MarioHeart

MarioHeart: Novel *In-Vitro* Flow Model for Testing Heart Valve Prostheses and Anticoagulant Therapies

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Mechanical heart valve (MHV) prostheses present a risk of thromboembolic complications despite antithrombotic therapy. Further steps in the development of more hemocompatible MHVs and new anticoagulants are impeded due to the lack of adequate *in-vitro* models. With the development of a novel *in-vitro* model (MarioHeart), a pulsatile flow similar to the arterial circulation is emulated. The MarioHeart design owns unique features as 1) a single MHV within a torus with low surface/volume ratio, 2) a closed loop system, and 3) a dedicated external control system driving the oscillating rotational motion of the torus. For verification purposes, a blood analog fluid seeded with particles was used to assess fluid velocity and flow rate using a speckle tracking method on high-speed video recordings of the rotating model. The flow rate resembled the physiological flow rate in the aortic root, in both shape and amplitude. Additional *in-vitro* runs with porcine blood showed thrombi on the MHV associated with the suture ring, which is similar to the *in-vivo* situation. MarioHeart is a simple design which induces well-defined fluid dynamics resulting in physiologically nonturbulent flow without stasis of the blood. MarioHeart seems suitable for testing the thrombogenicity of MHVs and the potential of new anticoagulants. *ASAIO Journal* 2023; 69;e192–e198

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Currently, the mandatory lifelong anticoagulant therapy for patients with a mechanical heart valve (MHV) prosthesis is far from perfect, with persisting thromboembolic complications and anticoagulant-related hemorrhages accounting for approximately 75% of all complications after heart valve replacement.[1] Even though the annual risk of thromboemboli remains 1%–2% despite anticoagulation and up to 25% of prostheses-related deaths is attributable to hemorrhages,[2–7] MHVs are favored over bioprostheses in patients with long life expectancy and no contraindication for antithrombotic therapy.[8,9]

The continuing demand for MHVs, in particular for younger patients with a higher risk of structural bioprosthetic valve deterioration,[10,11] and the challenges of contemporary oral anticoagulants based on vitamin K antagonists demonstrate the necessity of improving MHV hemocompatibility and revising the antithrombotic strategies. Studies on inhibiting coagulation factor XIIa,[12–15] and XI or XIa[16–18] as a new approach to anticoagulation present promising opportunities. To date, studies on the effect of these new anticoagulants on MHV prostheses are lacking. As thrombus formation on MHVs is a complex process in which the presence of foreign materials, altered flow patterns and shear stresses play a significant role,[19,20] adequate *in-vitro* models are scarce.

In the past decades, several *in-vitro* models involving MHVs were developed and used in successful thrombogenicity-related studies. One *in-vitro* setup comprised a left ventricular assist device (LVAD) to drive the circulation and two MHVs.[20] Although this setup was adequate for studying flow-induced platelet activation, it is unsuited for anticoagulation studies due to the LVAD and its inherent thrombogenic properties. Another study introduced a low-volume pulsatile flow facility which was used to assess the thrombogenic potential of implantable cardiac devices including MHV prostheses.[21] However, the small bypass to channel blood from one side of the MHV to the other adversely affect coagulation due to complex flow patterns. More recently, the Thrombosis Tester Helmholz Institute Aachen (THIA) 3 was presented as an approach for a standardized *in-vitro* method after improvements to previous testers (THIA 1 and THIA 2), which were all successfully used for comparing anticoagulants as well as evaluating the thrombogenic potential of heart valve prostheses.[22–25] Nevertheless, the THIA setups induce undesirable complex flow patterns as well, and involve a large foreign material surface.

To fill the gap in *in-vitro* models to test new anticoagulant therapies for MHVs, we aimed to develop and evaluate a novel *in-vitro* model that is suitable for testing the thrombogenicity of MHVs as well as potential anticoagulation therapies. Design objectives included a limited priming volume and contact surface, realistic anatomical dimensions of the ascending aorta and aortic annulus, and a generated flow that closely resembles physiological flow behavior in the aorta.

Materials and Methods

Model Design

The MarioHeart model was developed at the department of Cardiovascular Biomechanics of Eindhoven University of

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Technology, in collaboration with the University Medical Center Utrecht. The apparent simple design features one MHV in a hollow torus with a tube diameter of 22 mm, based on anatomical measurements of the proximal ascending aorta diameter and aortic annulus in human adults.[26] Similarly, the torus outer diameter of 130 mm is chosen such that the torus curvature is reasonably low and resembles the shape of the proximal aorta, resulting in an exceptionally low priming volume (155 ml) and contact surface area.

Two polycarbonate discs, each with half the torus milled out, serve as a smooth and rigid casing of the hollow torus. Both halves are secured to one another by screws at the outer rim, aided by a large screw in the center that also attaches the torus axis horizontally to its driving servomotor on a metal stand (Figure 1A). Silicone rubber, biocompatible O rings line the inner and outer border of the torus to prevent fluid leakage from the torus after assembly. The MHV (LivaNova Carbomedics reduced aortic bileaflet valve, 21 mm) is situated in a valve chamber that can be twisted with respect to the remainder of the model to connect the torus to two ducts for filling and draining (Figure 1B). Furthermore, this chamber allows for convenient evaluation of thrombus formation around the valve as this compartment can easily be removed while the rest of the model remains intact. As the valve chamber features a cavity for the suture ring of the MHV (Figure 2), it can be mounted inside the torus without compressive forces and the suture ring does not affect the flow. By positioning the valve with the leaflet hinges in the plane of the torus, flow through the valve is essentially symmetric with respect to the torus plane.

Flow Verification

A blood analog transparent fluid (0.69 g/L Xanthan gum [XG][27] in water solution with 0.5% glycerol) supplemented with orange tinsel particles small enough to float in the fluid yet large enough to be visualized (concentration 2 g/L) is used for flow verification purposes. Preliminary rheology tests confirmed blood analogous viscosity of the XG solution. Flow is generated by alternating clockwise and counterclockwise semirotational motion of the torus around its central axis, controlled by the servomotor with dedicated control system that involves a pulse trajectory as angle-based information for rotation. Rotational motion in counterclockwise direction allows flow through the valve (systole). The valve moves through the fluid while the fluid essentially maintains its original position due to the inertia of blood and the blood analog, and low friction with respect to the surrounding torus. In clockwise direction, the fluid is pushed by the closed valve (diastole), moving at the same speed as the rotating torus. Hence, the model generates pulsating motion of fluid through the valve, mimicking pulsatile flow.

Motion of the model starts by rotating in counterclockwise direction to a maximum angle of 140° from the starting position, to obtain a fictitious stroke volume of 60 ml. When the maximum rotation angle is reached, the direction of rotation reverses and the model rotates back to the starting position in clockwise direction. The rotation to maximum angle and back to the starting position is considered as one cycle. Cycle rate was set to 75 cycles per minute to simulate a heart rate of 75 beats per minute (bpm).

High-speed camera recordings (Phantom V9.0, Vision Research, Groningen, NL) were retrieved for one test during eight cycles with a frame rate of 500 Hz and, consequently, eight cycles generated a total of 3,200 images. Using Matlab R2018b, each image was rotated around the models center point. Thereby, the model appeared stationary, while fluid shows cyclic motion through the valve within the torus. The modified images were used as input for a speckle tracking algorithm[28–31] to determine two-dimensional particle displacement

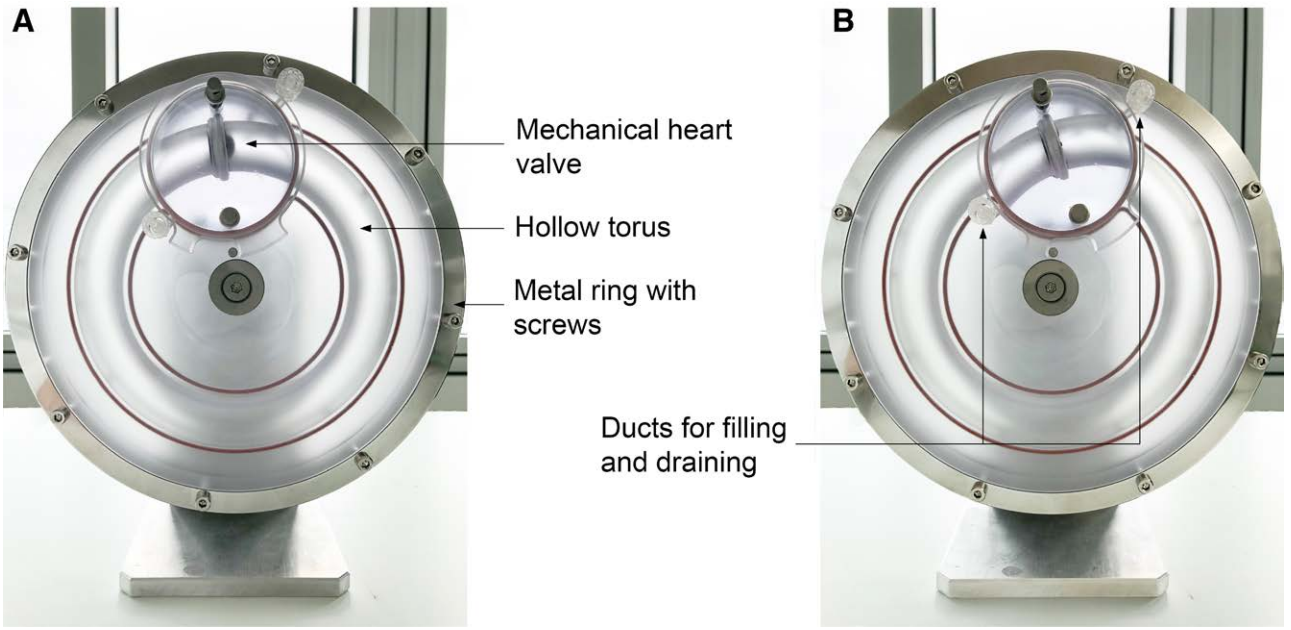

Figure 1. The MarioHeart *in-vitro* flow model comprises a mechanical heart valve and a hollow torus. The metal ring with screws and the screw in the center secure two compartments to one another and to the servomotor. **A**: Closed setting, torus is not connected to the ducts. **B**: Open setting, valve compartment is twisted to connect the torus to two ducts that enable filling or draining (indicated by the arrows).

Figure 2. Valve chamber front view (left) and side view (right) showing valve positioning. Note the extra cavity for the suture ring in the left image.

within a region of interest (ROI). Particle displacement in pixels per frame was converted to velocity (cm/s) and by assuming a flat flow velocity profile, fluid flow (L/min) was estimated, leading to an upper boundary estimate of actual fluid flow rate.

In-Vitro Blood Tests

To verify the model function, exploratory blood tests using porcine blood were included in this study. Experiments were conducted at LifeTec Group BV to assess the thrombogenic potential of the *in-vitro* model within a climate box at 37°C, both with and without the MHV. A silicone tube inlay for the empty suture ring cavity during the tests without MHV ensured smooth torus surface. Blood (500 ml) was collected in a low-heparinized bottle (Heparin LEO, i.v. injection fluid, heparin sodium, 5,000 IU/ml) to a final concentration of 1,300 IU/L during exsanguination of slaughterhouse pigs after electrical stunning.

Five blood batches, collected from five pigs on different days, were tested within the model consecutively with and without the valve. All tests involving blood were performed within 6 hours after collection. Before testing, the model and all other blood-contacting materials were preheated at 37°C and primed with phosphate-buffered saline (PBS) to facilitate complete filling and removal of air bubbles. Rotation was set to 75 cycles per minute, with stroke volume 60 ml and test duration 1 hour per experiment. Thrombus formation was evaluated by visual analysis of the disassembled separate valve chamber as well as the remainder of the torus after each test.

Results

Design Outcome

The MarioHeart design incorporates a smooth fluid-contacting surface and avoids geometries and superfluous components that potentially disturb the flow within the torus. Filling the torus requires 155-165 ml of liquid, to remove all air bubbles. In closed state, no fluid leakage from the torus was observed. The separate valve chamber design enabled examination of the valve when removed from the remainder of the model.

Model Performance

The control system showed good regulation of rotational motion by following the prescribed cycle rate, maximum rotation angle, and position-based pulse trajectory. In addition, the model was tested during a longer run of 15 minutes and showed unperturbed repetitive motion. The model has shown to perform in agreement with the design and prescribed control settings.

Velocity and Flow

Particle displacement with respect to the torus appeared to be similar at clock analogous positions 3, 6, and 9 o'clock, when the valve is positioned at 12 o'clock. Therefore, the speckle tracking algorithm has shown no artifacts for application on the rotated images of the torus. Results are elaborated for the 6 o'clock position only. The selected ROI is chosen in the center of the tube to exclude the blurry fields on the camera recordings, caused by internal reflection adjacent to the torus walls due to a refractive index mismatch, in which particles could hardly be distinguished. Transverse pixel displacement is found to be approximately zero, while axial pixel displacement varies in time. The axial displacements in pixels per frame were converted to velocities in cm/s, using frame rate and measured pixels per cm (Figure 3).

Velocity *versus* time results were obtained during eight cycles (Figure 4). Velocity peaks were similar for each cycle, with triangular systolic peak shape. The fluctuation of the velocity just after the systolic peak is due to moving fluid after the abrupt turn to change rotational direction. Analysis of the high-speed recordings showed that the valve was closed during that fluctuation.

As velocities are comparable for each pixel (Figure 3), these results represent plug flow and allow for calculation of fluid flow using the tube section area. Mean velocity and flow equal

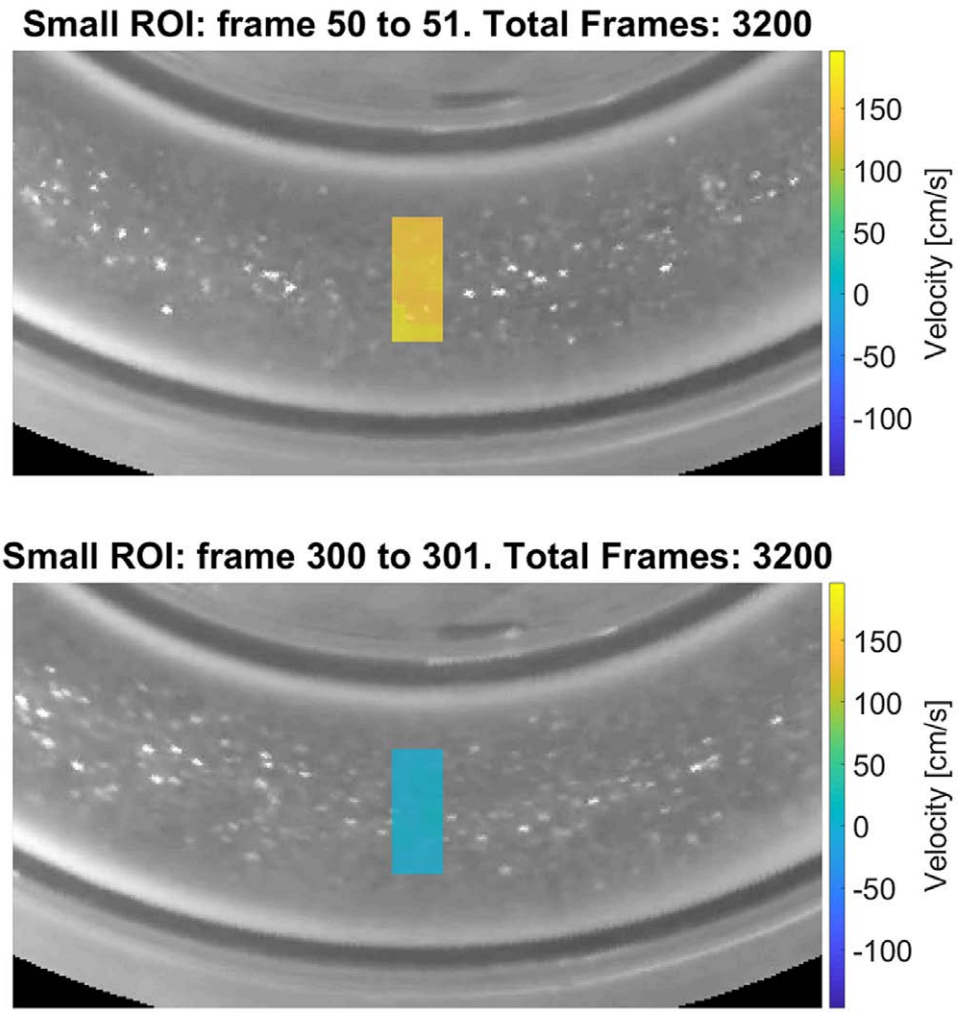

Figure 3. Velocity results are obtained from speckle tracking results for axial (horizontal) displacement. One frame in systole (frame 50, top) and one frame in diastole (frame 300, bottom) were captured. Each pixel of the region of interest (ROI) is colored according to a color range for velocities. Color scale for velocity is indicated on the right. Positive velocities correspond to axial movement from right to left, whereas negative velocities correspond to axial movement from left to right.

23 cm/s and 5.3 L/min, respectively (Figure 4, red horizontal line). On average, peak velocity was 180 cm/s and associated peak flow reached 41 L/min, systole duration was 270 ms (34% of cycle duration 800 ms), and AT, the time from onset of flow to maximal velocity, equaled 130 ms.

Thrombogenicity Assessment

In three of the five low heparinized (1,300 IU/L) blood tests with valve, thrombus formation was detected and associated with the rigid ring structure of the valve, close to or touching the suture ring (Figure 5). Thrombi were located either before or after the valve, and occurred both close to the bottom (inner border of the torus) and top (outer border of the torus). Unfortunately, thrombi were also observed in the torus after two of the five tests with valve and after three of the five tests without valve. In general, thrombus formation in the torus occurred at the seam of the torus, where the large O rings line the inner and outer border of the torus, and on the border where the torus is split when turning the separate valve compartment.

Discussion

The novel *in-vitro* MarioHeart model has proven its suitability for testing the thrombogenicity of prosthetic heart valves in a simulated pulsatile flow environment and shows potential for the evaluation of new anticoagulant therapies. It comprises one MHV (21 mm) in a closed torus with dimensions that simulate the anatomical geometry of the proximal ascending aorta and the aortic annulus in human adults. Unique model characteristics such as simplicity, low priming volume (155–165 ml), and a limited blood-contacting surface area distinguish this model from existing *in-vitro* models (Table 1). Cycle rate and maximum rotation angle settings determine fluid flow within the torus and enable simplified flow evaluation as compared with flow probe measurements. Moreover, the removable valve chamber allows for visual analysis of the valve and the adjacent tube section area without handling the valve and its surroundings directly.

Fluid velocity within the model demonstrated triangle-shaped systolic flow peaks that correspond to physiological flow peak shapes in large arteries. Furthermore, systole

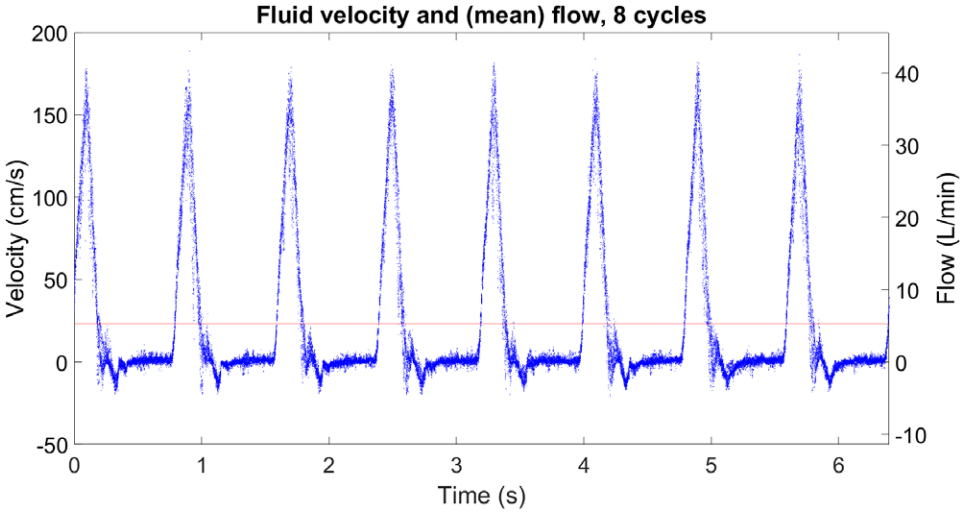

Figure 4. Fluid velocity and flow *vs.* time results for the region of interest shown in Figure 3. The red line indicates mean velocity (23 cm/s) and flow (5.3 L/min).

covered 34% of cycle duration, which closely resembles physiological systole to diastole ratio 1:2 and matches the demands for valve testing according to standard ISO5840.[32] After the onset of systole, the velocity increased from zero to peak velocity within 130 ms on average. This extremely rapid velocity increase is also observed in Doppler recordings of a bileaflet MHV *in vivo*.[33] Peak velocities were lower than the *in vivo* peak systolic velocity results for a Carbomedics 21 mm aortic bileaflet MHV (180 cm/s on average *versus* 248 ± 45 cm).[34] We suspect that different measuring locations may play a role here. In this study, velocities were measured at an axial distance of 20 cm from the valve, thereby excluding the increased jet flow through the central orifice of the bileaflet valve that was measured *in vivo*.

Homogeneous pixel velocity within the ROI allowed for calculation of the fluid flow using velocity and tube section area. Mean flow was higher than expected (5.3 *vs.* 4.5 L/min) considering the cycle rate of 75 per minute and maximal angular rotation of 140° for a fictitious stroke volume of 60 ml. This may have resulted from the fluctuations at the end of systole and the nonzero measured velocities during diastole. Both causes were observed to be present because of whirling tinsel particles as the closed valve pushed the fluid.

MarioHeart is a flow model that omits the potential influence of pressure on thrombogenicity of MHVs, based on the presumption that the thrombogenicity is mainly flow-induced due to nonphysiological flow patterns.[20,35] Generating flow through the valve within the torus is feasible with rotational motion in alternating directions, regulated by a dedicated control system. Thereby, the need for external driving systems affecting blood activation and flow is circumvented.

Evaluation of thrombus formation on the MHV and within the torus showed MarioHeart's suitability for further thrombogenicity experiments. All thrombi observed on the valve were located on the rigid ring and associated with the suture ring. Visual analysis within the torus was facilitated by the transparent casing and revealed thrombus formation associated with the seams in tests with MHV as well as in tests without MHV.

As the current study focused primarily on the verification of velocity and flow, additional porcine blood tests were performed and included in this study to demonstrate practical feasibility of thrombus formation and assessment. To achieve controlled

Figure 5. Thrombus formation on the mechanical heart valve after three different experiments with porcine blood in the model. Left: Test day 1, thrombus formation after the valve, mainly at 6 o'clock. Middle: Test day 2, thrombus formation before the valve, both around 12 o'clock and 6 o'clock. Right: Test day 5, thrombus formation after the valve, only at 11 o'clock.

Table 1. Advantages and Disadvantages of the MarioHeart Model and Current *In-Vitro* Flow Models

In-Vitro Model (Paper)	Advantages	Disadvantages
MarioHeart model	• Limited priming volume (155 ml) and contact surface • Flow within the torus is derived from rotational motion settings • Removable valve chamber • Geometry that does not disturb the flow: one blood compartment, low risk for stasis of the blood • Flow without internal pump, reducing the occurrence of hemolysis or pathological shear stress conditions	• Current setup is unsuited for flow probe measurements
LVAD circulation loop (Bluestein *et al*. 2004)[20]	• Limited priming volume (100 ml)	• Unsuited for coagulation experiments due to the thrombogenic LVAD and two MHV's within the circulation loop • Piston pump drives the fluid
THIA 2 (Linde *et al*. 2011)[24]	• Flow is measured by a flow probe	• Higher priming volume (220–270 ml) • Moving membranes and a bypass induce complex flow patterns
THIA 3 (Linde *et al*. 2019)[25]	• Flexible aortic root • Flow is measured by a flow probe	• Higher priming volume (450 ml) • Moving membranes and a bypass induce complex flow patterns • Piston pump drives the fluid
Pulsatile flow facility (Arjunon *et al*. 2015)[21]	• Limited priming volume (150 ml) • Flow is measured by a flow probe	• Small bypass affects coagulation due to complex flow patterns • Piston pump drives the fluid

LVAD, left ventricular assist device; MHV, mechanical heart valve; THIA, Thrombosis Tester Helmholz Institute Aachen.

thrombus formation on the valve within the model, experiments with standardized porcine blood in terms of initial coagulation parameters are planned for the future. Subsequently, similar experiments using human blood will be carried out before experiments with existing and new anticoagulants.

This study involves limitations regarding the implementation of the thrombogenicity experiments, primarily because blood coagulation parameters were not obtained. Hence, observed thrombus formation is possibly due to preactivated coagulation rather than activation by the mechanical valve. Second, a higher number of tests is required before controlled and reproducible thrombus formation can be established with standardized porcine blood.

The primary limitation of the current model is the customized rigid casing. Due to the specific design, the cavity for the suture ring fits a 21 mm LivaNova Carbomedics reduced aortic bileaflet valve only. In addition, the seams of the casing appeared to be a prominent location for thrombus formation within the torus. Therefore, an updated version of the MarioHeart is being developed, using disposable flexible tubes connected to the valve chamber and attached on a circular plate to replace the rigid torus. Adding an antithrombotic coating to the flexible tubes may further improve hemocompatibility of the setup and will be further investigated in the future. The use of flexible tubes in the new iteration of the design would also enable flow probe measurements and introduces solutions for taking blood samples during runs *via* needle puncture, which is not possible with the current model. With the development of multiple valve chambers that can easily replace the existing removable valve compartment, the model is suitable for experiments with various prosthetic heart valves and provides opportunities as setup for testing novel heart valve prostheses. The fluid velocity profile (*i.e.,* an essentially flat profile or plug flow) and flow peak shapes as generated by the current model with a 21 mm valve prosthesis would be similar for alternative prostheses, regardless of their size.

In conclusion, the MarioHeart model presents a new approach to *in-vitro* flow testing by avoiding external driving systems and superfluous moving components, generating flow through the valve by semirotational motion of a fluid-filled torus. Verification experiments with a transparent blood analog demonstrated that fluid velocity and flow within the model is very similar to physiological flow behavior in large arteries. Tests with minimally anticoagulated porcine blood showed valve thrombosis associated with the rigid ring and suture ring of the valve. These thrombus localizations are also identified in MHV recipients *in vivo*.[36] In general, the model seems suitable for evaluating the flow-induced thrombogenicity of prosthetic heart valves and new antithrombotic therapies.

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