

PRECLINICAL TESTING OF A NEW VENOUS VALVE

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Abstract: Venous valvular incompetency is a debilitating disease affecting millions of patients. Unfortunately, the current physiologic and surgical treatments are prone to the extreme risk of post-operative thrombosis. A new design for venous valves has been proposed using biomimicry. The medical device has the shape of a natural valve with sufficient elasticity to maintain patency and competency in the leg veins. The venous valve was tested for patency, competency, cyclic fatigue, compressibility, and thrombogenicity. Patency is maintained with a low opening pressure of less than 3 mmHg. Competency is maintained with backpressures exceeding 300 mmHg. The valve is fatigue resistant to over ¼ million cycles. The valve can maintain its integrity when compressed in a stent and deployed without tilting or mal-alignment. Little thrombus forms on the valve with perfusion of whole blood under pulsatile flow conditions. The pre-clinical tests demonstrate efficacy as a new venous valve for treatment of chronic venous insufficiency.

1 BACKGROUND

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. 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. Symptoms include swelling, edema, pain, itching, varicose veins, skin discoloration, ulceration and limb loss. Post-thrombotic damage within the deep veins is the most significant cause of CVI, reported as high as 95%. Current clinical therapies are only moderately effective; and therefore, the need for a better solution remains.

Surgical treatment for CVI is avoided due to lack of accurate surgical technology and the extreme risk of post-operative thrombosis. Valvuloplasty is extensively time consuming and reserved for patients with a congenital absence of functional valves and severe cases of CVI. This surgical procedure involves a venotomy, where the valve cusps are plicated 20-25%. A singular valvuloplasty is usually sufficient to correct CVI except in systems that include occlusion of the femoral or popliteal vein, or absence/incompetence of the communicating leg veins.

Previous prosthetic vein valves have experienced complications due to either *in vivo* thrombosis, in-growth, foreign body reaction, or prosthetic disorientation (tilting). The umbilical vein valves and pellethane valves by Hill *et al.* failed due to thrombotic occlusion (Hill *et al.*, 1985). Two-thirds of the autogenous venous valves by Rosenbloom *et al.* failed from complications arising to thrombosis (Rosenbloom *et al.*, 1988). The mechanical valves by Taheri *et al.* failed after three months due to severe thrombotic occlusion (Taheri and Schultz, 1995). Partial thrombosis appeared in the valve cusps of the polyetherurethane valves by Uflacker (Uflacker, 1993). Thrombosis occurred immediately after deployment of the glutaraldehyde-fixed bovine external jugular by Gomez-Jorge *et al.* (Gomez-Jorge *et al.*, 2000). Glutaraldehyde-preserved bovine jugular valve-bearing venous xenograft show thrombosis by histology (de Borst *et al.*, 2003).

A second cause of failure is hyperplasia. 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 glutaraldehyde-fixed bovine external jugular by Gomez-Jorge *et al.* produced a granulomatous response and foreign body reaction (Gomez-Jorge *et al.*

al, 2000). Between 2000 and 2005 Pavcnik *et al* reported on a stent-based porcine small intestine submucosa prosthesis (Pavcnik *et al*, 2005); all failed valves were the result of prosthesis tilting. Overall, eight out of ten of the reported valve designs experienced complications due to thrombosis.

A new design for venous valves has been proposed using biomimicry. The medical device has the shape of a natural valve with sufficient elasticity to maintain patency and competency in the leg veins. This paper describes the pre-clinical verification and validation testing of this new venous valve.

2 METHODS

The venous devices were subjected to a battery of tests to demonstrate sufficient function as a one-way valve, propensity for thrombosis, and suitability for minimally invasive delivery. The new “GT” vein valve prosthetic is presented in Figure 1.

2.1 Pulsatile Flow System

This pulsatile system was designed to mimic the physiologic flow conditions present in the lower extremity venous system. During normal walking, compression occurs about 40 times a minute (0.67 Hz). Fresh, whole, porcine blood with heparin (6.0 ± 0.2 U/mL) was transferred into a blood donor collection bag. The collection bag was raised 30 cm above the test section, rotated on an orbital mixer, and attached to 90 cm of vinyl tubing, 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 pulsatile pump 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 by occlusion or the contents of the fluid reservoir were emptied.



Figure 1: GT vein valve. Upper left, isometric view. Upper right, cross sectional view. Lower, downstream view.

2.2 Test Section

The test section included a vein valve, a flexible venous-like tube, and suture material. The valves were manufactured according to the procedure outlined in reference (Sathe, 2007). The valve material was made from a PVA hydrogel biomaterial. 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.

2.3 Dacron Lined Valve

A Dacron-lined valve acted as the positive control. The lining was constructed from a commercially available cardiovascular Dacron patch often used clinically of approximately $14 \text{ mm} \pm 1 \text{ mm}$ by $9 \text{ mm} \pm 0.5 \text{ mm}$, which was then sutured to the GT valve. One stitch was placed on each Dacron piece on the upstream side, these sutures held the Dacron against the GT valve.

2.4 Pressure Tests

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. Pressure was applied with the syringe and read upstream of the test section. For opening pressure, the prosthetic vein valve was orientated with the distal end closest

to the syringe, and the proximal end facing ambient atmosphere. For backpressure, the prosthetic valve was reversed in orientation.

2.5 Thrombosis of Whole Blood

Whole blood samples were harvested from pigs and quickly anti-coagulated with porcine heparin to a final concentration of 6.0 ± 0.2 U/mL. The samples were mixed with a nutating rocker 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.

2.6 Histology

Samples were fixed in 10% formalin (VWR International, West Chester, PA) for at least 72 hours. 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 were collected from each sample. Alternating samples were stained with Haematoxylin and Eosin stain (H&E), and Carstairs' stain (specific for platelets). Sections were analyzed microscopically using a Nikon E600 microscope, a digital camera and Q-capture software.

2.7 Flat Compression

The valves were evaluated for plastic deformation with respect to compression time. Initially, they were evaluated for opening pressure and backpressure conditions. At periodic time points the valves were allowed to expand and were re-evaluated for opening pressure and backpressure conditions.

2.8 Radial Compression

The GT valves were inserted into balloon expandable Palmaz stents, 10mm diameter and 20-25mm in length, (Cordis Endovascular, Miami, FL; and IntraTherapeutics, St. Paul, MN), and sutured into place. The valves were evaluated for opening pressure and backpressure.

2.9 *In Vivo* Placement

Placement inside an actual vein has been problematic for some previous designs. The valves

might tilt or dislodge in the vein. Thus, our valves were surgically placed in the correct anatomic position in animals. The external jugular veins (EJV) were exposed on four previously deceased, 2.5 year old, 50-60 Kg Dorset ewe sheep. A vertical incision was performed on the EJV and the prosthetic was placed inside the vein. The vessel diameter was measured and the prosthetic valve was manipulated to evaluate potential misplacement. This procedure was repeated on the iliac veins.

3 RESULTS

The GT vein valve was evaluated for patency, competency and cyclic life (Sathe, 2006). 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. The patency and competency endpoints were statistically unchanged after 500,000 cycles of cyclic testing.

3.1 Thrombosis from Pulsatile Blood Flow

Blood was perfused through five GT vein valves; a graphical representation is shown in Figure 2. All five valves remained patent after 20 minutes of blood flow without significant flow rate deterioration. The average blood flow rate was 11.8 ± 0.4 mL/min. The upstream pressure fluctuated between 15 – 21 mmHg. Once the system was exhausted of blood, the pressure dropped off to just above 10 mmHg. When the flow system depleted the blood reservoir the roller pump tried to pull blood through the valve, the upstream and downstream sections of the valve would collapse due to the negative pressure. There was no gross thrombus visible on any of the valve leaflets. The leaflets remained functional and the valves remained competent against backpressure.

The Dacron-lined valves initially produced the same velocity profiles as the GT 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 Dacron lined valves occluded after 6 ± 3.6 min of perfusion. The upstream pressure fluctuation prior to occlusion was between 15 – 21 mmHg, and after occlusion, the pressure was constant at 24 ± 1 mmHg. The frequency of occlusion for the Dacron lined valves in this assay was significant to $p < 0.02$. 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. The Dacron valves were visually inspected at the end of the experiment. The polyester fibers were covered with red blood and were visibly matted down. After occlusion, some red clot remained in the lumen of the tubes. The GT valves and Dacron lined valves were preserved for histological analysis.

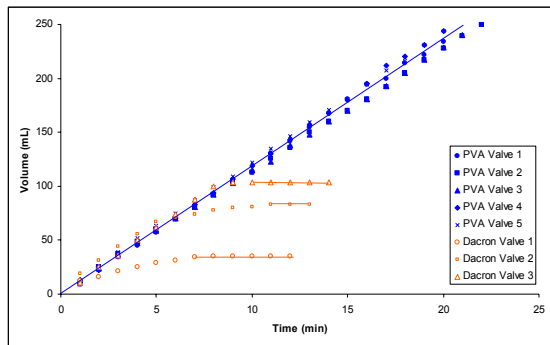


Figure 2: Perfused blood volume over time in the GT and Dacron-lined valves. The GT valves produced a constant flow rate; whereas, the Dacron lined valves produced a gradual cessation of flow.

Histology was performed on both the GT and Dacron lined valves to identify cell accumulation and the cause for cessation of flow. The histology stains used were Haematoxylin and Eosin stain (H&E), and Carstairs' stain (specific for platelets). 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 revealed that platelet aggregation with fibrin strands was a key component in the red debris. The presence of platelets on the Dacron leaflets, and the complete absence of platelets on the GT valves confirmed that the *in vitro* blood flow assay had the potential to thrombose, yet the GT valves do not exhibit any thrombosis or clot in this system.

3.2 Plastic Deformation – Flat Compression.

Valves were subjected to flat compression. Prior to compression the valves demonstrated an opening pressure of 3 mm Hg \pm 1 mm Hg, and a

backpressure of at least 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 maintained competency with a backpressure of 100 mm Hg.

3.3 Radial Compression

The average initial outside diameter of the valve-stent system was 8.8 mm \pm 0.1 mm. Prior to compression exposure the valves demonstrated an opening pressure of 3 mm Hg \pm 1 mm Hg, and a minimum backpressure of 100 mm Hg. The average compressed outside diameter of the valve-stent system was 6.5 mm \pm 0.1 mm. They were compressed for 1hr and subsequently expanded, shown in Figure 3. Visually the expanded valves retained their original configuration. The valves exhibited an opening pressure of 4 mm Hg \pm 1 mm Hg and withstood a backpressure of 100 mm Hg. The average expanded outside diameter of the valve-stent system was 9.5 mm \pm 0.5 mm. All valves met the original design criteria of opening pressure below 5 mm Hg and a backpressure up to 100 mm Hg.

3.4 *In Vivo* Placement

The prosthetic was positioned inside the external jugular veins and iliac veins of four sheep as depicted in Figure 4. The 10 mm prosthetic vein valve was of appropriate size for the EJVs of sheep.

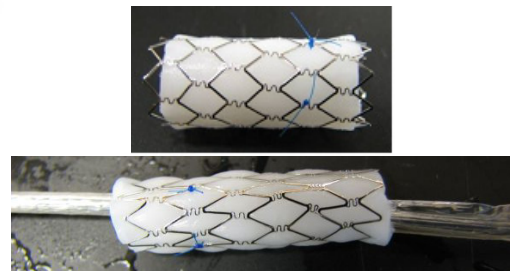


Figure 3: Above, Genesis Palmaz Stent (Cordis) with GT vein valve. Below, radially compressed valve and stent on a balloon catheter.

The 10 mm prosthetic vein valve was too small for the iliac vein. Vigorous manipulation of the prosthetic *in situ* did not cause any misplacement, tilting, or orientation problems. Tilting was of no concern due to the long profile of the prosthetic. A suite of valve sizes ranging from 10mm to 4 mm in 2 mm increments were created to account for varying vessel sizes, as seen in the iliac of sheep.

4 DISCUSSION

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 expensive *in vivo* studies.

The GT venous valve demonstrates low thrombus formation in the whole blood perfusion system, as it remained patent after 20 minutes of perfusion with no adherent platelets. In contrast, the Dacron valves occluded after 6 ± 3.6 min of perfusion. Histology revealed adherent fibrin, RBCs and platelet thrombus under histological analysis. The time of occlusion for the Dacron lined valves in this assay was significantly shorter than the GT valves ($p < 0.02$).

When designing an *in vitro* model it is most relevant for the model to be as close to physiologic conditions as possible. The *in vitro* model perfuses whole porcine blood through a prosthetic vein valve. The pulsatile frequency of the system, 0.75Hz, approximates the normal walking cadence of an adult. A potential limitation 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 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 a worse-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.

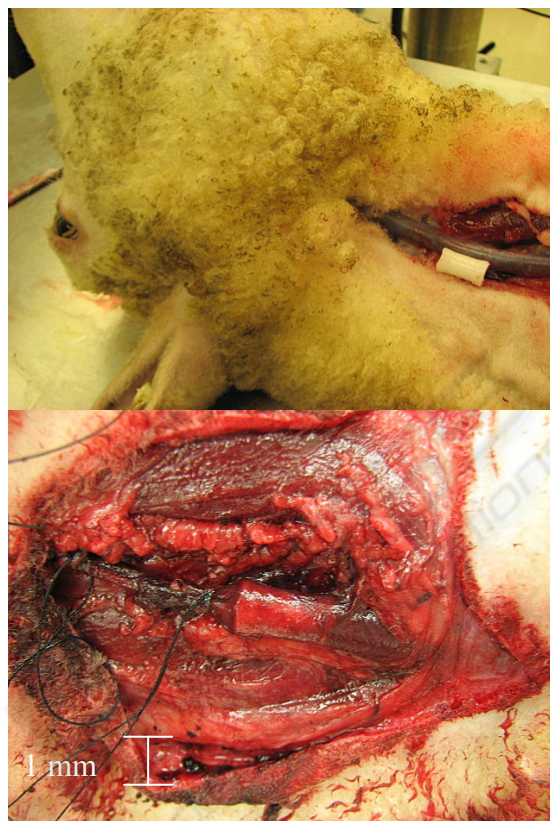


Figure 4: Above, GT valve positioned beside right external jugular vein. Below, GT valve implanted into right external jugular vein.

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. An appropriate delivery route may be from the external jugular vein down through the heart to the femoral or iliac vein. Reduction of the crimped valve profile may be achieved by decreasing the thickness of the cylindrical supporting material. Future improvements could include incorporation of antithrombotics or other eluting drugs into the valve to limit thrombosis, inflammation or foreign body response mechanisms. Due to the low *in vitro* thrombotic potential and the successful previous clinical use of the material as a medical implant material, clinical trials may be considered.

Given the successful implementation of pre-procedure crimping of percutaneous heart valves (Edwards Life Sciences), a similar technique was pursued for the GT vein valve to allow it to be compressed within 6 hours of implantation. The portable stent crimper makes this possible. Evaluating the thrombotic potential of a prosthetic

vein valve in an *in vitro* blood set-up is a novel process. The most common practice to test the thrombotic potential is in an animal model, where eight out of ten studies reviewed failed due to *in vivo* thrombosis. The two most successful vein valve studies use acellular tissues: the SG-BVV was constructed from porcine small intestine submucosa (SIS), and the PVVB used glutaraldehyde-preserved bovine jugular valves (Moll, 2003), (Gale et al, 2004). The GT vein valve provides several advantages over SIS and glutaraldehyde-preserved bovine jugular valves. Zoonosis from animal tissue prosthetics is possible and the use of animal derived prosthetics may be culturally or religiously controversial, therefore a synthetic material would alleviate these concerns. The PVVBs are fixed with glutaraldehyde, which is a toxic substance that will prevent cells from integrating into the material *in vivo*. This glutaraldehyde preservation process will cause a limited cyclic life due to the cross-linking of the collagen fibers, and ongoing biocompatibility issues due to the glutaraldehyde 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 is not an issue with the GT vein valve because of the long axial dimension. In addition, GT vein valve can be mass-produced and the design is easily modified. This is unlike acellular tissues which require extensive tissue preparation and processing times and modification of the tissue valve design could create concerns regarding suturing locations and tissue-to-stent attachment sites.

Another feature of the new GT Valves is that they may be processed to include embedded drugs, which could promote cell growth and/or reduce thrombus formation. It has superior biocompatibility and structural integrity, may be mass-produced, and has the potential to utilize new drug delivery technologies.

Providing relief to chronic venous insufficiency is a worthwhile 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 may provide a cure for this debilitating disease. With successful animal and human trials this valve could provide a useful therapy the 7 million people suffering from chronic venous insufficiency. The GT valve exhibits excellent flow, full competency, fatigue-resistance,

low-thrombogenicity, material flexibility, and *in situ* placement consistency.

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