Figure 56. The appropriate centimeter magnification scales are depicted in Figure 57. A representative sample of the H&E staining is found in Figure 58 for a Dacron lined valve, and in Figure 59 for a PVA valve. A representative sample of the Carstairs' staining is found in Figure 60 for the Dacron lined valve, and in Figure 61 for the PVA valve. As the PVA was processed the leaflets shrank; and therefore, they appeared separated on the histological stains. As can be seen in the PVA valve slides, there was no cellular accumulation on the PVA valves.

The PVA material is represented as pink in the H&E stain and a faint blue-grey in the Carstairs' stain. With regard to the Dacron lined valve slides, the gray circular structures

represented the Dacron fibers. The red debris located between the Dacron leaflets represented the cellular material that was preventing blood from passing through the leaflets in the *in vitro* model. Further analysis with Carstairs' stain reveals that platelet aggregation was a key component in the red debris. The presence of platelets on the Dacron leaflets, and the complete absence of platelets on the PVA valves confirmed that the pulsatile blood flow set-up had the potential to thrombose, yet the PVA valves do not clot in this system.

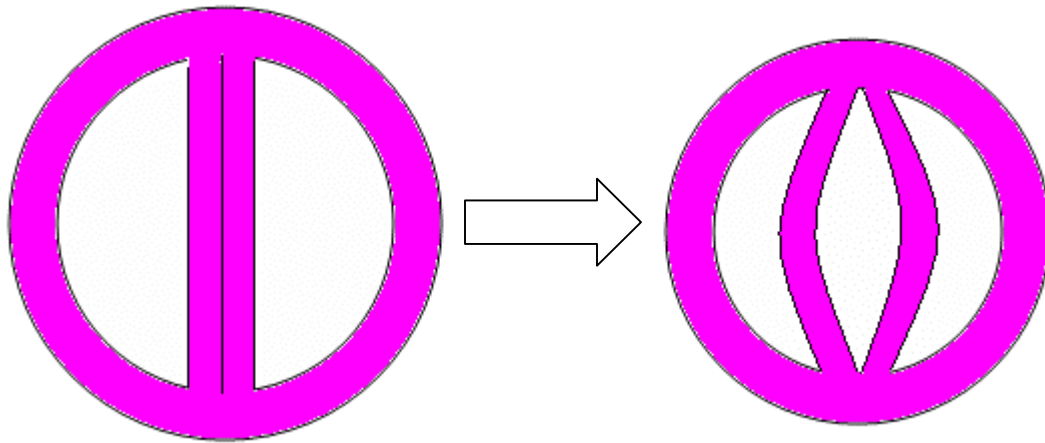


Figure 56: Pictorial representation of the shrinking effects of histological processing on the PVA vein valves; left, profile prior to processing; right, profile after processing.

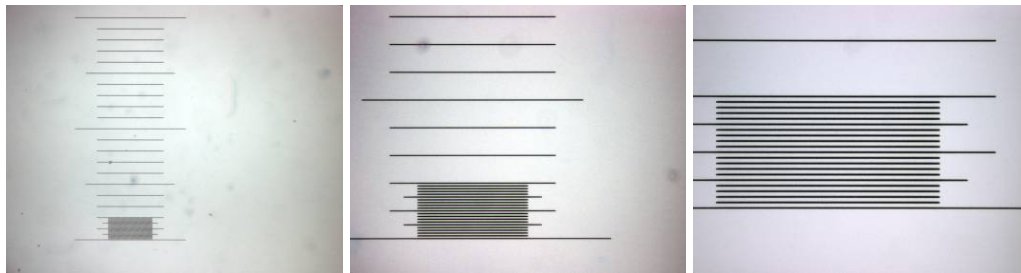


Figure 57: Centimeter magnification scales for histology images; (A) 4x, (B) 10x, and (C) 20x.

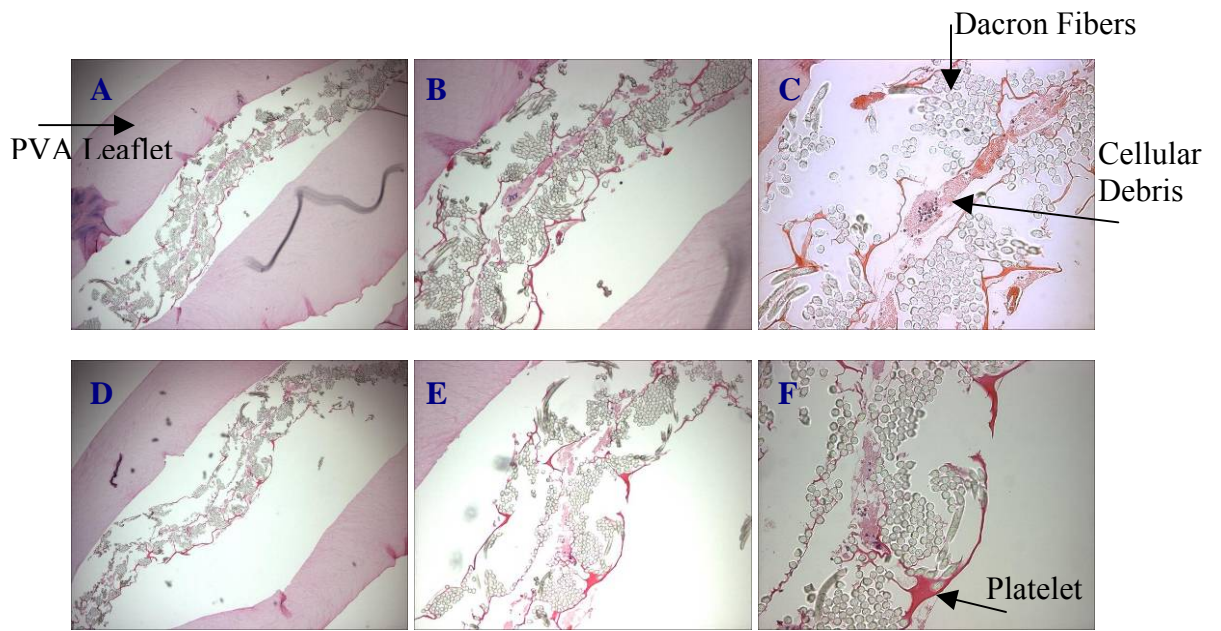


Figure 58: H&E Staining of Dacron lined valves; (A,B,C) valve 1, (D,E,F) valve 2; magnifications of (A, D) 4x, (B, E) 10x, (C, F) 20x

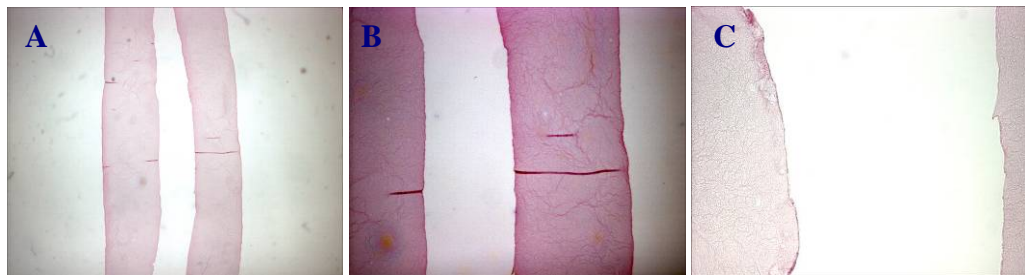


Figure 59: H&E Staining of PVA valve surfaces, magnifications of (A) 4x, (B) 10x, and (C) 20x.

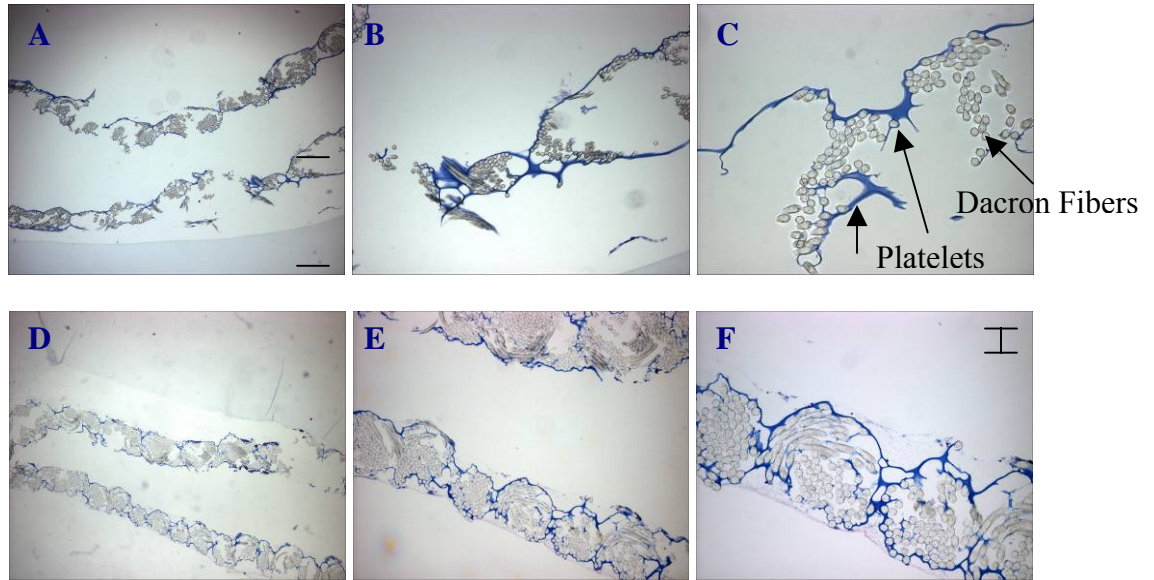


Figure 60: Carstairs' Staining of Dacron lined valves, (A,B,C) valve 1, (D,E,F) valve 2 magnifications of (A,D) 4x, (B,E) 10x, and (C,F) 20x.

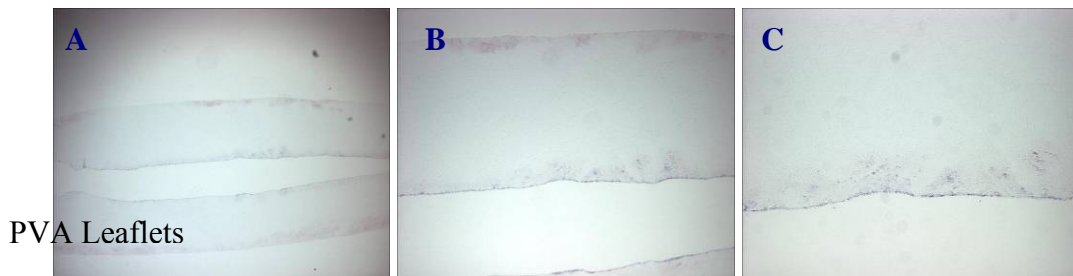


Figure 61: Carstairs' Staining of PVA valve, magnifications of (A) 4x, (B) 10x, and (C) 20x.

Shear Rate

The shear force required to activate platelets can be estimated as $4 \times 10^5 \text{ Ns/m}^2$, which is derived from equation 1. The shear force present in the orifice of the prosthetic vein valve is $1.9 \times 10^{-3} \text{ Ns/m}^2$. Reynolds number for the prosthetic vein valve was 134. The shear rate was 150, calculated from equation 2, using 6.64 for a and 39.4 for b [92].

$$\tau_{wall} = \frac{32\mu Q}{\pi D^3} \quad 1$$

$$\gamma_{max} = a \text{Re}_D^{0.5} + b \quad 2$$

CHAPTER 4

METHODS: DELIVERY SYSTEM DESIGN

Experimental Design

Analysis was performed to design a percutaneous delivery system for the PVA valve. The valves were tested for their compressibility and plastic deformation by compression time. Maximum PVA valve compression time was determined and appropriate stents, catheters and sheaths were selected to complete the delivery system.

Radial Compression (Long Term)

The PVA valves were inserted into balloon expandable stents, and a balloon catheter was placed through the orifice so that the balloon protruded from each end of the stent, as seen in Figure 62. The PVA valve was sutured to the stent and collapsed by applying pressure with rolling the stent between the forefinger and thumb. Placing a slipknot with string around the stent and tightening the knot further compressed the stent. The stent-valve-catheter system was maintained in the compressed state for 2 weeks, and then expanded through balloon catheter expansion. The degree of plastic deformation was visually evaluated.



Figure 62: Radially compressed valve-stent

Flat Compression

To identify how long the valves may be placed under compression and not experience plastic deformation, 3 valves were analyzed in a flat compression experiment. The valves were initially evaluated for opening pressure and backpressure conditions. The valves were not exposed to a backpressure beyond 100 mm Hg to prevent valve damage. The valves were photographed. The PVA valves were then placed between two cover slides and flattened with the orifice in the open position, as seen in Figure 63 and Figure 64. Three valves were compressed for 2 hours, while immersed in water; removed from compression; and returned to a water bath for 15 minutes to allow for re-expansion. The valves were photographed and evaluated for opening pressure and backpressure conditions. The same valves were subsequently re-compressed for 4hrs, 6hrs and 9hrs, and re-evaluated. To provide the valves additional time to fully relax back to their original configuration the uncompressed valves were placed in water for 12 hours and re-evaluated.



Figure 63: Flat compression of vein valve

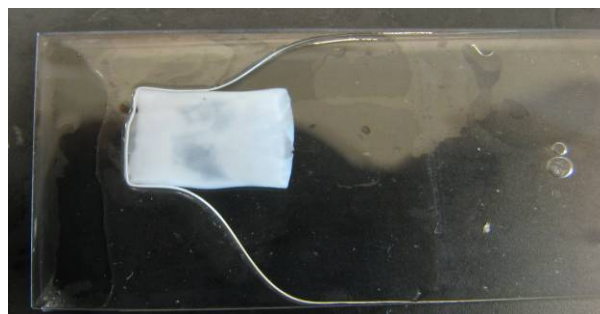


Figure 64: Flat compression of vein valve, emphasizing the open orifice

Radial Compression (Short Term)

The PVA valves were inserted into balloon expandable Palmaz-like stents, 10mm diameter and 20-25mm in length, (Cordis Endovascular, Miami, FL; and IntraTherapeutics, St. Paul, MN), and sutured into place. The initial stent-valve diameter was recorded. The valves were evaluated for opening pressure and backpressure. A balloon catheter (10 mm balloon diameter, Express Billiard LC, Boston Scientific, Natick, MA) was placed through the orifice, so that the balloon protruded from each end of the stent. The valves were crimped with an HH100 PTCA R&D Handheld Crimping Tool (Machine Solutions Inc., Flagstaff, AZ) to 100 lbs. of force, as seen in Figure 65. The crimping tool is shown in Figure 66. The crimped diameter was recorded. The valves were immersed in water for 1 hr or 2 hrs. A 10 mL syringe (Becton Dickinson and Co, Franklin Lakes, NJ) was filled with 10 mL of air, attached to the end of the balloon catheter, and the air was injected into the balloon. The balloon was maintained expanded for 30 sec; following the air was removed and the catheter was removed from the valve orifice. The final diameter was recorded. The valves were immersed in water for 24 hours, photographed, and evaluated for opening pressure and backpressure.



Figure 65: Radially Compressed Valve and Stent on a balloon catheter



Figure 66: HH100 PTCA R&D Handheld Crimping Tool (Machine Solutions Inc., Flagstaff, AZ)

CHAPTER 5

RESULTS: DELIVERY SYSTEM DESIGN

Stent Selection

Stents are divided into two categories: self-expanding and balloon expanding. The self expanding stents are constructed from Nitinol (equal parts titanium and nickel), which is a shape-memory and superelastic material. A valve was inserted into a self-expanding stent and attempted to be crimp with commercial grade crimping machines from Machine Solutions Inc. The valve-stent system could not crimp, therefore the self-expanding stent was eliminated as an option at this time.

Two classes of balloon-expanding stents were considered: open cell and closed cell. Balloon-expanding stents are typically constructed out of steel, and is therefore unfortunately not as flexible as the Nitinol stent. The open cell stent available was an Express biliary LD stent (Boston Scientific). The closed cell stent available was a Palmaz stent (Cordis). The open cell stent poorly expanded and contracted, leaving the stent struts deformed, as seen in Figure 67. These open cell stents had a specific open diameter, for instance an open diameter of 10 mm. As oppose to the Palmaz stent which had a range of open diameters, for instance 9 – 12 mm, making the Palmaz stent a more versatile design. The Palmaz stent expanded and contracted without strut deformation, as seen in Figure 68 and Figure 69. The Palmaz stent was selected as the most appropriate stent available based on the elimination of the Nitinol stents due to their crimping difficulties, the poor performance of the open cell stents, and the satisfactory performance of the Palmaz stents re-crimping ability.



Figure 67: Express Biliary LD stent (Boston Scientific)

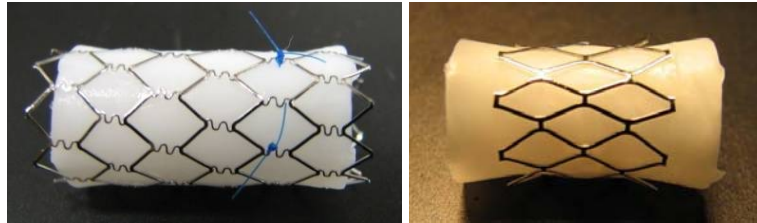


Figure 68: Left, Genesis Palmaz Stent (Cordis). Right, Palmaz Stent (Cordis)

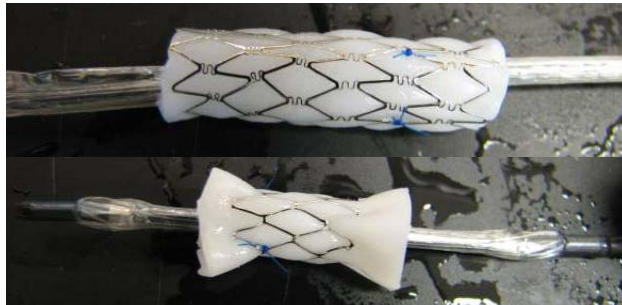


Figure 69: Top, Crimped Genesis Palmaz Stent. Bottom, Crimped Palmaz Stent

Radial Compression: Short Term

Valves were analyzed by radial compression with a handheld crimper; the results are presented in Table 4. The average initial outside diameter of the valve-stent system was $8.8 \text{ mm} \pm 0.1 \text{ mm}$. Prior to compression exposure the valves demonstrated an opening pressure of $3 \text{ mm Hg} \pm 1 \text{ mm Hg}$, and a backpressure of 100 mm Hg . To prevent valve damage, the valves were not pressurized beyond 100 mm Hg . The average compressed outside diameter of the valve-stent system was $6.5 \text{ mm} \pm 0.1 \text{ mm}$. They were compressed for 1hr and subsequently expanded. Visually the expanded valves retained their original configuration, as illustrated in Figure 70. The valves exhibited an opening pressure of $4 \text{ mm Hg} \pm 1 \text{ mm Hg}$ and withstood a backpressure of 100 mm Hg . The average expanded

outside diameter of the valve-stent system was $9.5 \text{ mm} \pm 0.5 \text{ mm}$. All valves met design criteria of opening pressure below 5 mm Hg and a backpressure up to 100 mm Hg.

Table 4: Results from Flat Compression Experiment

Valve	Initial Diameter (mm)	Opening Pressure (mmHg)	Back Pressure (mmHg) (up to 100 mmHg)	Compressed Diameter (mm)	Time Compressed (hr)	Expanded Diameter (mm)	Opening Pressure (mmHg)	Back Pressure (mmHg) (up to 100 mmHg)
1	8.8	4	100	6.48	1	9.9	5	100
2	9.0	2	100	6.40	1	9.9	3	100
3	8.8	3	100	6.50	1	9.0	3	100
4	8.9	3	100	6.50	2.5	9.5	3	100
5	8.6	2	100	6.43	1	9.0	5	100

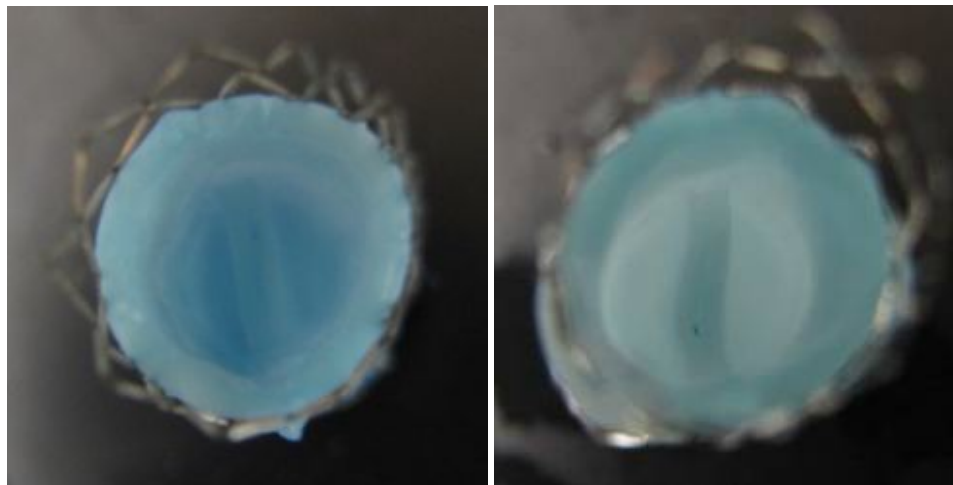


Figure 70: Left, valve prior to radial compression; right, valve after 1 hr of radial compression and re-expansion.

Complete Delivery System

Based on the “Radial Compression: Short Term” results the maximum crimped diameter was used to select the appropriate sheath size. The diameter was converted to French with the conversion of $1 \text{ mm} = 3 \text{ Fr}$. The sheath size was the corresponding French value.

A sheath was selected that will fit the maximum crimped diameter of $6.5 \text{ mm} \pm 0.1 \text{ mm}$. The result is a complete delivery system including: a 20-25 mm long and 10 mm diameter Palmaz stent (Cordis Endovascular, Miami, FL), a balloon catheter (10 mm

balloon diameter, Express Billiard LC, Boston Scientific, Natick, MA), and a 20 Fr sheath.

Flat Compression

Valves were analyzed by flat compression. Prior to compression exposure the valves demonstrated an opening pressure of 3 mm Hg \pm 1 mm Hg, and a backpressure of 100 mm Hg. To prevent valve damage, the valves were not pressurized beyond 100 mm Hg. Subsequently at 2 hrs, 4 hrs and 6 hrs after compression the valves exhibited an opening pressure of 3 mm Hg and a backpressure of 100 mm Hg, as shown in Table 5. At 9hrs under compression, the prosthetics were deformed into an open orifice position, even after 15 minutes in an immersed non-compressed state. The valves were subsequently left in a water bath for 12 hrs and re-evaluated. After 12hrs in an immersed non-compressed state, the valves visually relaxed back to their original configuration, as illustrated in Figure 71, and exhibited an opening pressure of 3 mm Hg and a backpressure of 100 mm Hg.

Table 5: Results from Flat Compression Experiment

Test	Valve	Time					
		0 Hrs	2 hrs	4 hrs	6 hrs	9 hrs *	9 hrs **
Opening Pressure (mm Hg)	1	4	3	3	3	0	3
	2	3	3	3	3	0	3
	3	3	3	3	3	0	3
Back- Pressure (mm Hg) up to 100 mm Hg	1	100	100	100	100	0	100
	2	100	100	100	100	0	100
	3	100	100	100	100	0	100

* tests performed 15 min after valves were removed from compression, valves did not have enough time to relax back to their original configuration

** tests performed 12 hrs after valves were removed from compression, valves had enough time to relax back to their original configuration

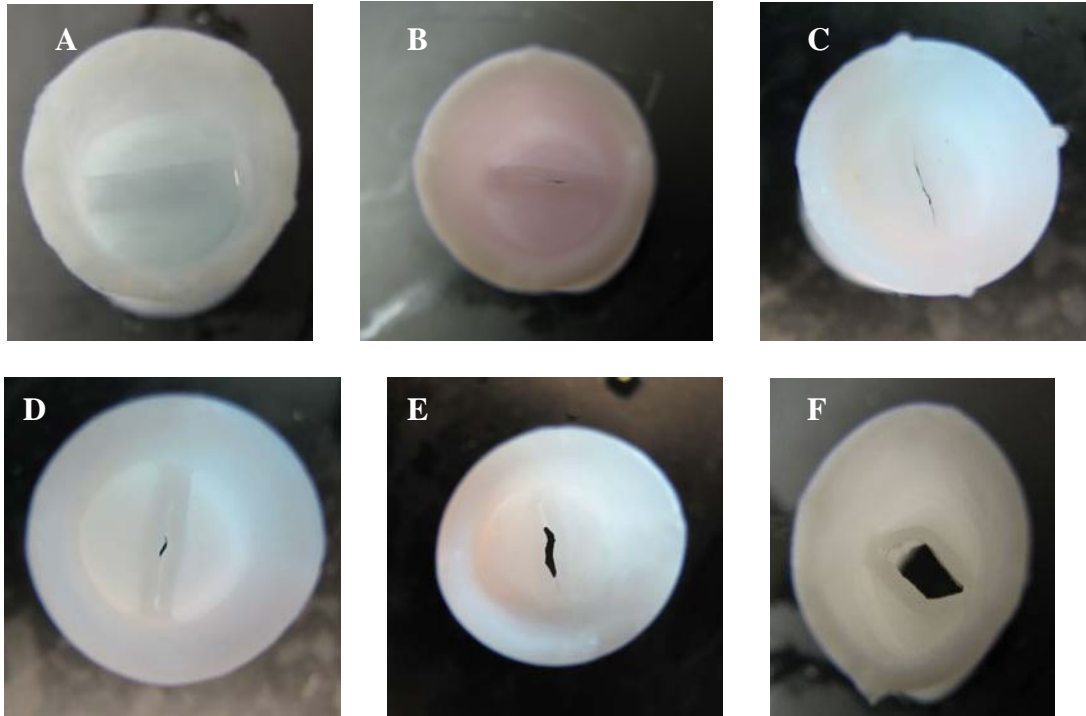


Figure 71: Visual deformation of valves after corresponding times of compression: A) control valve at 0 hrs of compression, (B) 2 hrs, (C) 6 hrs, (D) 12 hrs, (E) 18 hrs, and (F) 4 days.

Radial Compression (Long Term)

During stent crimping the stent became deformed, as is evident in Figure 62, though the valves were successfully reduced from 8 mm to 5 mm \pm 1 mm. Valves were visually analyzed after expansion to reveal a permanently open orifice, unmistakable creases in the exterior diameter, and expanded stent deformation. The PVA material plastically deformed from the long-term compression exposure, as shown Figure 72.

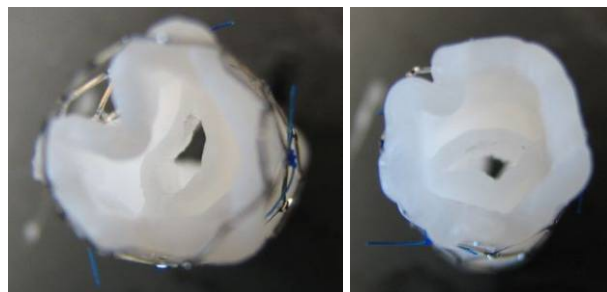


Figure 72: Plastically deformed valves, after 2 week radial compression

CHAPTER 6

DISCUSSION

Thrombotic Potential

Evaluating the thrombotic potential of a prosthetic vein valve in an *in vitro* set-up is a novel process. The most common practice to test the thrombotic potential is in an animal model. In the “Prosthetic Vein Valves : Animal Studies” section the majority of studies failed due to *in vivo* thrombosis [51, 55-59]. They concluded that their valves had the potential to form thrombus *in vivo*. If they had performed an *in vitro* thrombosis potential study they might have saved time, money and animal lives.

When designing an *in vitro* model it is most relevant for the model to be as close to physiologic conditions as possible. A potential draw-back to this *in vitro* set-up is that the flow through the prosthetic valves was 11.8 ± 0.4 mL/min, yet the blood flow through the femoral vein is around 70 mL/min. The flow was lower than physiologically observed valves because the frequency and collection time were selected, but the tubing diameter was restricted. The pulsatile frequency was chosen to mimic the normal walking cadence of 0.67 Hz. The tubing diameter could not be increased to reduce flow as it was limited to the pulsatile pump tubing specifications. Even though platelet adhesion in a stenosis happens at high velocities, vein thrombosis typically is thought to occur at low velocities. Therefore modeling a low flow rate may be more appropriate, since it is the physiologically worst case scenario. For instance, when one sits for a long period of time on a transatlantic flight and the calf pump is not actively engaged, the blood is traveling at a lower velocity back to the heart.

Histological analysis with H&E revealed that the red debris preventing blood from passing through the Dacron leaflets was cellular material. Further analysis with Carstairs' stain revealed that platelet aggregates were a key component of the red debris. The presence of platelets on the Dacron leaflets and the complete absence of platelets on the PVA valves confirmed that the pulsatile blood flow set-up had the potential to thrombose, yet the PVA valves do not clot in this system. A challenge faced when preparing the valve for histological analysis was that the fixation process shrank and deformed the valve, therefore the orifice was not occluded as the Dacron leaflets separated. However the presence of platelets on each leaflet was indicative of thrombosis.

Delivery System

Due to the plastic deformation revealed in the "Radial Compression: Long Term" results, it was concluded that compressing the GT vein valve for an extended period of time was not feasible, and alternative methods needed to be explored. The initial obstacle to address was determining how long the valves could remain compressed prior to experiencing plastic deformation; this led to the flat compression experiments. The flat compression experiments revealed that the PVA material may be compressed for short periods of time, less than 6 hrs, without experiencing plastic deformation. Concurrently, it was discovered that the Edwards Life Sciences' percutaneous heart valve, currently in clinical trials, is compressed at the surgical hospital just prior to implantation. Given the successful implementation of pre-procedure crimping, a similar technique was pursued for the GT vein valve to allow it to be compressed within 6 hours of implantation. Therefore an accurate and portable stent crimper was identified. The valves performed to specifications once expanded. The result is a complete delivery system including: a stent, a balloon catheter, and a sheath.

To provide minimal vessel trauma the sheath diameter for any percutaneous device needs to be as small as possible. It would be ideal to place the GT vein valve through a small vessel and up to the implant location. One significant disadvantage to the delivery system design presented is that the maximum crimped diameter is $6.5 \text{ mm} \pm 0.1 \text{ mm}$, which is large in terms of vessel size. The sheath required for this prosthesis is 20 Fr, which could not be placed from the saphenous vein up to the femoral vein. Therefore, the most appropriate route would be from the external jugular vein down through the heart to the femoral or iliac vein, as depicted in Figure 18. There would be additional stress on the heart when passing a catheter through the superior vena cavae to the inferior vena cavae. The GT vein valve is manufactured by an injection molded process; therefore, a second generation GT vein valve mold could easily be streamlined to reduce the valve profile and make the crimped diameter smaller.

As currently designed the crimped valve diameter of 20 Fr is too large to implant into humans percutaneously. An appropriate percutaneous device is within 6 to 10 Fr. Future work should include the redesign of the valve to reduce the thickness of the cylindrical supporting material.

Due to the flexibility of Nitinol, it is worthwhile to reconsider Nitinol stents as oppose to the selected steel stents. It was unfortunate that crimping limitations prevented the application of the Nitinol stent. As crimping technologies develop and through the redesign of the valve prosthetic, a Nitinol stent is expected to be a more appropriate stent selection.

Comparison to Previous Prosthetic Animal Valves

The two most successful vein valve studies use acellular tissues: the SG-BVV study used porcine small intestine submucosa (SIS), and the PVVB study used gluteraldehyde-

preserved bovine jugular valves. The GT vein valve is constructed from a synthetic polymer, which provides several advantages over SIS and gluteraldehyde-preserved bovine jugular valves. The PVVBs are fixed with gluteraldehyde, which is a toxic substance that will prevent cells from integrating into the material *in vivo*. This gluteraldehyde preservation process will cause a limited cyclic life due to the cross-linking of the collagen fibers, and ongoing biocompatibility issues due to the gluteraldehyde toxicity. The SIS tissue appears to be an appropriate material for vein valve prosthetics, with regards to its biocompatibility. However, despite revisions to the SIS vein valve, the SG-BVV continues to experience *in vivo* tilting. Tilting would not be an issue with the GT vein valve because of the long axial dimension. In addition, GT vein valve is manufactured by injection molding; therefore, the valve can be mass produced and the design is easily modified. This is unlike acellular tissues which require extensive time to process the tissues and modification of the tissue valve design would create concerns regarding suturing locations and tissue to stent attachment sites. Another advantage is that PVA may be processed to include embedded drugs, which could promote cell growth and/or reduce thrombus formation. By designing the GT vein valve out of PVA it has superior biocompatibility and structural integrity, may be mass produced, and has the potential to utilize new drug delivery technologies.

Future work: Animal Trials

Performing an animal study will evaluate the biocompatibility of the GT vein valve. The low thrombotic potential of the GT vein valve has been confirmed in an *in vitro* analysis; therefore, the appropriate next step is to perform an animal study and confirm the vein valve's biocompatibility. PVA has previously been used as a medical implant material therefore, biocompatibility is expected.

An ovine trial has been designed to test *in vivo* valve patency and quantify *in vivo* thrombotic potential. Five sheep will surgically receive implants for a duration of 4 weeks. As an initial feasibility study, only a few animals will be used to gain insight into the performance of the valve. Five animals will be used to account for potential biologic variance. Previous experimental studies have been performed on populations between 4-10 animals [51, 52, 55, 56]. Four prosthetic vein valves will be implanted into each animal; one in each of the external jugular veins, and one in each of the iliac veins.

Species Selection

Ovine were chosen because their cardiovascular geometry and physiology is similar to human physiology and anatomy. To test the patency of any valve, the hemodynamics and rheology of the animal should be as representative of humans as possible. They are also the most common animal for vein valve studies. Sheep are also robust animals, recovering fairly quickly from surgical operations, and are fairly easy to handle.

Surgical Procedure

The prosthetic valves will be implanted via percutaneous delivery. The external jugular vein (EJV) will be exposed by a longitudinal incision. To implant the valve, a sheath will be placed into the EJV. The flexible valve will be placed into a sheath delivery system prior to the time of surgery. The valve will be directed into the common iliac vein under fluoroscopic guidance. The valve will be deployed in this location with a balloon-expandable stent mechanism. The same procedure will be repeated for the EJV on the same side. Placement of the PVA valve is demonstrated in Figure 73. The venotomy in the EJV will be closed with interrupted sutures. The same procedure will then be repeated in the other EJV in order to deliver an additional valve/stent mechanism into the common iliac vein and EJV.

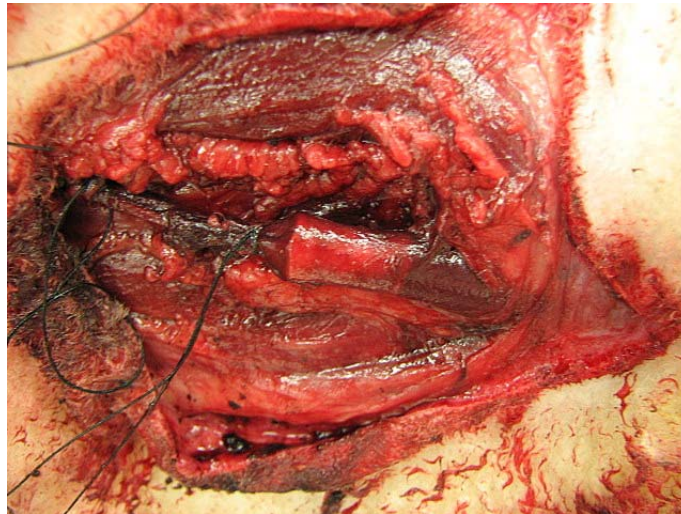


Figure 73: (Top) PVA valve positioned beside right external jugular vein. Bottom, PVA valve implanted into right external jugular vein.

Imaging

Patency will be demonstrated using two measurement techniques: venogram and Doppler ultrasound. A venogram consists of IV injection of iodine containing, radiopaque contrast media, followed by a fluoroscopic x-ray. The second method for demonstrating patency will be using an external hand-held ultrasound machine to detect forward flow of blood.

Study Endpoint

Animals will be sacrificed 4 weeks after implant, and segments of the external jugular veins and iliac veins containing the valves will be excised and examined with histomorphological techniques. A four week study is an appropriate study time to demonstrate biocompatibility.

Study Outcomes

Due to the low *in vitro* thrombotic potential and the long track record of PVA as a medical implant material, positive trial results are expected. In the event of negative biocompatibility results, the product design would be re-evaluated. In the case of positive results, the next step would be to initiate a larger animal trial and/or clinical trial.

CHAPTER 7

CONCLUSIONS

Evaluating the thrombotic potential of a prosthetic vein valve in an *in vitro* set-up is a novel process, as the thrombotic potential is typically evaluated in an animal model. Animal studies require the long process of approval from animal care and use committees, the trials are costly, the study itself is time consuming, and animal lives are sacrificed. *In vivo* models are necessary to determine the biocompatibility of the prosthetic device, and an important step towards clinical trials; yet using an *in vitro* thrombosis model provides an appropriate intermediate step between valve development and *in vivo* analysis.

The *in vitro* model presented perfuses whole porcine blood through a prosthetic vein valve. The pulsatile frequency of the system, 0.75 Hz, approximates the normal walking cadence of an adult. A Dacron lined valve provided an appropriate positive control to confirm the thrombotic potential of the system. This *in vitro* set-up was used to evaluate the GT venous valve.

The GT venous valve demonstrates low thrombus formation in the perfusion system. The GT vein valves remained patent after 20 minutes of perfusion with no adherent platelets of fibrin thrombus under histological analysis. In contrast, the control valves lined with polyester occluded after 6 ± 3.6 min of perfusion. Histology revealed adherent fibrin, RBCs and platelets on the positive controls. The time of occlusion for the Dacron lined valves in this assay was significant ($p < 0.02$). The GT valve exhibited low flow resistance, strong competency, fatigue-resistance, low-thrombogenicity, and material flexibility.

Given the positive results from the *in vitro* evaluation, the GT vein valve has excellent potential. By choosing to manufacture the GT vein valve out of a synthetic material, PVA, it has outstanding structural integrity, may be mass produced, and is easily modified. Furthermore, PVA has demonstrated exceptional biocompatibility in a wide range of other medical devices and it has the potential to incorporate new drug delivery technologies.

The future of medical implants lies in percutaneous devices; therefore, to create a marketable and less invasive implant, a percutaneous delivery system has been designed for the GT vein valve. As designed, this system will be utilized in an ovine trial of the valve. A stent will be placed around the GT vein valve and crimped onto a balloon catheter; it will then be loaded into a sheath. The profile of the collapsed valve is 6.5 mm \pm 0.1 mm, which indicates that a large vessel, such as the external jugular vein or common iliac, will be required for delivery. Reduction of the crimped valve profile will allow the valve to be delivered from smaller vessel locations, therefore a second-generation GT vein valve should aim to reduce this profile. Future improvements could include embedding drugs into the PVA which would limit thrombosis, inflammation or foreign body response mechanisms. Due to the low *in vitro* thrombotic potential and the strong history of PVA as a medical implant material, positive trial results are expected.

Providing relief to chronic venous insufficiency is a worth while pursuit as patients experience swelling, edema, pain, itching, varicose veins, skin discoloration, ulceration and limb loss. Current clinical therapies are only modestly effective; and therefore, a prosthetic vein valve can provide a cure for this debilitating disease. With successful animal and human trials this valve can provide a potential intervention for the 7 million people suffering from CVI.

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