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Protocol for Relative Hydrodynamic Assessment of Tri-leaflet Polymer Valves

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Corresponding Author:	Sharan Ramaswamy Florida International University Miami, Florida UNITED STATES
First Author:	Sharan Ramaswamy
Order of Authors:	Sharan Ramaswamy
	Manuel Salinas
	Rob Carrol
	Karla Landaburo
	Xavier Ryans
	Cynthia Crespo
	Ailyn Rivero
	Faris Al-Mousily
	Curt Degroff
	Mark Beiweis
	Hitomi Yamaguchi

Cover Letter

FIU FLORIDA INTERNATIONAL UNIVERSITY
Biomedical Engineering

10555 W. Flagler St.
EC 2612
Miami, FL 33174

Tel: 305-348-2532
FAX: 305-348-6954
sramaswa@fiu.edu
http://www.bme.fiu.edu

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Rachelle Baker
Associate Editor
Journal of Visualized Experiments
+1.617.500.7101 | rachelle.baker@jove.com

Re: JoVE Submission 50335 and Submission of *REVISED* manuscript entitled "Protocol for Relative Hydrodynamic Assessment of Tri-leaflet Polymer Valves" to *Journal Of Visualized Experiments (JOVE).*

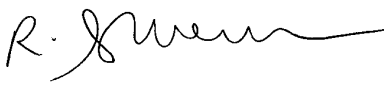
Dear Rachelle,

Regarding our JoVE submission 50335, we thank the reviewers for providing valuable suggestions and comments that have greatly strengthened the quality of our manuscript. We have addressed all of the comments and suggestions that were made and have incorporated them in the revised manuscript, which we are pleased to be submitting for your consideration.

Should you have any questions please feel free to let me know. I look forward to hearing from you.

Thank you for your kind consideration once again.

Sincerely,



Sharan Ramaswamy, PhD, F.A.H.A
Assistant Professor
Department of Biomedical Engineering
Florida International University
10555 West Flagler Street, EC 2612
Miami, Florida 33174
E-mail: sramaswa@fiu.edu
Tel: (305)-348-2532
FAX: (305)-348-6954

Protocol for Relative Hydrodynamic Assessment of Tri-leaflet Polymer Valves

Sharan Ramaswamy[1*], Manuel Salinas[1], Rob Carrol[2], Karla Landaburo[1], Xavier Ryans[1], Cynthia Crespo[1], Ailyn Rivero[1], Faris Al-Mousily[3,4], Curt DeGroff[3], Mark Bleiweis[3],Hitomi Yamaguchi[2]

[1]Tissue Engineered Mechanics, Imaging and Materials Laboratory (TEMIM Lab), Department of Biomedical Engineering, Florida International University, Miami, FL.
[2]Department of Mechanical and Aerospace Engineering, University of Florida, Gainesville, FL.
[3]College of Medicine, University of Florida, Gainesville, FL.
[4]King Faisal Specialty Hospital and Research Center (KFSH&RC), Jeddah, Saudi Arabia.

***Corresponding author:**
Sharan Ramaswamy, Ph.D., F.A.H.A
Florida International University
Department of Biomedical Engineering
College of Engineering and Computing
10555 W. Flagler Street, EC 2612
Miami, FL 33174
Tel: (305)-348-2532
FAX: (305)-348-6954
Email: sramaswa@fiu.edu

Keywords: valve disease; valve replacement; polymer valves; pulse duplicator; modification; tri-leaflet geometries; hydrodynamic studies; relative assessment.

Short Abstract

There has been renewed interest in developing polymer valves. Here, the objectives are to demonstrate the feasibility of modifying a commercial pulse duplicator to accommodate tri-leaflet geometries and to define a protocol to present polymer valve hydrodynamic data in comparison to native and prosthetic valve data collected under near-identical conditions.

Abstract

Limitations of currently available prosthetic valves, xenografts and homografts has prompted a recent resurgence of developments in the area of tri-lealfet polymer valve prostheses. However, identification of a protocol for initial assement of polymer valve hydrodynamic functionality is paramount during the early stages of the design process. Traditional *in vitro* pulse duplicator systems are not configured to accomodate flexible tri-leaflet mateirals; in addition, assessment of polymer valve functionality needs to be made in a relative context to native and prosthetic heart valves under identical test conditions so that variability in measurements from different instruments can be avoided. Accordingly, we conducted hydrodynamic assessment of i) native (n = 4, mean diameter, D = 20 mm), ii) bi-leaflet mechanical (n= 2, D = 23 mm) and iii) polymer valves (n = 5, D = 22mm) via the use of a commercially available pulse duplicator system (Vivitro Labs Inc, Victoria, BC) that was modifed to accomodate tri-leaflet valve geometries. Tri-leaflet silicone valves developed at the University of Florida comprised the polymer valve group. A mixture in the ratio of 35:65 glycerin to water was used to mimic blood physical properites. Instaneous flow rate was measured at the interface of the left ventricle and aortic units while pressure was recorded at the ventricular and aortic positions. Bi-leaflet and native valve data from the literature was used to validate flow and pressure readings. The following hydrodynamic metrics were reported: forward flow pressure drop, aortic root mean square forward flow rate, aortic closing, leakage and regurgitant volume, transaortic closing, leakage and total energy losses. Representative results indicated that hydrodynamic metrics from the three valve groups could be successfuly obtained by incorporating a custom-built assembly into a commercially available pulse duplicator system and subsequently, objectively compared to provide insights on functional aspects of polymer valve design.

Introduction

Heart valve disease often results from degenerative valve calcification [1], rheumatic fever [2], endocarditis [3,4] or congenital birth defects. When valve damage occurs, causing stenosis and/or regurgitation valve prolapse and cannot be surgically repaired, the native valve is usually replaced by a prosthetic valve. Currently available options include mechanical valves (cage-ball valves, tilting disk valves, etc.), homograft, and bioprosthetic valves (porcine and bovine valves). Mechanical valves are often recommended for younger patients based on their durability; however the patient is required to remain on anticoagulant therapy to prevent thrombotic complications [5]. Homograft and Biological prosthetic valves have been effective choices to avoid blood thinner therapy; however, these valves have elevated risk for fibrosis, calcification, degeneration, and immunogenic complications leading to valve failure [6]. Tissue-engineered valves are being investigated as an emerging technology [7-9], but much still remains to be uncovered. Alternative durable, biocompatible, prosthetic valves are needed to improve the quality of life of the heart valve disease patients. Again, this valve design could replace the bioprothesis used in transcatheter valve technology, with transcatheter approaches showing the potential for transforming the treatment of selected patients with heart valve disease [10].

As stated by current standards, a successful heart valve substitute should have the following performance characteristics: "1) allows forward flow with acceptably small mean pressure difference drop; 2) prevents retrograde flow with acceptably small regurgitation; 3) resists embolization; 4) resists hemolysis; 5) resists thrombus formation; 6) is biocompatible; 7) is compatible with in vivo diagnostic techniques; 8) is deliverable and implantable in the target population; 9) remains fixed once placed; 10) has an acceptable noise level; 11) has

reproducible function; 12) maintains its functionality for a reasonable lifetime, consistent with its generic class; 13) maintains it functionality and sterility for a reasonable shelf life prior to implantation." [11]. Some of the shortcomings of existing valve prostheses may potentially be overcome by a polymer valve. Biocompatible polymers have been considered top candidates based on biostability, anti-hydrolysis, anti-oxidation, and advantageous mechanical properties such as high strength and viscoelasticity. In particular, elastomeric polymers may provide material deformation resembling native valve dynamics. Elastomers can be tailored to mimic soft tissue properties, they may be the only artificial materials available that are bio-tolerant and that can withstand the coupled, *in vivo*, fluid-induced, flexural and tensile stresses, yet, move in a manner resembling healthy, native valve motion. Moreover, elastomers can be mass produced in a variety of sizes, stored with ease, are expected to be cost-effective devices and can be structurally augmented with fibrous reinforcement.

The concept of the use of polymer materials to assemble a tri-leaflet valve is not new and has been the subject of several research investigations over the last 50 years [12], which were abandoned largely due to limited valve durability. However, with the advent of novel manufacturing methodologies [13,14], the reinforcement of polymer materials [15,16] and potentially seamless integration of polymer valve substitutes with transcatheter valve technology, there has recently been a renewed interest and activity in developing polymer valves as a potentially viable alternative to currently available commercial valves. In this light, a protocol for enabling testing of these valves to assess hydrodynamic functionality is the first step in the evaluation process; yet commercially available pulse simulator systems generally do not come equipped to accommodate tri-leaflet valve designs and contain an annular spacing to insert commercially

available heart valves (e.g. tilting disc, bi-leaflet mechanical heart valves). Secondly, polymer valves are an emerging technology whose hydrodynamics can only be assessed in a relative context. Even though native heart valve pressure and flow data is available, it is important to conduct testing of native aortic porcine valves, which are biologically similar to human valves, using the same pulsatile simulator that is used to evaluate the polymer valves so as to account for measurement differences that may be system dependent. Thus, the goal of this study was to demonstrate how a commercially available pulse simulator can be fitted with an assembly to accommodate tri-leaflet valve constructs and to systematically evaluate polymer valve hydrodynamic metrics in a relative context in comparison to mechanical and native porcine heart valve counterparts. In our case, novel tri-leaflet silicone polymer valves previously developed at the University of Florida [13] comprised the polymer valve group.

Protocol

1. Preparation

1.1 Design and fabricate an assembly to accommodate a tri-leaflet valve geometry. This will at minimum include a valve holder to suture-in the valve leaflets, a tube to house the valve holder and surrounding accessories to secure the assembly onto the pulse duplicator system. In our case, we utilized a commercially available pulse duplicator system available from ViVitro Labs Inc. (Victoria, BC). Valve holder design as well as pre and post assembly configurations are depicted in Fig. 1.

1.2 The entire loop will need to be primed prior to usage. This involves two steps: i) cleansing of the entire loop system using soap solution and water, including replacement of any degraded tubes prior to use and ii) calibration of instruments connected to the loop, namely the pump being used, the flow probe and the pressure transducers (generally measured at atrial, aortic and ventricular locations). Calibration can initially be performed using 1% saline solution and should be repeated prior to using blood-analog glycerin solution.

2. Native aortic valve dissection

2.1 Obtain 4 fresh pig hearts with the aorta intact from a USDA approved slaughter

house (Institutional Animal Care and Use Committee (IACUC) approval may be required). In our case, our dissection protocol was approved by the IACUC at Florida International University (Protocol Approval Number: 11-020). Rinse the heart with deionized water and place it in a receptacle filled with the 1% antimycotic/antibiotic and sterile phosphate buffered saline (PBS) solution and transport on ice to the hydrodynamic testing laboratory.

2.2 Place hearts in a dissecting pan and carefully remove the pericardium. Position the heart such that ventral side is facing you. Visually inspect and identify the four chambers of the heart and locate the aortic arch on the intact aorta.

2.3 Separate the heart into two halves by cutting across horizontally at approximately ¾" below the annulus, i.e., the junction between the aorta and the left ventricle. Carefully isolate the intact aorta still attached to the left ventricular tissue segment.

2.4 Examine the aortic valve located in the aortic root, the region between the ascending aorta and the lower annulus, ensuring that there is no damage or any signs of calcification.

2.5 Split the aorta at ~ 1" above the annulus and separate the left ventricular tissue segment below the annulus to isolate the aortic valve (Fig.2a).

3. Polymer and Native Valve Suturing process

3.1 Place the heart valve inside the valve holder such that the base of each valve aligns with the base of the post holder. Secure the valve in place at each post temporarily with a paper clip, but be careful not to damage the commissures or the cusps.

3.2. Insert the suture in the needle. Begin suturing at the bottom of the valve holder by passing the needle through the first hole, from the outside to the inside such that the needle may be easily pulled from the bottom. In a looping fashion, start suturing the valve vertically up the posts of the valve holder.

3.3 Progress with suturing (Fig. 2b) along the circumference of the holder and secure with additional suture around the tips of the holder posts. Paper clips (Fig. 2c) can be removed when the valve is completely secured using sutures to the 3 posts and at the circumference of the valve holder (Figs. 2d and 2e).

4. Hydrodynamic evaluation (note actual protocol will vary depending on specific pulse duplicator system being used; all information contained herein used the Vivitro Pulse duplicator system, (Vivitro Labs Inc., Vancouver, BC).

4.1 Bi-leaflet valve

4.1.1 Set heart rate of pulse duplicator system to 70 beats/min.

4.1.2 Select a flow waveform to drive the pump (in the case of the Vivitro system the S35 waveform was chosen for all hydrodynamic tests). The specific waveform utilized in our experiments is illustrated by Lim *et al* (2001) [17].

4.1.3 Turn on amplifier and piston pump. Warm up for 15 min.

4.1.4 Place bi-leaflet valve (Fig. 2f) in the aortic position.

4.1.5 Smear vacuum grease on all junctions of the device where leaks could occur.

4.1.6 Pour glycerin/saline liquid in the atrial compartment. Note that the pulsatile duplicator system runs on 2 L of liquid with: 35%/0.7 L Glycerin and 65%/1.3 L of Saline solution. The saline solution is prepared using common salt well-dissolved in deionized water at a concentration of 9 mg/ml (weight/volume).

4.1.7 Turn on the flow transducer that has been placed in the aortic position.

4.1.8 Calibrate the pump.

4.1.9 Proceed with the flow transducer calibration followed by the pressure transducers. Similarly to the pump, simply follow the instructions given by the Vivitest software (Vivitro Labs Inc.) for each flow and pressure under the calibrate tab.

4.1.10 Once calibration is complete, start the pump at a low rpm until the fluid fills the aortic compartment. Check for leaks. Use additional vacuum grease if necessary.

4.1.11 Turn the two stop-cocks (aortic and ventricular transducers) to open position.

4.1.12 Increase the rpm of the pump until the stroke volume reaches 80 ml/beat.

4.1.13 Permit the system to run for 10 minutes until flow has stabilized. Flow stabilization can be verified by observing the flow and pressure waveforms displayed in the screen. Low to none variation between cycles is a good indicator of system stabilization.

4.1.14 In the Vivitest software select acquire mode.

4.1.15 Click on collect 10 cycles.

4.1.16 From the analyze mode, click on table and save the file. Also save an image of the waveforms using the photo-snap option in Vivitest.

4.2 Native and Polymer valves

4.2.1 For polymer and animal valve, follow the same steps 3.1.1 to 3.1.3 from the bi-leaflet valve instructions.

4.2.2 Place the valve holder with the sutured valve inside the glass tube from the custom made assembly. Sandwich the tube with the top and bottom pieces and secure in-place with lateral screws and nuts.

4.2.3 Place assembly between the aortic chamber and the original aortic valve holder.

4.2.4 Continue with steps 3.1.5 to 3.1.16 from the bi-leaflet valve instructions.

5. Post Processing

5.1 Flow and Pressure Waveforms

5.1.1 Average the data collected for each of the waveforms collected, i.e., aortic pressure (AP), ventricular pressure (VP) and flow rate (Q).

5.1.2 For each group of valve (polymer, porcine native aortic valve and bi-leaflet), plot the corresponding AP, VP and Q versus time relationships on the same plot.

5.1.3 For the AP, superimpose normal, native aortic valve [18] and bi-leaflet prosthetic valve [19] plots from the literature for validation purposes.

5.2 Hydrodynamic metrics

5.2.1 For each valve tested, the following hydrodynamic metrics should be computed: a) Forward flow pressure drop and maximum transvalvular pressure (TVP), b) the aortic root mean square (RMS) forward flow rate, c) aortic forward flow, closing, leakage and total regurgitant volume, d) valve end orifice area (EOA), e) transaortic forward flow, closing, leakage and total energy losses.

5.2.1.1 Forward flow pressure drop is computed from TVP readings and can be categorized into 3 time intervals, P: interval that starts and ends with 0 TVP, F: interval with forward flow and H: interval starting with 0 TVP and ending with 0 flow. Maximum TVP is the maximum pressure gradient recorded across the valve from the aortic and ventricular pressure readings.

5.2.1.2 The RMS forward flow rate (Q_{rms}) provides a useful metric for quantifying the magnitude of forward flow rate as follows:

$$Q_{rms} = \sqrt{\frac{1}{n}(Q_1^2 + Q_2^2 + Q_3^2 + Q_n^2)} \quad (1)$$

Where 'n' is the total number of time points collected, 'Q_i' is the instantaneous flow rate measurement collected in order '*i*'.

5.2.1.3 The aortic forward, closing and leakage volumes are computed based on the following time intervals, Forward: beginning of forward flow through the valve (t_o), to the end of forward flow (t_1); Closing: from t1 till the instance of valve closure (t2); Leakage: from t2 till the end of the cardiac cycle (t3). Total regurgitant volume is simply the sum of closing and leakage volumes.

5.2.1.4 The EOA based on blood properties can be computed for the 3 intervals, P, F and H from the mean TVP during each of these periods as[20]:

$$EOA = \frac{Q_{rms}}{51.6\sqrt{mean\ TVP}} \quad (2)$$

5.2.1.5 Energy losses are defined as follows[21]:

$$\Phi_{Forward\ flow} = \int_{t_o}^{t_1} Q_{valve} \cdot \Delta P \, dt \quad (3)$$

$$\Phi_{Closing\ flow} = \int_{t_1}^{t_2} Q_{valve} \cdot \Delta P \, dt \quad (4)$$

$$\Phi_{Leakage\ flow} = \int_{t_2}^{t_3} Q_{valve} \cdot \Delta P \, dt \quad (5)$$

Representative Results

Representative flow and pressure waveforms are shown in Figs 3, 4 and 5. The plots were averaged over the sample size of valves tested for each group, which was, n = 5, 4 and 2 valves for polymer, native porcine and bi-leaflet groups respectively. The mean hydrodynamic metrics and the standard error of the mean for these sample sizes are presented in Table 1.

Tables and Figures

Figure 1: (a) Schematic of the Vivitro pulse duplicator system showing the primary components that implement a Windkessel model for physiologically relevant flows (figure presented here with permission from Vivitro Systems, Inc, BC, Canada). (b) Rapid prototyped valve holder configuration to suture and secure silicone or native porcine valves in-place. (c) (b) Modification of the Vivitro pulsatile loop to accommodate tri-leaflet valve constructs;

Figure 2: (a) Native porcine valve. (b) Top view of polymer valve leaflets. (c) Side view of polymer valve after suturing and securing in-place within valve-holder. (d) Saint Jude Bi-leaflet mechanical valve.

Figure 3: Mean instantaneous flow rates of the 3 valves tested (n = 5, 4 and 2 valves for polymer, native porcine and bi-leaflet respectively). Flow rate was measured using an electromagnetic flow meter connected to a noninvasive flow probe placed at the interface location of the ventricle and aortic chambers (see Fig. 1a).

Figure 4: Mean instantaneous ventricular pressure of the 3 valves tested (n = 5, 4 and 2 valves for polymer, native porcine and bi-leaflet respectively). Ventricular pressure was measured in the ventricle chamber using a micro-tip pressure transducer. Superimposed literature ventricular pressure values for native and bi-leaflet valves (Diameter: 29 mm) were obtained from [18] and [19] respectively.

Figure 5: Mean instantaneous aortic pressure of the 3 valves tested (n = 5, 4 and 2 valves for polymer, native porcine and bi-leaflet respectively). Aortic pressure was measured just downstream from the aortic valve position using a micro-tip pressure transducer. Superimposed literature aortic pressure literature values for native and bi-leaflet (Diameter: 29 mm) valves were obtained from [18] and [19] respectively.

Table 1: Mean and Standard Error of the Mean (SEM) Hydrodynamic metrics computed for the heart valves tested (n = 5, 4 and 2 valves for polymer, native porcine and bi-leaflet respectively). The following intervals should be noted: P: interval that starts and ends with 0 TVP, F: interval with forward flow and H: interval starting with 0 TVP and ending with 0 flow. Mean diameters of the valves were as follows: Polymer valve (n=5): 22 mm; Native porcine valve (n=4): 20 mm; Bi-leaflet (n=2): 23mm. Small sample size for bi-leaflet valve was due to limited samples available for research use; the two bi-leaflet valves tested were previously donated to the Biomedical Engineering Department at Florida International University by Saint Jude Medical (Saint Paul, MN).

Table of Materials: Table of specific reagents and equipment used in this protocol

Discussion

In this study, we have demonstrated the utility of modifying a commercially available pulsatile duplicator unit to accommodate tri-leaflet valve geometries so that hydrodynamic testing of polymer and native porcine valves can be performed. Specifically in our case, the system modified was a Vivitro left heart and systemic simulator system (Fig. 1a) controlled via the ViViTest data acquisition system (Vivitro Systems, Inc, Victoria, BC, Canada). However, the system is not unlike several *in vitro*, pulsatile flow loops which all utilize a 2-component Windkessel model to mimic flow and pressure waveforms of relevance to the human circulation [22-25]. These 2-component Windkessel systems typically consist of a pulsatile pump, a compliance chamber that mimics the distensibility of the arteries, and a peripheral resistance controller that can be used to regulate the vascular resistance. The equation that describes the 2-component Windkessel model is:

$$C\frac{dP}{dt} = Q(t) - \frac{P}{R} \tag{6}$$

where C is the compliance, R the resistance, $Q(t)$ is the volumetric flow rate as a function of time and P is the arterial pressure (i.e., either in the pulmonary artery or aorta). In this context, we believe that a similar modification can be made to accommodate tri-leaflet valves in other pulsatile simulators as well. Specifically in our case, to house a tri-leaflet valve structure in the aortic valve location, an assembly primarily of acrylic plastic (plexiglass) casing that contained a rapid prototype valve holder and sutured tri-leaflet valve (Figs. 1b and 1c) could be easily integrated and removed from the primary Vivitro system. Hydrodynamic testing was subsequently conducted similar to other studies performed by Baldwin *et al.* [26] and Wang *et al.* [27]. Instantaneous flow rate was measured using an electromagnetic flowmeter system (Fig. 3).

Real-time measurement of pressure was recorded at the ventricular and conduit location using microtip transducers at a set heart rate of 70 beats/min (Figs. 4 and 5). The testing fluid was a blood-analog liquid, comprising, deionized water to glycerin in a 65% to 35% ratio and 9 g/l of NaCl, mimicking blood viscosity (~ 3.3 cP).

We initially tested a mechanical bi-leaflet valve and the obtained mean pressure wave forms were compared to literature values [19]. Some ventricular pressure variability was observed possibly owing to different pump mechanisms in place to drive fluid flow as well as geometry and specific settings of different pulse duplicator systems such as size of the ventricular chamber, specific valve mimicking the mitral valve location, heart rate chosen, physiological flow waveform selected, etc. On the other hand, the aortic waveforms were found to be very similar and system-independent. This exercise was repeated for native porcine valves and again, larger variability in ventricular pressure was observed when comparing our results to the literature [18]. However, it is important to note that within our system, instantaneous flow rates as well as both ventricular and aortic pressures were similar regardless of the valve that was tested, i.e., polymer and native with assembly or bi-leaflet without assembly. This exercise is important to perform because one needs to ensure that modifications to the duplicator system with an assembly do not considerably alter local flow and/or pressure conditions. Secondly, these results indicate that as a means of system validation, at minimum, comparable aortic pressures need to be derived across pulse duplicator platforms or the valve being tested. The interpretation of the hydrodynamic variables themselves is a matter of individual polymer valve design specifics. Standards such as ISO (International organization for standardization) 5840 used in the evaluation of cardiac valve

prostheses can serve as a guide to assess various parameters associated with the polymer valve geometry, manufacturing and material properties. These parameters can be further optimized and hydrodynamic testing subsequently re-visited to ensure that the standards needed for FDA submission are met.

For example, in our polymer valves, comparable energy losses and lower regurgitant volumes versus native and bi-leaflet valves suggested acceptable workloads on the left ventricle [21] and efficient valve closure (Table 1). However, the closing dynamics resulted in a relatively higher polymer valve maximum TVP gradient (versus bi-leaflet valves) which in our case, warrants further mechanical evaluation of silicone material being used to fabricate the valves to ensure that the higher stress does not cause leaflet rupture, and that a sufficient factor of safety can be put in place. In conclusion, we have demonstrated that an assembly consisting of a housing unit, glass tube and a valve holder can be fabricated to accommodate tri-leaflet structures such as polymer valves which can be sutured in-position. Comparative flow and pressure waveforms across native, prosthetic and polymer valve that is being developed needs to be obtained. Secondly the pressure waveforms need to be validated with literature values. A limitation of our approach is that ventricular waveforms are pulse duplicator system specific and are likely to show differences; however aortic pressure waveforms should be comparable across platforms or valve being tested if sufficient valve functionality exists. A future direction of this work is to further optimize the polymer valve material, manufacturing process and geometry. Hydrodynamics tests will subsequently be repeated under identical conditions so as to determine if functional improvements are quantitatively observed by comparing the current and previous hydrodynamic metrics computed.

Disclosures

The authors have nothing to disclose.

Acknowledgments

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*Figure

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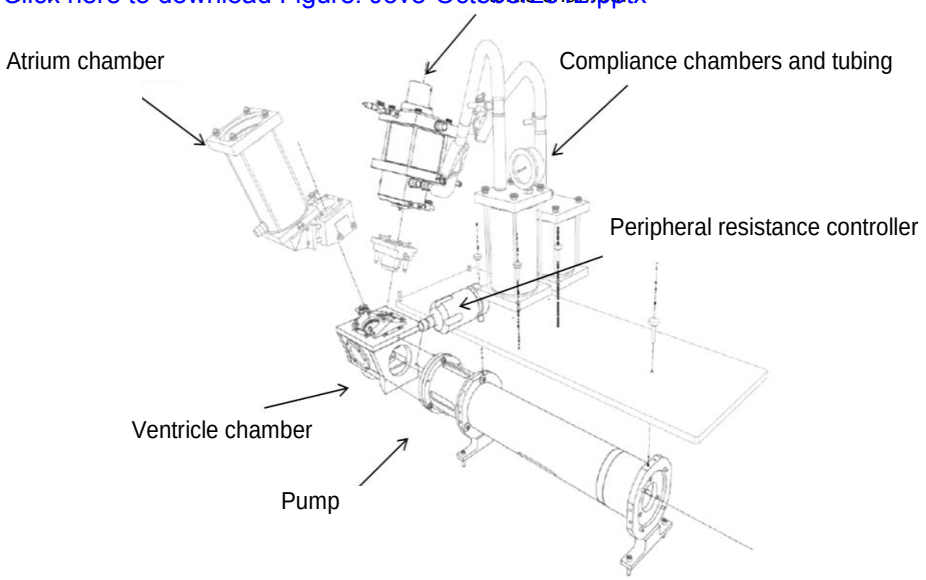
Figure 1

(a)



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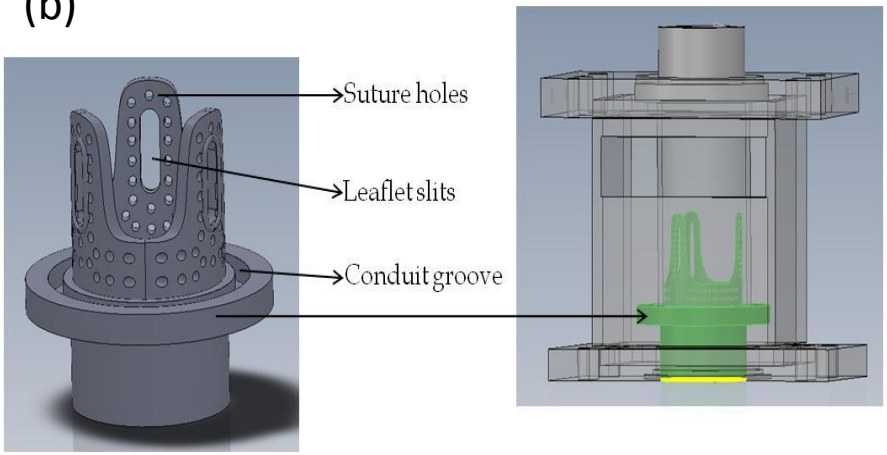

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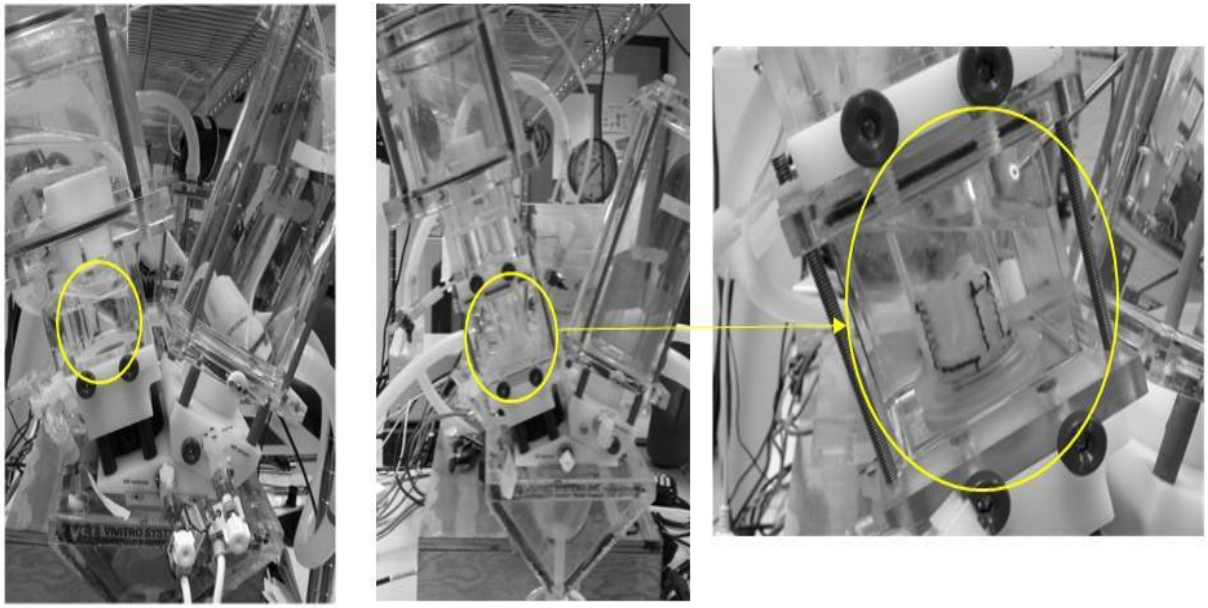

Figure 2

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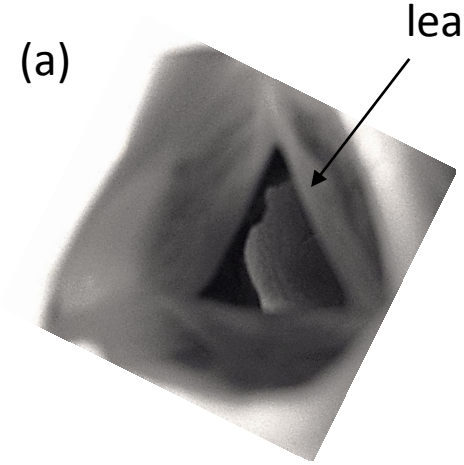

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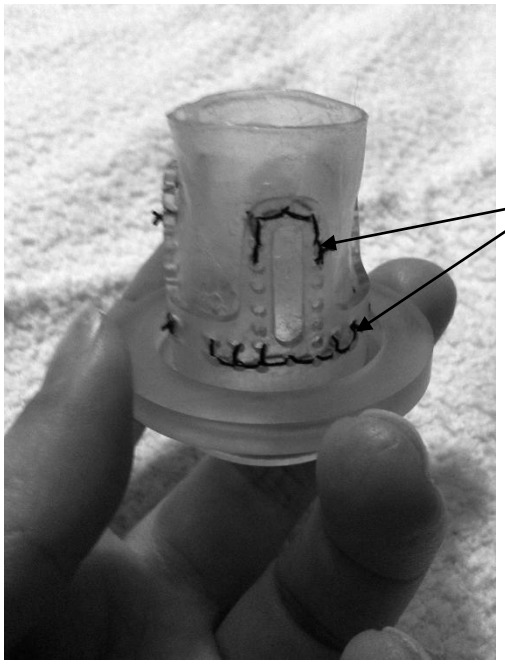

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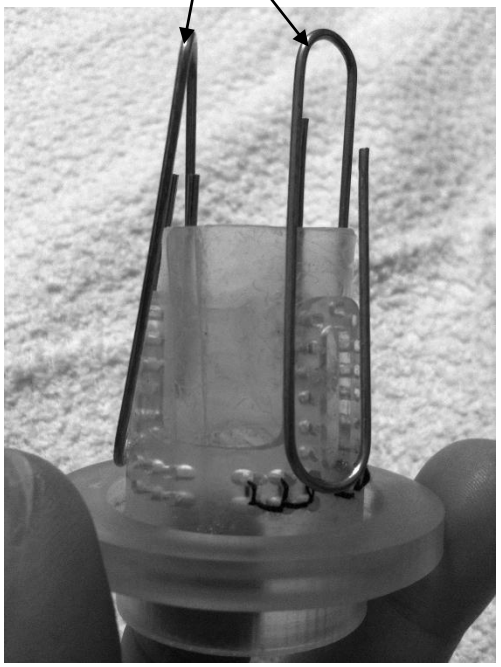

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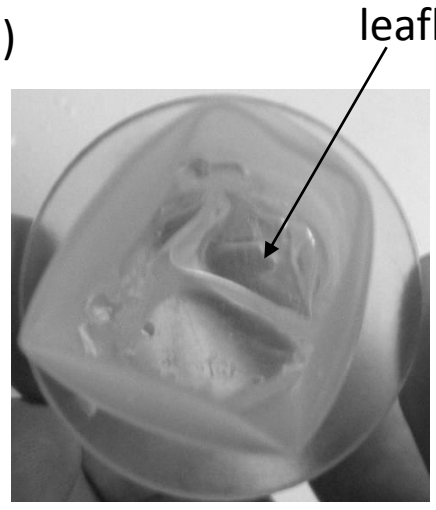

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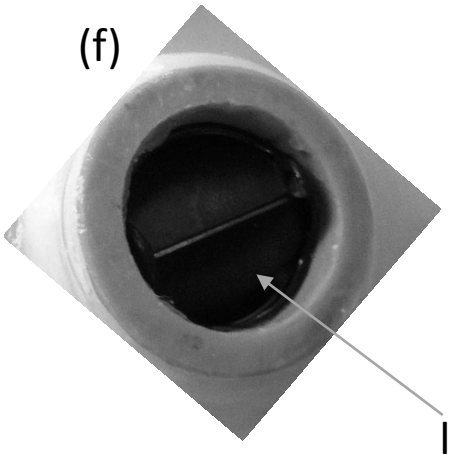

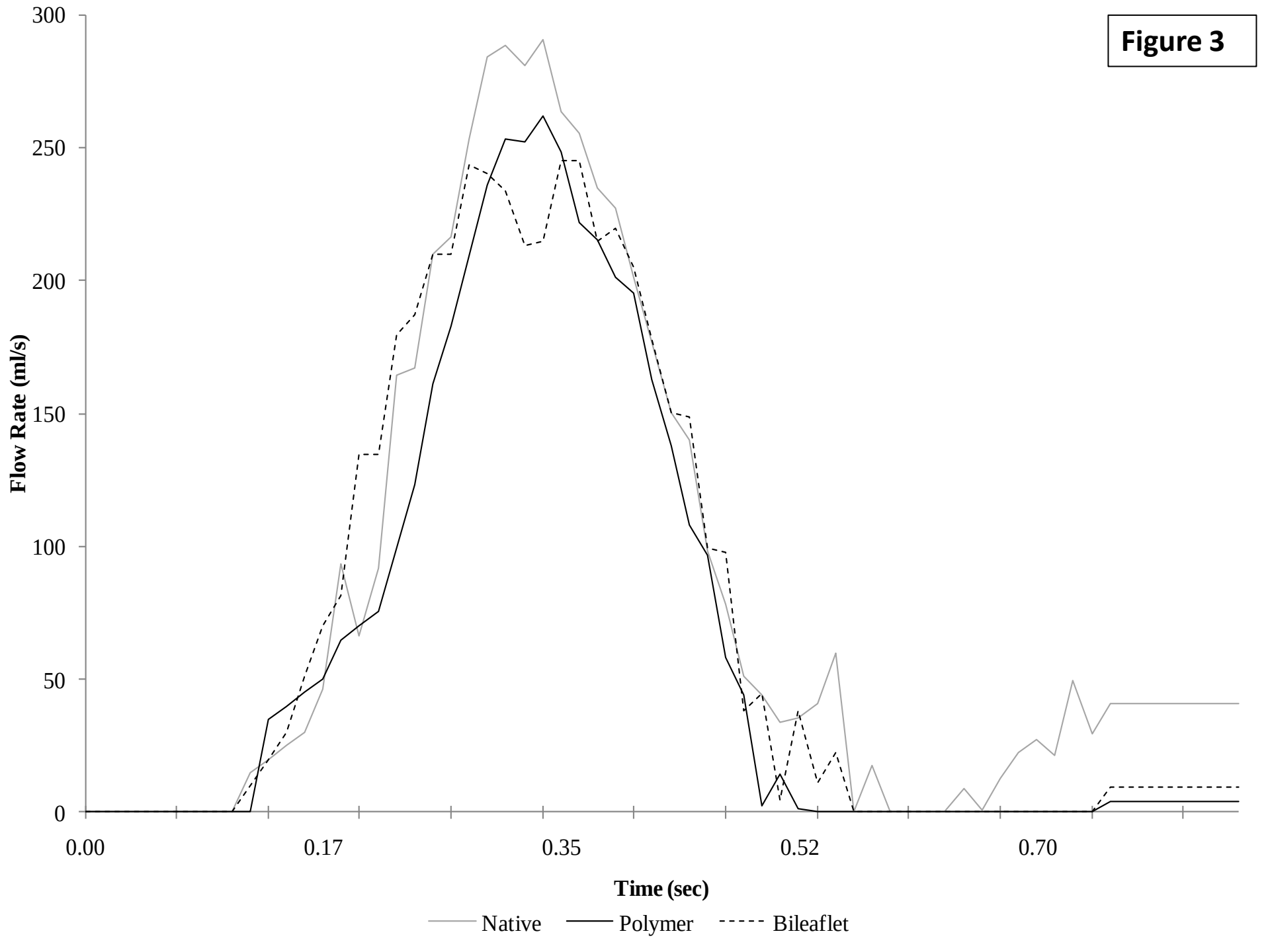
Figure 3
300
250
200
150
100
50
0
Flow Rate (ml/s)
0.00
0.17
0.35
0.52
0.70
Time (sec)
Native
Polymer
Bileaflet

Figure 4

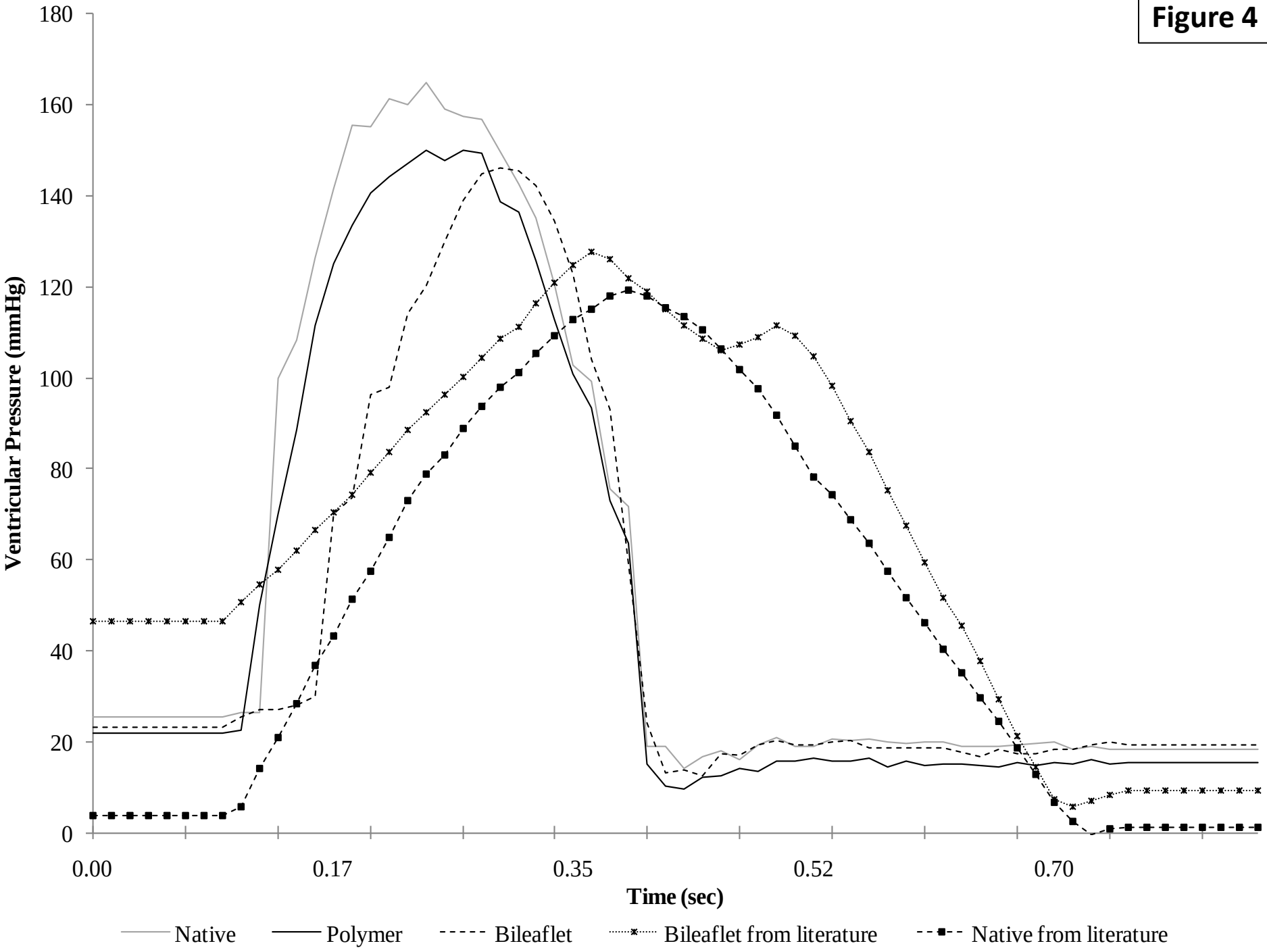
180
160
140
120
100
80
60
40
20
0
Ventricular Pressure (mmHg)
0.00
0.17
0.35
0.52
0.70
Time (sec)
Native
Polymer
Bileaflet
Bileaflet from literature
Native from literature

Figure 5

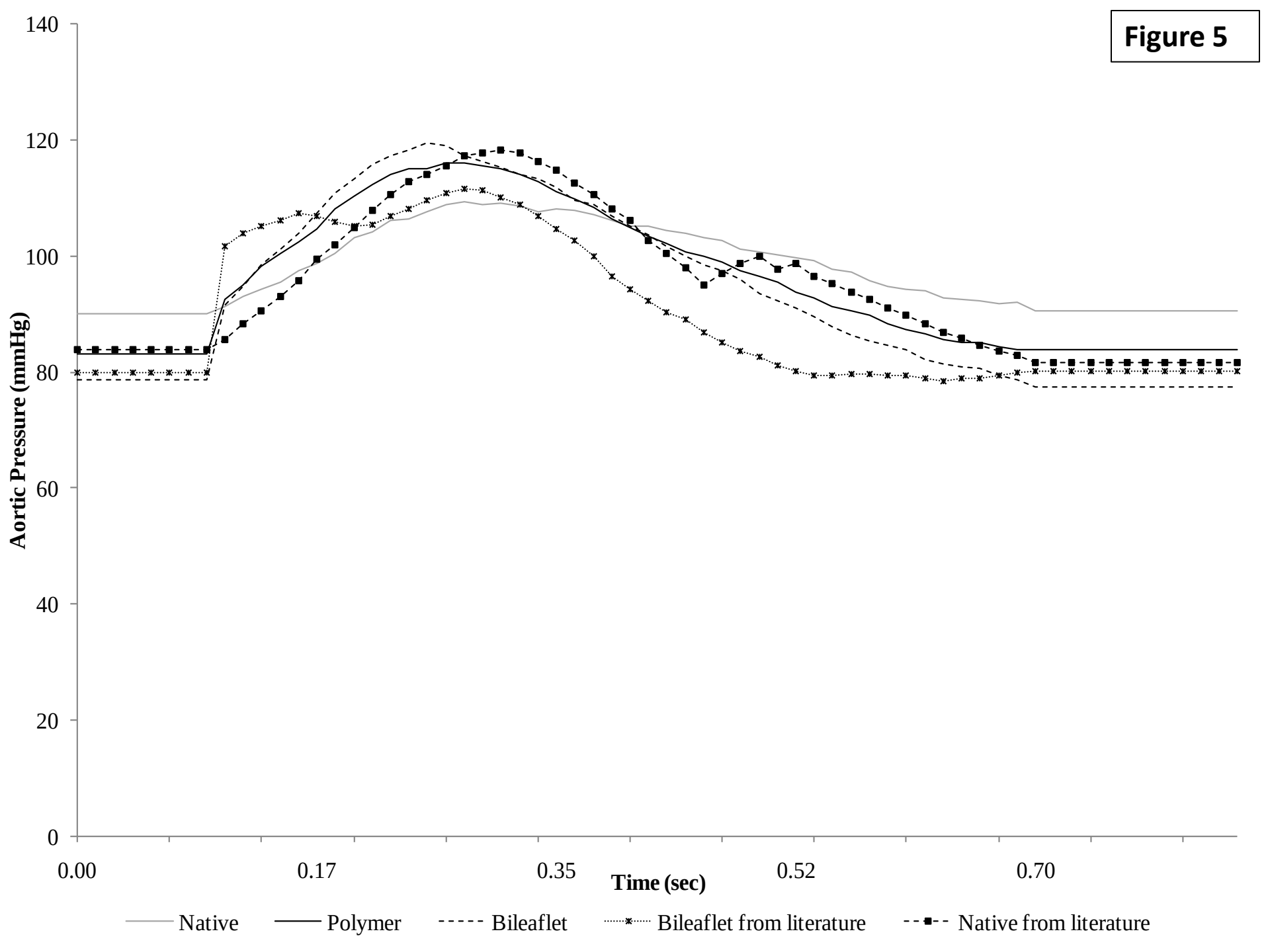
140
120
100
80
60
40
20
0
Aortic Pressure (mmHg)
0.00
0.17
0.35
0.52
0.70
Time (sec)
Native
Polymer
Bileaflet
Bileaflet from literature
Native from literature

Table 1

	Bileaflet (n=2)		(Polymer n=5)		Porcine (n=4)	
Data Description	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Aortic Orifice Area [P] (cm2)	3.143	2.697	2.920	1.306	2.516	1.258
Aortic Orifice Area [F] (cm2)	7.940	1.286	4.613	2.063	3.975	1.988
Aortic Orifice Area [H] (cm2)	7.516	1.633	4.575	2.046	3.942	1.971
Forward Flow Pressure Drop [P] (mmHg)	17.000	0.054	22.284	12.007	40.795	11.670
Forward Flow Pressure Drop [F] (mmHg)	0.410	0.210	30.424	9.235	29.766	9.733
Forward Flow Pressure Drop [H] (mmHg)	26.520	0.120	50.790	4.230	5.610	4.970
Trans-Aortic Max Pressure (mmHg)	15.850	12.400	60.930	20.470	75.250	17.470
Aortic RMS Forward Flow Rate [P] (ml/s)	88.280	11.110	162.120	24.970	189.080	32.610
Aortic RMS Forward Flow Rate [F] (ml/s)	193.570	3.820	204.560	6.680	177.310	2.630
Aortic RMS Forward Flow Rate [H] (ml/s)	197.790	0.630	174.760	11.530	182.680	3.160
Aortic Forward Volume (ml)	68.180	6.430	55.390	3.660	64.200	1.750
Aortic Closing Volume (ml)	62.260	0.860	32.990	9.820	45.260	11.990
Aortic Leakage Volume (ml)	60.140	3.470	33.090	9.220	56.130	11.260
Total Regurgitant Volume (ml)	122.400	4.320	66.080	17.200	101.390	23.160
TransAortic Forward Flow Energy Loss (mJ)	80.321	4.65	115.287	17.354	184.325	12.354
TransAortic Closing Energy Loss (mJ)	25.231	0.589	29.52	6.872	12.354	4.874
TransAortic Leakage Energy Loss (mJ)	87.219	13.242	84.02	12.205	97.029	25.047
TransAortic Total Energy Loss (mJ)	192.771	23.51	228.827	47.254	293.708	36.483

*Table of Reagents/ Materials Used

Click here to download Table of Reagents/ Materials Used: JoVE_Materials.xlsx

Name of Reagent/Material	Company	Catalog Number	Comments
Pump	Vivitro labs	n/a	http://vivitrolabs.com/products/superpump/
Flow Meter and Probe	Carolina Medical	Model 501D	http://www.carolinamedicalelectronics.com/documents/FM501.pdf
Pressure Transducer	Vivtro Labs	HCM018	
ViVitro Pressure Measuring Assembly	Vivtro Labs	6186	
Valve holder	WB Engineering	n/a	Designed by Florida International University; Manufactured by WB Engineering
Pulse Duplicator	Vivitro labs	PD2010	http://vivitrolabs.com/wp-content/uploads/Pulse-Duplicator-Accessories1.pdf
Pulse Duplicator Data Acquistion and Control System, Including, ViViTest Software	Vivitro labs	PDA2010	http://vivitrolabs.com/products/software-daq/
Porcine Hearts and Native aortic Valves	Mary's Ranch Inc	n/a	
Bi-leaflet mechanical valves	Saint Jude Medical	n/a	http://www.sjm.com/
High Vacuum Grease	Dow Corning Corporation	n/a	http://www1.dowcorning.com/DataFiles/090007b281afed0e.pdf
Glycerin	McMaster-Carr	3190K293	99% Natural 5 gal
Phosphate Buffered Saline (PBS)	Fisher Scientific	MT21031CV	100 ml/ heart
Antimycotic/ Antibiotic Solution	Fisher Scientific	SV3007901	1 ml in 100 ml of PBS/ heart; 20 ml for Vivitro System
NaCl	Sigma-Aldrich	S3014-500G	9 grams/ 1 L of deionized water
	EMD Millipore		Millipore Deionized Purification System;

Deionized Water	EMD Millipore Chemicals	n/a	1.3L for Vivitro System, 200 ml for heart valve dissection process

17 Sellers Street
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tel. +1.617.945.9051
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Author(s): Sharan Ramaswamy, Manuel Salinas, Rob Carrol, Karla Landaburo, Xavier Ryans, Cynthia Crespo, Ailyn Rivero, Faris Al-Mousily, Curt DeGroff, Mark Bleiweis, Hitomi Yamaguchi

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AUTHOR:

Name: Sharan Ramaswamy

Department: Biomedical Engineering

Institution: Florida International University

Article Title: Protocol for Relative Hydrodynamic Assessment of Tri-leaflet Polymer Valves

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RESPONSE TO REVIEWERS **October 16th 2012**

Reviewer #1

1) Page 1: "When valve damage occurs, causing (stenosis and/or regurgitation valve prolapsed), and cannot be surgically repaired, the native valve is usually replaced by a prosthetic valve."
   - Run on sentence. The author may want to reword this sentence or try removing parenthesis and the second comma

We thank the reviewer for this suggestion. We have corrected this sentence as suggested.

2) Page 4: Need a line space between sections 2.3 and 2.4

We have corrected as suggested by the reviewer.

3) Page 4: Figure 2b and 2c
   - These figures do not show clear pictures of the sutures and clips mentioned. The author should reconsider these pictures.

We thank the reviewer for this suggestion. We have updated Fig. 2 with new Figs. 2b and 2c showing the sutures and the clips respectively.

4) Page 7: Figures 4 and 5
   - Need to label which is aortic pressure and which is ventricle pressure in the y axis

We have updated the y-axes as suggested.

5) Page 7: "? n = 5, 4 and 2 valves for polymer, native porcine and bi-leaflet groups respectively."
   - The author should consider adding more valves to the bi-leaflet group. N=2 is a very weak sample size, consider increasing to n=4 or 5.

We thank the reviewer for this suggestion. The two bi-leaflet mechanical heart valves utilized in this study were a previous donation to the Biomedical Engineering Department at Florida International University several years ago. These valves are not typically available for research use. We were fortunate in that we had access to these two valves. We definitely would have like to have tested more bi-leaflet valves; however we were practically limited by access to these devices. Thus, we have added the followings statements to the caption of Table 1 reflecting this as follows:

"Small sample size for bi-leaflet valve was due to limited samples available for research use; the two bi-leaflet valves tested were previously donated to the Biomedical Engineering Department at Florida International University by Saint Jude Medical (Saint Paul, MN)."

6) Page 7: Table 1
   - What are the second numbers in the Aortic Orifice Area rows under the Polymer SEM and the Porcine Mean and SEM?

The Aortic Orifice Area was computed from the ViviTest software. The sample size for the polymer, native and bi-leaflet groups were as follows: n = 5, 4 and 2 valves. The mean reflects the average orifice area from the total number of samples tested per group. The SEM is the "standard error of the mean" which is the (standard deviation/$\sqrt{n}$) typically reported for a relatively small sample size. We have now clarified the abbreviation "SEM" in the caption for Table 1.

Reviewer #2

1) There is no new information in the paper even if the authors claim polymer valve was used. That is how we used this system long before. It is not really important what design of the valve can be used in the system.

We thank the reviewer for this comment. We completely agree that the type of valve being tested does not add a factor of novelty to the study. However we respectfully disagree that there is no new information in this paper. The two new pieces of information are as follows:

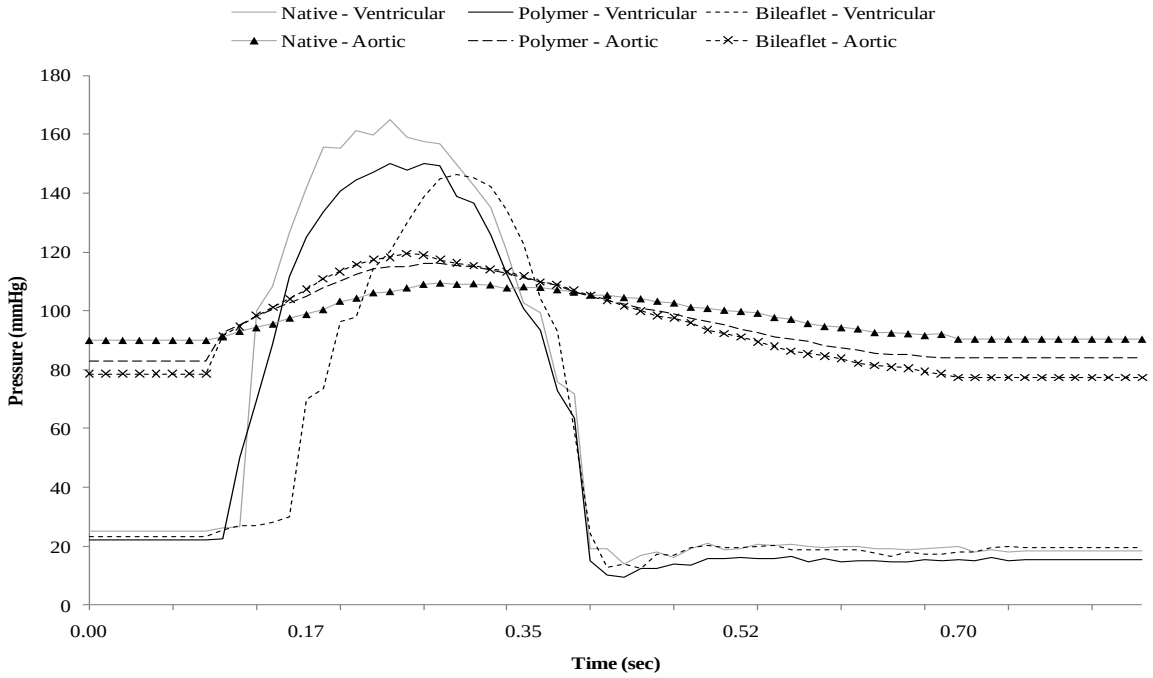
*a) The Vivitro system like most commercial systems are intended to test commercially available mechanical and bio-prosthetic valves that are mounted into place from their Dacron ring configuration. These systems are highly useful in that real time pressure ae and flow data can be monitored/recorded and hydrodynamic metrics calculated from the software system. However, these systems **cannot** accommodate tri-leaflet valve structures. An assembly has to be built and validated to accommodate tri-leaflet valves. As a component of this assembly, in the case of soft materials, like elastomers, a valve holder design needs to be available so that the valve leaflets can be sutured in place. We built such an assembly (Fig. 1) and validated it against bi-leaflet mechanical valve pressure and flow data thereby demonstrating that tri-leaflet valve configurations could be tested by building an assembly and integrating it with an existing Vi-vitro System.*

b) Since polymer valves are an emerging technology, it would be useful to make comparisons to native porcine valves which are biologically similar to native human valves. Native aortic porcine valves are also tri-leaflet in structure and would thus readily be accommodated in our modified Vi-vitro unit. It is important to make direct comparisons between polymer and native valves using the same testing equipment because making indirect comparisons with valve pressure waveforms obtained clinically may include system-dependent differences and not true differences of the valves themselves. Thus a second novelty of our work was to introduce the concept of objectively comparing polymer versus native valve hydrodynamic metrics.

Finally, we note that the objective of this manuscript is mainly to provide additional insights on the overall protocol for hydrodynamic testing via video, a component of JoVE and that JoVE does not require novel results. Nonetheless we wanted to provide the above responses to also highlight that there were novel elements in this study.

2) I also found that figs 2, 3, 4, 5 were not reasonable for a physiological cases because the flow measurement resolution was low and not normal in the backward flow and pressures were not normal for bad compliance control. You can see these pressure problems if you put ventricular pressure and aortic pressure in the same graph.

We thank the reviewer for this excellent comment and we have followed up as suggested. The aortic and ventricular plots are superimposed as shown below:



As shown in the above figure, we have eliminated noise in the curves particularly during the early phase of ventricular pressure increase. We have also corrected for the issues related to compliance and have verified that the rise and decrease in ventricular and aortic pressures are synchronous. All the figures for flow (Fig. 3), ventricular (Fig. 4) and aortic (Fig. 5) pressures have been thus updated accordingly. We continue to keep ventricular and aortic pressures as separate plots in the manuscript for clarity. Here however, we have superimposed the plots as suggested by the reviewer to demonstrate that the noise in the data has been minimized and there is now synergy between the aortic and ventricular pressures. Finally, the resolution of the flow waveforms has also improved owing to the smoothing of the curves.

3) Equation 1 need "2" as square form of Q

We thank the reviewer for catching this error. We have now corrected.

4) How to choose pressure or flow value in the calculation in the equation 1-5

Pressure and flow values are instantaneous measurements and are chosen based on the intervals specified, and subsequently averaged, in order to compute the equations. For equations 3 – 5 the intervals are defined as follows: Forward: beginning of forward flow through the valve (t_o),

to the end of forward flow (t_1); Closing: from t_1 till the instance of valve closure (t_2); Leakage: from t_2 till the end of the cardiac cycle (t_3). The pressure drop in the equations is the difference between the instantaneous ventricular and aortic pressures measured at corresponding locations by pressure transducers.

5) In table 1, why was forward pressure negative? How to control the same forward flow volume?

Indeed the pressures should not be negative and this was a calculation error. We have now re-done our calculations and the new values can now be found in the updated Table 1. The forward flow volume can be regulated by adjusting the heart rate in the system or alternatively, by regulating the peripheral resistance component (Fig 1a) if forward flow volume needs to be adjusted without affecting the pressure measurements.