Design of a Pulsatile Flow Facility to Evaluate Thrombogenic Potential of Implantable Cardiac Devices

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Due to expensive nature of clinical trials, implantable cardiac devices should first be extensively characterized in vitro. Prosthetic heart valves (PHVs), an important class of these devices, have been shown to be associated with thromboembolic complications. Although various in vitro systems have been designed to quantify blood-cell damage and platelet activation caused by nonphysiological hemodynamic shear stresses in these PHVs, very few systems attempt to characterize both blood damage and fluid dynamics aspects of PHVs in the same test system. Various numerical modeling methodologies are also evolving to simulate the structural mechanics, fluid mechanics, and blood damage aspects of these devices. This article presents a completely

hemocompatible small-volume test-platform that can be used for thrombogenicity studies and experimental fluid mechanics characterization. Using a programmable piston pump to drive freshly drawn human blood inside a cylindrical column, the presented system can simulate various physiological and pathophysiological conditions in testing PHVs. The system includes a modular device-mounting chamber, and in this presented case, a 23 mm St. Jude Medical (SJM) Regents® mechanical heart valve (MHV) in aortic position was used as the test device. The system was validated for its capability to quantify blood damage by measuring blood damage induced by the tester itself (using freshly drawn whole human blood). Blood damage levels were ascertained through clinically relevant assays on human blood while fluid dynamics were characterized using time-resolved particle image velocimetry (PIV) using a blood-mimicking fluid. Blood damage induced by the tester itself, assessed through Thrombin-anti-Thrombin (TAT), Prothrombin factor 1.2 (PF1.2), and hemolysis (Drabkins assay), was within clinically accepted levels. The hydrodynamic performance of the tester showed consistent, repeatable physiological pressure and flow conditions. In addition, the system contains proximity sensors to accurately capture leaflet motion during the entire cardiac cycle. The PIV results showed skewing of the leakage jet, caused by the asymmetric closing of the two leaflets. All these results are critical to characterizing the blood damage and fluid dynamics characteristics of the SJM Regents® MHV, proving the utility of this tester as a precise system for assessing the hemodynamics and thrombogenicity for various PHVs. [DOI: 10.1115/1.4029579]

1 Introduction

It is well established that blood element damage and platelet activation that are caused by flow-induced shear stresses result in thrombus formation in cardiovascular systems [1–5]. This affects the performance of a broad range of cardiovascular devices such as prosthetic valves, stents, bypass pumps, and flow-assist devices [6–19]. All these hardware are designed to mimic the function of the heart either totally or partially. Ventricular assist devices are increasingly being used in congestive heart failure patients and have been proven to be more effective than treatment through drug therapy alone [6–11,20]. PHVs are widely used as a replacement for diseased heart valves. In spite of increasingly successful usage of implantable devices for treating cardiovascular ailments, these devices still show significant complications in terms of hemolysis, platelet activation, and thromboembolism. In particular, studies on bileaflet MHVs have emphasized the significant risk of thromboembolic complications when blood elements are subjected to artificially high shear stresses levels [21–23]. MHVs are prone to complications including tissue overgrowth, thromboembolic complications, hemorrhaging, infection, paravalvular regurgitation, and hemolysis [24,25]. Patients implanted with MHVs must undergo lifelong anticoagulation therapy to offset the effect of coagulation complications. However, anticoagulation therapy runs the risk of causing hemorrhage. Additionally, dosing is a nontrivial patient-specific challenge in anticoagulation [26]. Blood clots formed at the site of implantation can break into micro-emboli, which may migrate into the cerebral circulation [27] resulting in transient ischemic attacks. The incidence, frequency and morphology of these micro-emboli have been characterized previously [28,29]. It has also been reported that MHV patients have a tendency for solid and gaseous cerebral emboli [30].

Clot initiation caused by a cardiovascular device can occur via three basic pathways: phospholipid and/or tissue factor exposure to blood during cell damage; platelet activation caused by exposure to excessive shear stress; and contact activation by foreign materials. Von Willebrand factor, a blood glycoprotein, and fibrin formed during coagulation process operate together to increase platelet adhesion and aggregation under high shear stress conditions [31–35]. The coagulation cascade consists of a group of coagulation proteins acting in synergy to form a solid clot. Each

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component of the coagulation cascade must be functioning properly and be present in sufficient quantity for normal blood clot formation. Since fluid forces and cellular level mechanisms act synergistically to form a solid clot, this should be studied from both hematology and fluid mechanics perspectives. Thrombus formation is a complex process involving shear stress activation of platelets, flow separation, and flow stagnation [1,2,32–35]. This should be studied from both hematology and fluid mechanics perspectives. This combined approach is essential given that isolated approaches are not strongly conclusive. For example, fluid mechanics studies have predicted shear stress levels for all MHVs to be higher than platelet activation levels [24,36,37], but not all MHVs have same levels of thromboembolism. Similarly, hematology studies indicating good performance in animal models do not always assure satisfactory performance in humans [38–40]. Given that the blood coagulation cascade in animals is different than in humans, it becomes imperative to test the implantable medical devices using human blood while testing for coagulation potential [41]. With respect to implantable cardiac devices, the flow fields in the vicinity of the device need to be accurately characterized so that there can be a better understanding of the thromboembolic complications associated with them. For instance, the Medtronic Parallel® valve cleared all in vivo and preclinical testing successfully, but failed in clinical trials due to excessive thromboembolism. Later investigation revealed that the Parallel® valve activated the platelets and increased blood element damage in the hinge regions of the valve in a time dependent manner. As inferred later [39], seemingly minute differences in design can lead to substantial differences in coagulation [42]. Although no bench top testing can replace clinical trials for efficacy and safety, innovative engineering approaches may be able to elucidate potential complications during the design, development, and validation processes of medical devices. The present study represents a significant step in this direction.

Many previous studies have attempted to elucidate the thrombosis pathway in cardiac devices [8,15,19], especially MHVs [1–3,43–47]. To assess the coagulation potential of these devices, studies have used various human-blood substitutes and models [48,49]. Bluestein et al. used a modified-prothrombinase method with washed platelets to investigate flow-induced platelet activation for two Björk–Shiley monoleaflet MHVs [50], wherein the activation state of the platelet sample was indicated by the amount of acetylated thrombin generated. It was observed that in a pulsatile flow system, platelet activation was dependent upon the restriction-level of the valve. These restrictions were used for simulating defective functioning of a compromised valve in vivo. The unrestricted valve resulted in no significant platelet activation while the severely restricted valve resulted in significant platelet activation (eightfold higher). Different valve positions for MHV (Mitral position [51]) and other damage-inducing fluid dynamics phenomenon (cavitation [51]) have also been studied. Recent microfluidic systems have attempted to isolate the effect of shear rates and shear gradients using stenosed microchannels [52]. Though these systems offer distinct advantages in terms of being real-time, microscope-free, and label-free, it cannot mimic physiological pressure and volume variations in modeling cardiovascular devices. Yin et al. used a modified prothrombinase assay to quantify platelet activation [4]. This study used a left ventricular assist device to measure the procoagulant properties of MHVs. Plasma free platelets were used in the study with no simulation of physiological pressures. Xenos et al. developed a hemodynamic shearing device to expose purified platelets to varying levels of shear stress fields using a combined cone-plate and Couette viscometer approach [53]. This system exposed platelets to precise levels of shear stress as measured from other models. Linde et al. developed a low-volume (220 ml) tester for MHVs, but used porcine blood in their studies. Porcine blood has been shown to differ from human blood in terms of coagulation rate and cascade [54]. The valve retainer used in their studies seemed to affect the blood damage outcome.

Experimental fluid dynamics have been extensively used to characterize intricate flow structures generated in flow through MHVs under steady and pulsatile conditions using techniques like PIV and laser Doppler velocimetry (LDV) [5,23,55–57]. Shear stress values, which directly correlate with blood element damage [58], can be computed from these flow field characterizations. Studies using PIV and LDV have identified the hinge regions of MHVs to be a major reason for blood coagulation [53,59]. Activated platelets, along with other protein factors such as plasma proteins and adhesive proteins, tend to form thrombi once they enter the flow stagnation regions of these MHV hinges [33,35]. Studies on MHVs have focused on specific segments of the cardiac cycle (closing phase, leakage phase) or subcomponents of the MHV structure (hinge region, b-datum line).

The system described in this study provides the capability for flow characterization (using PIV) as well as fresh human blood studies in the same facility. This system requires a small volume of human blood (150 ml) that can be drawn from a single donor. Other comparable testers use higher volume of blood [54,60]. Another feature of this system is the absence of tester-induced secondary flow structures in the blood volume, since the flow is driven by a rigid piston in a straight rigid tube. Inclusion of rigid components avoids the variations typically associated with flexible structures. This also maintains consistency and repeatability of operating conditions. Comparable testers [54] use flexible membranes to mimic ventricular pumping. This might give rise to secondary flow structures during pumping motion. Given that blood damage and platelet activation can possibly be triggered by these secondary flow structures, it is necessary to avoid those in a tester. With rapid advances in material science and polymer technology, new valve designs are emerging [42,61,62]. The tester presented here can accommodate these polymeric valves using the same mounting method as that of the test valve used in this study. Thus, the presented system provides a simple yet powerful facility to investigate various devices for both platelet activation and thromboembolic potential.

2 Materials and Methods

2.1 Design Principle. The design of this pulsatile facility was guided by the following requirements: (1) biocompatibility for using freshly drawn human blood, (2) low-volume of operation so that a single donor's blood can be used, (3) optical access for flow studies, (4) well-regulated physiological environment, and (5) high-resolution data acquisition capabilities. The system was conceptualized to include a modular mounting chamber that houses the cardiovascular devices, such as prosthetic valves and stents.

The schematic of the system is shown in Fig. 1. Figure 2 shows various modular chambers that can be plugged into the tester. The operating principle and control mechanism is versatile to allow simulation of appropriate physiological and pathophysiological conditions based on the cardiovascular device being tested in the pulsatile system. The modular chambers were manufactured out of biocompatible, transparent acrylic (polymethylmethacrylate—USP Grade VI) with optical access required for PIV studies. Careful measures were taken to make all the blood-contacting surfaces biocompatible, in terms of materials and surface-finish. All the machined surfaces were smoothed and polished to avoid any surface generated coagulation. All the components of this test-bed were carefully chosen to minimize the total volume of the system (150 ml), while maintaining the physiological relevance in terms of hemodynamics and blood damage values. As indicated earlier, the present study utilizes a SJM Regent MHV under aortic conditions for validation; however, the system can be easily adapted to accommodate various other cardiovascular devices.

2.2 Design Description. The various components of this system are shown in Fig. 3 and described in detail below.

2.2.1 Linear Actuator System. A master computer was used to set up the motion profile connected to the motor controller

Fig. 1 Schematic of the system design showing the pump (linear actuator and motor), blood containing test section, and the pressure regulation system

(CMMP-AS, Festo Corporation, Hauppauge, NY) of the linear actuator. The servo motor (EMMS-AS-55-S-TSB, Festo Corporation) was equipped with a digital single-turn displacement encoder with a resolution of 16 bit per revolution. The toothed belt driven linear actuator (DGE-25-300-ZR-R—Festo Corporation) has a working length of 300 mm. The motion profile is set up using the FESTO Configuration Tool software where the position of the actuator, velocity, and acceleration can be finely tuned in order to mimic the physiological pressures and timing of the cardiac cycle. The end of the linear actuator arm carries a piston, made of poly-ether ether ketone, which slides inside the titanium tube. A

biocompatible (USP class VI) silicone O-ring seals (Parker, Lexington, KY) the piston and the inner wall of the tube. The operation of entire system is synchronized on a LABVIEW™ environment, which samples the pressure traces of the transducers, position and velocity of the actuator, position of the leaflets, and synchronizes pulses for PIV experiments. Since the linear actuator is directly coupled with the piston, it enables precise control of flow rate, flow acceleration, and deceleration. This feature helps to mimic a variety of physiological and pathophysiological conditions by programming in the appropriate motion profile that corresponds to changes in flow rate, flow volume, and pulsatility rate.

Fig. 2 Modular chambers that can be plugged into the tester to simultaneously study fluid dynamics and blood damage levels. (a) Chamber to study flow in MHV leaflet-hinges, (b) an inferior vena-cava filter design and chamber to mount the filter, and (c) a MHV with transparent ring and its chamber with an axisymmetric aortic sinus.

Fig. 3 (a) Valve holder with SJM valve, (b) test system showing the actuator at the bottom, middle test section with bypass tube, flexible bag inside the compliance chamber at the top

2.2.2 Test Section and Modular Valve Chamber. The end of the linear actuator arm carrying the piston slides inside the cylindrical test section. In order to reduce the friction associated with the piston motion, custom surface-finished grade IV biocompatible titanium tubes were used for the test section. The internal diameter of the tubes was machined with a precise tolerance of 0.001 in. and the internal surface was highly polished to reduce piston friction. The titanium tubes were press fit into the acrylic chamber, where the internal diameter of the tube matched the diameter of the conduit inside the chamber. This was critical to avoid any secondary flow structures that may be created due to any interface mismatch. The modular acrylic sections were fabricated with clear planar surfaces to minimize the optical distortion of the view of the flow inside the internal tube for PIV experiments (Fig. 2). Between the two acrylic sections, the valve housing is placed and aligned with the internal conduit of the chamber using alignment pins.

2.2.3 Valve and Valve-Housing. A 23 mm MHV (St. Jude Regent valve, SJM, St. Paul, MN) was used for this study. A grade IV titanium holder was fabricated to support the heart valve. This design allows the assembly of the valve in the acrylic chamber using alignment pins and avoids bypass leakages. The valve housing is covered on both sides with silicone gasket material in order to ensure perfect sealing of the upper and lower acrylic sections of the chamber.

2.2.4 Pressure Sensors. Pressures on the ventricular and the aortic sides of the test chamber were measured using pressure sensors (Model: DPT-200, Utah Medical; Natural frequency >200 Hz) placed 4 cm from either side of the MHV. The pressure sensors are connected to a Wheatstone bridge based signal amplifier and to an analog-digital converter (National Instruments, Model: BNC-2120). The pressures are recorded using a custom LABVIEW™ program.

2.2.5 Pressure Regulation. A compressed-air pressure system, operating on the aortic side, helps to maintain a mean aortic pressure of 100 mmHg. During blood experiments, the blood is contained in a flexible cylindrical biocompatible bag (Qosina, Edgewood, NY), suspended from the top of the pressure chamber. This chamber is connected to the compressed air source. During PIV experiments, the fluid is exposed to free air from an air reservoir that is connected to the compressed air source. The volume of air enclosed in the pressure chamber (blood experiment) or the air reservoir (PIV experiment) provides the compliance in the system, which helped to achieve physiological pressures during the entire cardiac cycle.

2.2.6 Laser-Based Position Sensors. The leaflet dynamics were monitored individually using two Micro-epsilon (optoNCDT 1605) laser-based position sensors with a resolution of 30 μm and a frequency response of 3 kHz. The position sensor used the measurement principle of laser triangulation, in which a laser beam projects a laser spot on the measurement object and the reflected light was captured by the sensor at a certain angle depending on the distance. The distance to the object was calculated by the position of the scattered light on the sensor. Though the leaflets were not tracked at the leaflet tip, the leaflet motion can be considered as a solid body rotation and the measurement obtained from mid-leaflet could be extrapolated easily to the tip of the leaflet, where the displacement is largest. All the output signals were sampled using a National Instruments BNC-2090 data acquisition board and recorded using LABVIEW™.

2.3 Operation. To test the 23 mm MHV (St. Jude Medical Regents™) in aortic position, this low-volume system was operated by replicating the appropriate physiological pressure and flow conditions across the MHV (cardiac output 5 l/min, 70 ml stroke volume, 70 beats per minute, 120/80 mmHg systolic/diastolic pressure, 330 ms of systole, and 530 ms of diastole). The section upstream of the valve corresponded to the left ventricle of the heart and the downstream section represented the aorta. During the simulated systole of a cardiac cycle, the ventricular pumping action was achieved by the computer controlled linear actuator, which pumped the fluid from the ventricular side to the aortic side through the MHV. During diastole, the MHV remained closed because of the reversed transvalvular pressure gradient, allowing the fluid to return to the ventricular side through a bypass return tube (Pharmed BPT, Cole Parmer). The diameter of the bypass tube was carefully chosen to avoid any unwanted pressure drop, while keeping it small enough to minimize the volume of the system. Pressure on the aortic side was maintained using the regulated compressed air supply. A flow probe (Transonic systems Model: ME8PXL) measured the flow through the bypass tube in the system. The small leakage back flow during diastole through the leaflet-hinges, the B-datum line (gap between leaflets), and the gap between the leaflets and the valve ring was calculated by subtracting the bypass flow from the total flow as calculated from the linear actuator motion.

2.4 PIV Experiments. Time-resolved PIV was used to characterize the flow dynamics in the vicinity of the MHV. For the study presented here, the objective of the PIV experiment was to

characterize the flow structures near the valve leaflets on the ventricular side and to establish the validity of these results by comparing with previously observed flow features of the same valve [23]. This measurement technique utilized image pairs of the tracer particles, which seeded the flow, taken within a very short period of time (ΔT). The digitized particles of each image pair were cross-correlated to obtain a vector flow field of the area of interest. The seeding particles, which were 1–20 μm fluorescent polymethyl methacrylate particles coated with Rhodium-B, were illuminated using a continuous diode-pumped solid state laser (1 W, $\lambda = 532 \text{ nm}$) from Laserglow Technologies. A laser sheet was produced using a combination of spherical and cylindrical lenses, which illuminated the test bed along the centerline of the conduit (Fig. 4). The thickness of the sheet was approximately 1 mm and the minimum velocity resolved depended on the time between image pairs. The images were taken on a time series at 4 kHz and depending on the time location within the cardiac cycle, different ΔT 's were used to resolve the velocities within the flow field. In order to resolve the velocities during the different parts of the cardiac cycle, multiple ΔT 's had to be selected: from $\Delta T = 1000 \mu\text{s}$ to resolve the highest velocities expected to $\Delta T = 2500 \mu\text{s}$ to resolve the lower leakage flow velocities. Images were acquired using a Vision Research Phantom V12.1 complementary metal-oxide semiconductor high-speed camera with a resolution of 1280×800 pixels and a maximum frame rate of 6000 frames per second at full resolution. The field of view of the PIV velocity and vorticity measurements was $20 \times 17 \text{ mm}$ with a resolution of $20 \mu\text{m}/\text{pixel}$. Images were acquired at a frame rate of 4 kHz in a single-frame mode. Attached to the imager, a 105 mm Nikon lens was used. The camera was connected to a master computer and triggered using DAVIS software (LaVision, MI). The working fluid in the test section (a mixture of distilled water and glycerin—65% and 35% by volume, respectively) had a kinematic viscosity that matched that of blood (3.5 cSt). The specific gravity for this mixture was $1.10 \text{ g}/\text{cm}^3$. The particle density ($1.06 \text{ g}/\text{cm}^3$) helped to minimize the velocity phase shift during unsteady flow phases. The long-pass optical filter, mounted on the camera lens transmitted the orange fluorescent light from the particles while

filtering off the green light reflected from the surfaces. The programmable-timing unit (PTU) of the PIV system was triggered by a LABVIEW generated TTL signal that was synchronized to a particular event on the volume flow rate trace of the cardiac cycle. Once the PIV acquisition was triggered, the PTU sent a trigger pulse to the camera that initiated the capture of the images.

2.5 Blood Experiments. This study was approved by Institutional Review Board (IRB) for human subject research at Georgia Institute of Technology (IRB protocol # H07328). Blood was collected from randomly selected healthy volunteers (median age 24) after informed consent, by venipuncture using a 19 gauge needle into 60 ml syringes filled with 3.2% sodium citrate solution corresponding to 10:1 blood–citrate ratio [5,63]. It was ensured that volunteers were aspirin and ibuprofen free for 1 week before the blood collection. The total volume of blood used for each experiment was 150 ml from a single donor. All the blood contacting components in the loop, other than disposables were rinsed with buffered-saline and then washed with degreaser (Versa-clean at $10\times$ dilution, Fisherbrand, PA). Then, the parts were then washed with 70% ethanol, rinsed with deionized water, and dried completely before assembling. Preliminary timeline experiments were conducted to decide the duration of these experiments. The optimum duration was decided to be 60 min upon considering the saturation of the blood damage observed from the blood damage indicators. Over the course of the experiments, 1 ml samples were withdrawn in 3 ml ethylenediaminetetraacetic acid vacutainer tubes for the assays, in time intervals of 20 min. This sample volume was replaced by 1 ml buffered-saline to maintain test volume. These samples were centrifuged at 14,000 RPM for 15 min to obtain platelet poor plasma, which was subsequently used for quantifying blood damage through biological assays.

To quantify progression in coagulation levels in the pulsatile tester, clinical parameters such as TAT, PF1.2, prothrombin time (PT), activated partial thromboplastin time (aPTT), and hemolysis (Drabkins assay) were measured. The presence of TAT in samples was measured using Enzygnost TAT assay (Dade Behring, Deerfield IL). For this chromogenic assay, the intensity of color was measured with a microtiter absorbance plate reader (Model: SpectraMax plus 384) at a wavelength of 492 nm. The intensity was proportional to the concentration of TAT based on a standard curve obtained using the samples provided in the assay kit. For quantifying PF1.2, an Elisa kit was used (Life sciences advanced technologies, St Petersburg, FL). A spectrophotometer was used at a wavelength of 450 nm to quantify the absorbance of samples and compared to standards in the kit. Hemolysis was determined by mixing the samples plasma with Drabkins reagent at 1:9 ratio. The reagent was prepared by diluting concentrated reagent in 1000 ml of deionized water and 0.5 ml of 30% Brij L23 detergent solution. The reagent was then mixed with $20 \mu\text{l}$ of erythrocyte and plasma sample and allowed to react for 15 min. A spectrophotometer was used to indirectly indicate the level of hemoglobin by chromogenic absorbance under 540 nm wavelength and compared to standard absorbance.

To evaluate the blood damage caused by the tester itself, experiments were performed with and without the MHV in place ($n = 5$ for each set), using the same motion profile of the linear actuator and pressure settings that were used for “with MHV” experiments. Though, pressure and flow variations were different in the system without MHV, blood damage values from these experiments helped to quantify the damage inflicted by various components of the tester. A Student's *T*-Test was used for this experiment with an alpha value of 0.05, a confidence interval of 0.95, and two tails. This test was used to analyze differences between the mean values of all three markers at different time points after normalizing with the $t = 0$ values. Section 3 presents the results from all these validation experiments.

Fig. 4 Top figure illustrates the arrangement of the sensor, emitter, and leaflets during both opening and closing of MHV. Bottom flow-curve figure shows the part of the cardiac cycle that was studied using PIV.

3 Results

3.1 Hydrodynamic Performance. The pressures on the aortic and ventricular side of the valve were continuously monitored

Fig. 5 Pressure variation on aortic and ventricular side of the MHV, averaged over 15 cycles, are shown after filtering high frequency oscillations. The negative section of flow curve indicates closing and leakage volumes of the MHV.

during the course of the experiment. Figure 5 shows these pressure variations on either side of the valve during a single cardiac cycle. The valve begins to open when the ventricular pressure rises above the aortic pressure and this marks the beginning of systole, which lasts for about 330 ms. The valve begins to close after the ventricular pressure falls below the aortic pressure. The closing motion takes approximately 50 ms. The beginning of reverse flow marks the beginning of diastole. The closing volume was found to be around 4 ml and leakage volume (reverse flow after the valve is completely closed) was found to be around 4 ml averaged over 15 cycles. The profile of the flow through the MHV is also shown in Fig. 5. The system simulates finer aspects of flow like higher acceleration and slower deceleration during systole of the cardiac cycle, as observed in human heart. This precise control of the flow rate is made feasible by the programmable actuator, which is mechanically coupled with the driving piston. The negative velocity (flow) indicates the closing and the leakage phase (diastole) of the cardiac cycle.

Sample traces of the leaflets motion of a single cardiac cycle is shown in Fig. 6(a). It can be observed from the motion traces that both leaflets open at different instants but they close simultaneously. Variations in leaflet opening–closing motion were observed across cardiac cycles and an asynchronous motion could occur as shown in Fig. 6(b). An observation of 100 cycles indicated a variation in closing motion of the leaflet spanning around 70 ms. This dynamic resolution of the motion is extremely important to

characterize the leaflet behavior, especially given the variation in hemodynamic conditions under different heart rate, flow rate, and pressure conditions.

3.2 PIV Results. Figure 7 shows the instantaneous velocity and vorticity measurements, respectively, of the flow field near the leaflets on the ventricular side of the valve during closing-phase and leakage flows of two different cardiac cycles at several time points. It can be observed that the formation of a strong jet and a pair of counter-rotating vortices along the B-datum line of the MHV occurs during the closing motion of the valve. Figure 7 shows differences in the jet velocity, and thus formation, as observed in the first time point shown (394 ms). One can observe in Fig. 7 that there is a clear deviation of the b-datum jet away from the perpendicular line passing through the center of the b-datum line. A pair of counter-rotating vortices in cycle 2 can also be noticed. This phenomenon is due to an asymmetric closing of the leaflets (one closes faster than the other), tilting the jet in this case toward the right, which implies that leaflet 2 (see Fig. 4) closes faster than leaflet 1. It is also observed that the time elapsed between the formation of the jet through the B-datum line and the initial dissipation of the pair of counter-rotating vortices is around 30–40 ms (which corresponds to the time point of 425 ms in the cardiac cycle) and the strong vortex pair completely vanishes at around 200 ms after the B-datum line jet starts to form (598 ms

Fig. 6 (a) Leaflet position traces of leaflet 1 and leaflet during a single cardiac cycle. In the vertical axis, an angle of 7 deg corresponds to fully open position and an angle of 60 deg corresponds to fully closed position. (b) Spread of single-leaflet position traces sampled over 100 cycles.

Fig. 7 *Top two rows:* plot of PIV velocity magnitude in the ventricular side of the valve for two cardiac cycles. The change in direction of dotted lines (between cycle 1 and cycle 2) during the closing phase indicates the vectoring of the leakage jet due to asynchronous leaflet closing during cycle 2. *Bottom two rows:* PIV velocity vectors and vorticity contours for two cardiac cycles (top and bottom).

into the cardiac cycle). These vortex structures qualitatively match the vortex evolution as characterized by Pierrakos et al. [64].

3.3 Thrombogenicity Results. Thrombogenicity results of experiments ($n=5$) that were conducted “with” and “without” MHV in place (Fig. 8) show an increase in blood coagulation (TAT, PF1.2, hemolysis) with time increments. While testing MHVs, PT, and aPTT measurements did not vary through the course of the experiments and were eventually dropped from coagulation analysis. Significant differences were shown in the PF1.2 and Free Hemoglobin Assays at 20, 40, and 60 min. There was a difference seen in the TAT Assay values only at 20 and 60 min. Plasma hemoglobin levels indicated significant levels of hemolysis occurring in the test setup. All the coagulation indicators that were being considered, except TAT at 40 min, showed that the coagulation potential of the tester was less than that induced when the MHV was mounted in the tester. The damage imparted to the blood by the tester itself was very minimal compared to the cardiovascular device that is being tested.

4 Discussion

The mechanism underlying thrombus formation, especially when induced by nonphysiological flows, is not well understood. Many experimental and numerical studies have tried to understand

this complex phenomenon by trying to identify regions of high shear stress and flow disturbance. What has been missing, however, is the correlation of these fluid dynamics phenomena with thrombogenic markers in whole blood. Some researchers have established methodologies to combine *in silico* and *in vitro* methods by first evaluating the shear stress variation from a numerical model and then exposing the platelets to the same shear patterns to quantify the platelet-activation response [53,65]. Alternatively, the presented method attempts to quantify blood damage by exposing whole blood to relevant physiological conditions and coupling them with fluid-dynamics derived parameters by conducting both studies in the same *in vitro* setup. Different flow structures and patterns may induce varying levels of blood damage. For example, vortices observed in flow through MHVs [66–69] provide the combination of conditions (shear stress levels, residence time of activated platelets, and concentration of platelets at the core) that enable formation of microemboli. In the past, phase averaged PIV methods have been used to assess these flow structures [55,70,71]. However, it should be noted that platelet response is an instantaneous process. This necessitates the characterization of *instantaneous* flow structures that activates and extrudes the platelet pseudopodia, which in turn aggregates other platelets. The presented system tries to bridge this gap by providing a test-bed to characterize these intricate instantaneous flow structures as well as to quantify thrombogenic markers.

Fig. 8 Blood coagulation indicators: (a) TAT, (b) hemolysis, and (c) PF1.2 showing that the coagulation potential of the tester was less than that induced when the MHV was mounted in the tester

Another advantage of the tester is its ability to simulate different hemodynamic conditions for various cardiovascular devices. A subset of cardiovascular hardware that can be tested in this system is shown in Fig. 2. Each cardiovascular device has its own intricate in vivo hemodynamics associated with it. The strength of an in vitro platform lies in its ability to replicate these intricate conditions. For instance, the increase in rate of flow through aortic valve during valve-opening in vivo is different than decrease in flow rate during valve closure. This plays a significant role in the closing dynamics of the valve leaflets. Our high-fidelity leaflet tracking capabilities allowed the observation of these closing dynamics. Leaflet dynamics observations show variation in closing motion of the leaflets. This variation induces generation of flow structures downstream of the valve in size scales relevant to blood cell damage. Therefore, it is conceivable that in the flow field there are variations in the primary vortical structure formed by the leakage jet along the b-datum line during various time points in diastole. The fact that the leaflets do not close synchronously could vector the primary vortex with respect to a case when both leaflets close simultaneously. Figure 7 shows instantaneous velocity and vorticity observations from two different cardiac cycles at several time points and it can be clearly depicted that in cycle 1 the vortex pair is aligned vertically with the sidewalls of the conduit, while in cycle 2, the primary vortex is vectored to the right due to an asynchronous closing of the leaflets. This indicates that phase averaging would bias the results since the vortex pair could not be in the same location from one cycle to the next. Phase averaging in such a situation will also underestimate the instantaneous impact of flow structures on blood cells. To avoid such biasing, conditional averaging could be applied to cases with similar leaflet dynamics and pressure traces. Since, for each cardiac cycle, leaflet position and pressure traces are recorded, the PIV images could be sorted out to average

dynamically similar realizations. These high-fidelity fluid and leaflet dynamics measurements help to correlate these observations with thrombus formation.

Thrombus formation in cardiovascular hardware is a complex, multistep process starting with platelet activation and culminating in formation of solid clot [2,9,11,15,42,50]. Magnitude and duration of pathophysiological shear stress exposure have been found to be two dominating factors in thrombus formation, with cell lysis and tissue factors aiding the process [58]. Once platelets start aggregating, the intercellular spaces provide an environment for accumulation of various protein factors involved in formation of a stable, bigger thrombus [33,35]. Simplified in vitro methods cannot replicate these in vivo events accurately since it is not feasible to re-create the combinations of shear variation, exposure time, and spatial-biochemical factors. These methods use a flow rate, which is different than the in vivo flow rates. This, in turn, simulates a shear stress magnitude and exposure time on the platelets, which are different from the native conditions [72]. Some researchers have been successful in exposing the platelets to realistic shear stress profiles, but used reconstituted platelets [53]. Some researchers use isolated platelets [42,53], and such methods also negate any reduction in thrombin generation because of the absence of red blood cells. The coagulation patterns have also been found to be different between whole blood and blood-substitutes [73]. The presented system tries to overcome these challenges by replicating the in vivo hemodynamic conditions, and testing the cardiovascular hardware under these conditions. This study used freshly drawn human blood, rather than platelet-rich blood substitutes, so as to test the device as close as possible to the native state. A basic citrate system was used as the anticoagulant system. In previous studies from the authors [5], calcium influx into the blood stream was used to increase TAT to a detectable level, which was otherwise insignificant. TAT formation is a

positive indication of cumulative thrombin generation. It should be noted that, though TAT formation is a positive indication of cumulative thrombin generation, all thrombin and antithrombin may not be bound in a TAT complex. In the current study, the artificial calcium influx was avoided, given that the TAT levels were in the normal clinically observed range. This was a critical step in the model setup. The platelet activation also depends on nonactivated platelets coming into contact with other activated platelets expressing its peak calcium flux within a time frame of 0.6 s as observed in flow through glass microcapillaries [72]. In such a situation, residence time becomes significant [58], and it becomes critical for the tester to replicate the physiological conditions to predict thrombus formation. This tester captures the blood damage occurring through such physiological conditions, as evidenced by TAT and PF1.2 levels. This is supported by higher TAT levels from our study when compared to previously reported results on the same MHV [5]. In the case of the SJM MHV, flow through the hinge region and B-datum space has been found to inflict the most blood damage. This has been studied by Anna et al. and Dasi et al. [5,63] by using different geometrical variations of the SJM valve. The valve used in the current study corresponds to the standard valve used in their study. The TAT results for the standard valve at the 60 min time point from previous work seems to be one order lower than the values observed in the current study, though it should be noted that a steady-flow system was used in their studies. It is expected that the dynamics of flow in a fully pulsatile system as described here would increase the expression of these biomarkers. Hemolysis levels were insignificant in the study by Anna et al. The various blood damage parameters under consideration were saturating above clinically relevant levels after 60 min (TAT < 7 ng/ml, PF1.2 < 300 pmol/l, Hemolysis < 2 mg/ml) [74]. The timeline for this study was established particularly for this cardiac device. Any cardiovascular device will warrant a separate timeline study to establish the duration of the blood experiments, based on the saturation of blood damage parameters. All blood damage indicators were chosen after careful consideration of specific operational conditions.

5 Limitations

This system carries the inherent limitations of any in vitro tester. The tester is primarily made of rigid components, in contrast with the flexible and dynamic muscular structures in the body. Though the rigid components help to maintain the repeatability of operating conditions, the natural variations in hemodynamics in vivo are not present in this system. However, it is expected that the lumped compliance provided at the top of the aortic section can help achieve physiological hydrodynamic conditions. Other factors associated with blood vessels and blood vessel injury such as dilation, filtration, and inactivation mechanisms are absent as well. Since this study involved blood drawn from healthy set of volunteers, the red blood cell and platelet counts were not kept constant. The hematocrit values and platelet-count varied within 15% of the mean value for all the samples. Though the blood damage results were normalized using the values at $t=0$ to offset this variation in cell count, it may have caused some variation in the coagulation levels over the course of the experiment. All the experiments were performed under room temperature. Though the blood damage levels were in the clinically accepted blood damage range, it should be noted that the results from this tester should be investigated in comparison with different study conditions rather than looking at the absolute values of blood damage. To partially overcome such a limitation, shear stress values from PIV data can be used to identify high-probability-regions for blood damage. For example, in case of MHV, PIV data can be used to identify relative contribution of leaflet-hinge and b-datum line to total blood damage by calculating the shear stresses in those regions. This would also negate the in vitro limitations of the tester (e.g., temperature effects), since all the experiments will have the same operating conditions. It should also be noted that platelet

activation and hemostasis factors associated with thrombus formation have not been found to be affected significantly at temperatures lower than 37 °C [75,76]. Qualitative visual inspection and histological analysis were not performed in this study. Another limitation of this system is the inability to isolate blood damage caused by specific components of the cardiovascular hardware. For the case of MHVs, the blood damage caused by the leaflet hinge cannot be isolated from the damage caused by the B-datum line. The periodic blood samples drawn from the system give the total blood damage caused by the MHV. The PIV measurements can be efficiently used to overcome this by characterizing the flow fields of hinges and B-datum lines separately.

6 Conclusion

Blood clot initiation, formation, and stabilization in any cardiovascular device is a multiscale phenomenon. The system presented here has been demonstrated to be a good test-bed for testing cardiovascular devices, with distinct advantages over some of the thrombogenic testers found in literature. This system is completely biocompatible and has very low volume of operation (150 ml). This low volume is critical when using freshly drawn whole human blood. The modular test chambers have optical access for PIV studies, which allows the possibility of performing fluid dynamics and thrombogenicity studies in the same facility. The pumping action is simulated by a programmable linear actuator pump, which gives the ability to precisely control the flow rate and pulsatility corresponding to different test conditions. This tester should serve well in testing cardiac devices before proceeding into animal studies or clinical trials, at a fraction of the cost associated with such work.

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