**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.*

1. Page 4: Need a line space between sections 2.3 and 2.4

*We have corrected as suggested by the reviewer.*

1. 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.*

1. 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.*

1. 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).”*

1. 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/√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:*

1. *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.*
2. *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.*

1. 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.*

1. 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 (to), to the end of forward flow (t1); Closing: from t1 till the instance of valve closure (t2); Leakage: from t2 till the end of the cardiac cycle (t3). 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.*