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High-Resolution Measurement of the Unsteady Velocity Field to Evaluate Blood Damage Induced by a Mechanical Heart Valve

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We investigate the potential of prosthetic heart valves to generate abnormal flow and stress patterns, which can contribute to platelet activation and lysis according to blood damage accumulation mechanisms. High-resolution velocity measurements of the unsteady flow field, obtained with a standard Particle Image Velocimetry system and a scaled-up model valve, are used to estimate the shear stresses arising downstream of the valve, accounting for flow features at scales less than one order of magnitude larger than blood cells. Velocity data at effective spatial and temporal resolution of 60 µm and 1.75 kHz, respectively, enabled accurate extraction of Lagrangian trajectories and loading histories experienced by blood cells. Non-physiological stresses up to 10 Pa were detected, while the development of vortex flow in the wake of the valve was observed to significantly increase the exposure time, favouring platelet activation. The loading histories, combined with empirical models for blood damage, reveal that platelet activation and lysis are promoted at different stages of the heart cycle. Shear stress and blood damage estimates are shown to be sensitive to measurement resolution.

Keywords: cardiovascular device, particle image velocimetry, Lagrangian tracking, platelet activation, platelet lysis

1. Introduction

Any investigation of the performance of cardiovascular devices must face the problem that blood damage is modulated by the action of the flow on cells, at a scale orders of magnitude smaller than the device and surrounding anatomy which determine the flow field. In turbulent and transitional flow, in particular, the microscale flow has a complex structure that cannot be completely determined from measurements of the macroscale flow. Non-physiological flow plays a major role among the causes of thromboembolic complications associated with prosthetic mechanical heart valves (MHVs)[1-4], exposing blood cells to shear stresses higher than the values found in native valves. Geometry and dynamics of artificial occluders promote abnormal features such as jets, separation, and vortex shedding, which can subject cell trajectories to prolonged or intermittent exposure to large shearing forces[5,6]. It has been suggested that flow structures in these fields are effective at scales down to tens of μm[7,8] or even less[9]. These estimates stem from Kolmogorov theory for isotropic, homogeneous, statistically stationary turbulence, which is questionable for the strongly unsteady short-duration flow involved. These calculations hint at the existence of cellular-scale flow structures, but to date, the smallest flow structures in MHV flow have not been directly measured, because of limitations on the resolution of experimental methods.

Predicting blood damage as a function of macroscopic flow conditions requires that a quantitative model of cellular response to mechanical loads be integrated with detailed knowledge of the flow field that determines the mechanical loads. Numerous authors have subjected blood (or suspensions of blood cells) *in vitro* to controlled shear stress and measured resulting hemolysis[10-12], platelet lysis[13-15], or platelet activation[16-18]. It has been shown that both hemolysis and platelet activation are dependent not only on instantaneous stress, but on the history of stress, suggesting that blood elements can accumulate sublethal damage. Such findings have been used to develop a variety of empirically based

mathematical models[15,19-22]. Since cellular response to stress is dependent on history, such models must be integrated over the Lagrangian trajectory of a cell through the flow field of a given device in order to predict flow-induced blood damage. The flow field should be characterised at sufficient spatial resolution to enable all relevant flow features to be measured, and at sufficient temporal resolution to allow stress to be recorded over the history of a cell as it is convected through the field. To date, blood damage index in MHVs has been calculated only from numerical data, either for hemolysis[19] or thrombosis[23,24]. Although Particle Image Velocimetry (PIV) has been used extensively to measure the flow field in MHVs[5,25-29], its temporal and spatial resolution has not been sufficient to allow the integration of blood damage models along Lagrangian cell trajectories.

Non-physiological flow patterns can arise at different locations near a valve. Blood leakage through the gaps between leaflets, between leaflets and valve housing and in the hinge mechanism is beneficial for reducing thrombosis, but on the other hand produces high-speed jets associated with large shear stresses and turbulent fluctuations which are considered responsible for triggering hemolysis[30]. The main forward flow through the valve provides gentler hemodynamic conditions than either leakage or hinge flow[9]. Nevertheless, for the most part, blood flow interacts with the valve when it is fully open, so even moderate thrombogenic conditions occurring during the main forward flow can potentially affect the patient's health. This is the reason why the main forward flow is still paid so much attention in recent experimental[25-28,31,32] and numerical[6,23,28,31,33-35] investigation.

In this paper we present the results of experiments that exploit scale-up to enhance the current resolution limits for MHV flow measurements. The combination of PIV diagnostics with a scaled-up model valve has been recently demonstrated as a promising approach to increase spatial and temporal resolution simultaneously[36]. Planar PIV data collected downstream of a

scaled-up MHV in the aortic position are used to evaluate the mechanical loading history of blood elements. That enables the application of the Lagrangian tracking approach used to date almost exclusively in numerical investigations. We evaluate two blood damage models which have been proposed to predict platelet activation accounting for the non-linear dependence on exposure time in a physically sound fashion[19-22]. The integration of those models along almost 25,000 trajectories provides an estimate of platelet activation in the bulk forward flow during systole and moreover allows for evaluation of the relevance of the increased measurement resolution.

2. Materials and Methods

2.1. Experimental apparatus

The test rig is designed to reproduce and investigate the unsteady main forward flow through a large-scale model valve in aortic position and has been described in more detail elsewhere[36]. The valve design is loosely based on the geometry of St. Jude Medical™ bileaflet valve, scaled up by a factor of 5.8 to 140 mm diameter. The model valve has two semi-elliptical leaflets, each with two rectangular protru- sions which sit in a butterfly-shaped hinge recess. The butterfly constrains the leaflet between closed and fully open positions.

The test section is a cylindrical tube as a model for the root of the aorta. The tube wall is 5 mm thick and is made of transparent PMMA to allow optical access. The valve is mounted in the cylindrical tube, just downstream of the reservoir. This mimics the anatomical arrangement, in which blood flows through the valve from the relatively large left ventricle of the heart. The inlet is flared with a radius equal to 0.3 of the internal diameter, to prevent flow separation as the fluid enters the test section from the reservoir. A transparent viewing box, with 5-mm-thick PMMA walls, surrounds the test section and is filled with water. The

purpose of the viewing box is to reduce optical distortion that occurs between the working fluid and the cylindrical test section.

A sketch of the experimental system is shown in Fig. 1. Upstream of the test section and valve, there is a 160-L polyethylene tank, which is vented to the atmosphere and acts as a reservoir. Downstream, a 50.8-mm hose connects the test section to a computer-controlled piston pump. The stainless steel piston rod is driven by a stepper motor controlled using National Instruments™ MAX and LabVIEW software, via a NI™ motion control card.

The flow system is not operated in continuous mode. In order to reproduce the unsteady systolic waveform at the inlet of the aorta, the piston travels from one end to the other of the pump cylinder at varying speed. After the end of systole the pump is reset to the start position and the fluid is kept at rest for 1 minute prior to the next run in order to eliminate residual flow from the previous cycle.

According to dimensional analysis[36], fluid dynamic similarity between model and physiological scale is assured by preserving the Reynolds number

$$Re = \frac{UD}{\nu}$$

and the Womersley number

$$\alpha = \frac{D}{2}\sqrt{\frac{2\pi f}{\nu}}$$

where U is the flow velocity, D is the diameter, ν is the kinematic viscosity and f is the heartbeat frequency. Since the viscosity of water, used as the working fluid, is 3.5 times smaller than blood, flow velocity is reduced by a factor of about 19.1 and the time scale of the experiment is increased by a factor of 118 to preserve Reynolds and Womersley numbers.

The fluid is seeded with tracer particles and illuminated by a Nd:YAG laser. The laser beam is shaped into a light sheet by a 15 mm cylindrical lens and focused by a 200 mm spherical lens. These lenses yield a light sheet whose thickness we estimated as 100 µm at its waist. This estimate is based on digital images of a black diffuse target illuminated by the light sheet at an angle of 10º.

The PIV system used to conduct the experiments comprises a double-pulse Nd:YAG laser (PIV Gemini model Y100-15, 610127, TSI Inc.), a digital camera with a 1000×1016 pixel CCD (PIVCAM 10-30, TSI Inc.), an articulated light delivery arm, a synchroniser and a PCI frame grabber.

Images for PIV were acquired at a rate of 15 Hz and processed with non-overlapping 16×16 pixel interrogation regions, yielding a grid of 63×62 velocity measurements. Post-processing of the data includes a local median filter, which rejects about 4% of the vectors. To control the experimental noise which can affect the first derivatives of the velocity data used to calculate the shear stresses, Gaussian smoothing is applied on 3×3 masks.

2.2. Test conditions and measurement resolution

The velocity field was measured downstream of the model valve in the mid-plane of the aortic root. The flow is unsteady, replicating the realistic aortic waveform[37] shown in Fig. 2. The leaflets are locked in the fully open position.

To avoid any ambiguity, all values of flow rate (Fig. 2), distance, time and shear stress henceforth are expressed in equivalent physiological scale. The systole duration is 370 ms and the peak Reynolds number (6200) is reached after 93 ms. The unsteady flow was measured at 650 time steps throughout the systolic phase, which according to the equivalent acquisition rate of the PIV system (1.75 kHz) yields a temporal resolution of 570 µs.

The flow field was investigated in 15 regions downstream of the valve. For each frame the camera was configured to achieve a spatial resolution of 60 µm, resulting in a field of view 3.8 mm × 3.7 mm. The 15 frames cover an overall area of about 11 mm × 19 mm, located downstream of one of the valve leaflets, as displayed in Fig. 3. The x coordinate is aligned with the main flow direction and the y coordinate is normal to the plane defined by the x axis and the leaflets' rotation axis. The origin is placed on the datum line defined by the tips of the leaflets (see Fig. 3).

2.3. Lagrangian tracking

PIV provided planar measurements of the two velocity components, u and v, in an Eulerian framework. For each node of the spatial grid, instantaneous shear stresses are calculated as $\tau = \mu(\partial u/\partial y + \partial v/\partial x)$ using a second-order accurate central difference scheme. Since blood damage is related to the history of mechanical loading experienced by cells, our interest is to investigate the flow field in a Lagrangian fashion. Therefore we integrate the velocity maps obtained with PIV to calculate a number of possible trajectories. Each trajectory is initiated from a selected start time t_0 and start location (x_0, y_0). For any location (x_i, y_i) at the i-th time point, the values of the 2 velocity components u_i and v_i are integrated over the time step $\Delta t = 570$ µs to calculate (x_{i+1}, y_{i+1}), using a forward Euler scheme[38]. Bicubic spline spatial interpolation[39] of the discrete velocity data at the i-th time point provides the values (u_{i+1}, v_{i+1}), to be used in the next integration step, as well as the local shear stress τ_{i+1}.

Trajectories are integrated across a spatial domain defined by the 15 frames: at the beginning of every integration step i, the frame used for the spatial interpolation is selected as the one including the point (x_i, y_i). The temporal domain for integration consists of 650 time points that cover the entire systole. Integration stops when the tracked particle leaves the area of the 15 frames. However, the trajectories initiated at the later stages of systole experience a very

slow motion, so it can happen that they do not exit the investigated area before the end of systole. It is assumed that those cells remain in the same region during the whole diastolic phase (not investigated here), when velocity is low. If a tracked particle has not left the measurement area at the end of systole, the integration of its trajectory resumes at the beginning of the next systole.

Trajectories are initiated at $x_0 = 0$, i.e. at the upstream edge of the measurement area. They are calculated for 186 start locations y_0 (taken between $y = 0$ mm and $y = 11$ mm) and 130 start times t_0 (every fifth measurement time, between $t = 0$ and $t = 370$ ms), resulting in a total of 24,180 calculated cell paths.

2.4. Blood damage models

The objective of this investigation is to identify flow features causing intense and/or prolonged shear stress on cells, with the potential to trigger blood damage. To determine the loading history of representative individual cells, shear stresses are evaluated along the calculated trajectories. The knowledge of the mechanical loading of cells as a function of time allows for the calculation of the cumulative blood damage. In this paper we chose to use the platelet damage models proposed by Goubergrits and Affeld[19] and Grigioni et al.[20]. Both models respect the principle of causality[20,40], reproduce the original empirical model introduced by Giersiepen et al.[15] under constant shear stress and account for the loading history previously sustained by the blood cell[20,21].

The model proposed by Grigioni et al.[20] is based on the idea that the blood damage index BDI is a certain function of the mechanical dose $D = t \times \tau^{b/a}$ supplied to the cell. In particular for each integration step the cumulative mechanical dose contributes to the *i-th* damage increment $\Delta(BDI)_i$, yielding the discrete formulation

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$$BDI(t_i) = \sum_{k=1}^{i} C\, a \left[\sum_{j=1}^{k} \tau\left(t_j\right)^{b\!/\!a} \Delta t + D(t_0) \right]^{a-1} \times\ \tau\left(t_k\right)^{b\!/\!a} \Delta t \tag{1}$$

where $D(t_0)$ is the mechanical dose already accumulated by the cell at the integration start

time and (a, b, C) are empirically tuned parameters. The total damage $BDI(t_i)$ can be

calculated by summation of all the discrete increments accumulated along a trajectory from t_0

to t_i.

The model introduced by Goubergrits and Affeld[19] introduces an additional virtual time to

account for the fact that the damage index is not a linear function of time. This correction

leads to the following formulation[21]:

$$BDI(t_i) = C \left[\Delta t + \left(\frac{BDI(t_{i-1})}{C \cdot \tau(t_i)^b} \right)^{1\!/\!a} \right]^{a} \tau(t_i)^{b} \tag{2}$$

Both Eq. 1 and Eq. 2 embed three empirically tuned parameters. Here we take in

consideration two sets of values proposed for these parameters. The former set was proposed

by Giersiepen et al.[15] to fit measurements of the LDH enzyme (a marker of platelet lysis)

released by platelets exposed to uniform shear stress in a Couette viscometer:

$$(a_1, b_1, C_1) = \left(0.77,\ 3.075,\ 3.31 \times 10^{-6}\right) \tag{3}$$

The model in Eq. 1 has been used by Nobili et al.[22], who tuned the three parameters

according to experimental measurements of thrombin generation due to platelet activation

induced by controlled dynamic shearing:

$$(a_2, b_2, C_2) = \left(1.3198,\ 0.6256,\ 10^{-5}\right) \tag{4}$$

As a consequence, the results produced by the two sets of parameters will be regarded as predictions of two different events, platelet lysis (Eq. 3) and platelet activation (Eq. 4).

2.5. Summary of data processing

A methodology is proposed to use experimental measurements of a macroscale flow field for evaluating the mechanical loading history experienced by blood cells as the travel downstream of a mechanical heart valve. The method can be more generally applied to a number of cardiovascular devices, the crucial point being the availability of space- and time-resolved data.

The signal chain from flow-field images to blood damage estimates is summarised in the flowchart reported in Fig. 4. The top row of the flowchart covers the procedures used to process the raw images to calculate the velocity maps and, from these, the instantaneous shear stress maps. With regards to the cross-correlation of the raw images, the effect of scale-up and optical distortion (due to refractive index mismatch) on the measurement error has been extensively discussed[36]. The calculation of crude velocity data is followed by a validation stage, consisting of median filtering, which rejects about 4% of the vectors. Gaussian smoothing with a 3×3 stencil is then applied, followed by numerical differentiation with a second-order accurate central difference scheme to estimate velocity gradients. The combination of Gaussian smoothing with this finite difference scheme has been used by Ge et al.[27], among others, for similar measurements. It has been shown by Luff et al.[41] that this approach is superior to more complex finite differences. In tests on synthetic PIV data, is has been demonstrated that the smoothing step can reduce noise in the gradients from 68% to about 4%[41].

Lagrangian tracking (to calculate trajectories and loading histories from shear stress maps) is accomplished by temporal integration of the velocity data and subsequent spatial

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interpolation. Temporal integration by a forward Euler scheme, already proposed for the treatment of similar numerical data[38], introduces an approximation, since it assumes uniform velocity in the time interval between to PIV acquisitions. However the high temporal resolution achieved is beneficial for this integration, as the maximum displacement between two measurement times is about 2% of the device length scale. The spatial interpolation with a bicubic spline function[39] is necessary to overcome the fact that the velocity data is available at discrete locations. The same spatial interpolation provides the value of the instantaneous shear stress for every point of the trajectory, thus yielding the loading history. Finally, the integration of the blood damage models over the loading histories gives the damage parameter here referred to as *BDI*.

3. Results

As the interest is on instantaneous shear stresses, the results presented in this paper are taken from one realisation of the experiment for each of the 15 frames. While experiments were designed to minimise cycle-to-cycle variability, variations of the velocity field are intrinsic to unsteady flows with moderately high Reynolds numbers. In preliminary repeatability tests, we collected velocity data and computed shear stress in the frame closest to the leaflet tip in 25 realisations. Root-mean-square (rms) variation in shear stresses is plotted in Fig. 5 for peak systole ($t_0 = 93$ ms), the time at which highest variations occur. In this plot the highest rms is around 3.5 Pa, the average value of the shear stress being about 9 Pa.

3.1. Eulerian and Lagrangian representation of the instantaneous shear stresses

An illustrative instantaneous shear stress map, taken at peak systole ($t_0 = 93$ ms), is shown in Fig. 6. The largest shear stresses can be observed just downstream of the valve, as the flow close to the upper surface of the leaflet maintains a steep velocity gradient $\partial u/\partial y$. Away from

the leaflet tip, in the bulk of the lateral orifice jet, we observe a relative paucity of large stresses with respect to the central orifice jet.

The integration of the velocity data from PIV provided possible trajectories of cells transported through the fully open valve during the unsteady systolic flow. In Fig. 7, temporal profiles are shown for shear stress along two typical trajectories, initiated at the start time $t_0 = 74$ ms (about 20 ms prior to peak systole) from the locations $y_0 = 5.1$ mm and $y_0 = 6.4$ mm. The trajectory initiated at $y_0 = 5.1$ mm gets entrapped in a vortex shed from the trailing edge of the leaflet, so the stress repeatedly oscillates between 0 and about 9 Pa. The other trajectory selected, initiated at $y_0 = 6.4$ mm, exhibits exposure to shear stress peaking at 10 Pa.

Cells travelling across the valve at different times are exposed to dissimilar loading. In Fig. 8 the path-averaged stress for trajectories initiated at $y_0 = 6.1$ mm is plotted as a function of the start time t_0. Trajectories started before $t_0 = 130$ ms are exposed to stresses that, averaged over the entire trajectory, are larger than 1 Pa. In particular the average stress can be as high as 3 Pa when cells pass through the valve close to the peak systole ($t_0 = 93$ ms). At later stages, for t_0 up to about 200 ms, average stresses can slightly exceed 1 Pa, and later on they range between 0.5 and 1 Pa. For comparison, trajectories initiated in the lateral orifice at $y_0 = 10.1$ mm (also plotted in Fig. 8) exhibit average stresses between 0.5 and 1 Pa for any start time.

For the trajectories started at $y_0 = 6.1$ mm considered in Fig. 8, the time needed to exit the region under observation is less than 100 ms for start times before peak systole. For later start times, however, transit times are up to 250 ms. Cells travelling in the lateral orifice ($y_0 = 10.1$ mm) exit the observed region in less than 50 ms for any start time before $t_0 = 200$ ms.

3.2. Preliminary test of the blood damage models

Equations 1 and 2 were tested by selecting two simple stress patterns as input loading. In one test we used a square waveform, with stress alternately equal to 1 or 20 Pa at 1 Hz frequency. The duration was 2000 ms and the time step Δt was set to 1 ms. For the other test, we used a sinusoidal waveform, oscillating between 0.01 and 20 Pa at 1 Hz frequency. In these tests for both Eq. 1 and Eq. 2 we use the set of parameters proposed for platelet lysis (Eq. 3).

The results of the tests indicate that, in spite of the seemingly different functional form, the models proposed by Grigioni et al.[20] (Eq. 1) and Goubergrits and Affeld[19] (Eq. 2) produce very similar predictions with either square or sinusoidal waveform. Fig. 9 shows the response of the two models to the sinusoidal input stress.

In a further test, Eq. 1 and Eq. 2 were used to predict the response to a constant shear stress of 1 Pa for a duration of 1 s. Both equations correctly predict the *BDI* of 3.31×10^{-6}, consistent with the original model by Giersiepen et al.[15]. Varying the time step Δt between 10^{-5} s and 10^{-1} s, we found that Eq. 2 gives the expected value, whatever time step is used. However, the result of Eq. 1 asymptotically approaches the expected value as $\Delta \tau \to 0$.

3.3. Blood damage predictions from PIV data

Combining the PIV capabilities with the benefits of scale up, in our experiments the effective time step between measurements is 570 μs. With this time step the gap between the response of Eq. 1 and Eq. 2 to uniform shear stress is just 0.13%. However, we used Eq. 2 for the following calculations, as it is independent of the choice of the time step. We integrate Eq. 2 using the parameters for platelet lysis[15] (Eq. 3) and activation[22] (Eq. 4).

The blood damage index *BDI* is calculated integrating the loading histories for every trajectory, so that 24,180 values of this parameter are available as a function of t_0 and y_0.

These values are used to build up contour plots for platelet lysis and activation. Fig. 10 shows the results obtained for platelet lysis. The model predicts that the blood damage is higher where the cells are exposed to large shear stresses, which occurs in the middle (M) frame during the acceleration stage of systole. The values of *BDI* calculated for platelet lysis peak at about 8×10^{-5}. Most of the damage is concentrated in the trajectories initiated closest to the leaflet. The *BDI* for these trajectories before systole peak is typically around 3.5×10^{-5}.

Cells passing through the valve after the systolic peak ($t_0 > 93$ ms) undergo less intense stress, and the model predicts a quick decay of blood damage to less than one third of the values predicted before systolic peak. Moreover, during the deceleration stage the damage is lower but more diffused, as it spreads to a larger interval of y_0 values. At this stage only, blood damage is detected in the top (T) frame too, though less intense. For trajectories initiated in the bottom (B) frame the predicted *BDI* is uniformly low and never exceeds 5% of the highest value detected in the middle frame.

The predictions obtained for platelet activation are reported in Fig. 11. The scale of blood damage index cannot be compared to the results in Fig. 10, as the peak *BDI* is close to 1.5×10^{-6}, which is about 50 times smaller than the highest value obtained using the parameters of Giersiepen.

Similarly to what was observed in Fig. 10, most of the blood damage predicted for platelet activation is concentrated in the trajectories initiated closest to the leaflet. However lower but comparable peak values are found also in the top (T) and bottom (B) frame and the temporal distribution of the damage is quite different from the other model. Prior to systolic peak the values of *BDI* are low everywhere, with the exception of the trajectories initiated close to the leaflet tip. But differently from platelet lysis, the largest values for platelet activation are detected after the systolic peak. The value of *BDI* for most of the trajectories initiated close to

the leaflet for 120 ms $< t_0 <$ 200 ms is above 8×10^{-7}, peaking at 1.5×10^{-6}. Despite the fact that during flow deceleration cells experience lower shear stresses, at this stage the exposure time is longer, as cells are transported away from the leaflet wake more slowly. The platelet activation model (Eq. 4) estimates larger damage for long exposure to moderate stress than for brief exposure to high stresses. That also explains why comparable damage is predicted also in the bottom (B) and top (T) frame, where only moderate stress values are detected.

3.4. Effect of measurement resolution

In order to evaluate the effect of measurement resolution on blood damage prediction, we compare the predictions obtained from the same flow field at different spatial resolutions. This analysis was conducted for the measurement frame closest to the leaflet tip. To obtain low-resolution velocity maps of the same flow, we reprocessed the images using 32×32 pixels interrogation windows, instead of 16×16 pixels as for the results presented so far. The new processing reduces the spatial resolution of the velocity field by a factor of 2, resulting in an effective spatial resolution of 120 µm. The new data are then integrated to calculate cell trajectories, and subsequently the blood damage index is evaluated.

For the low-resolution data, trajectories are initiated at 130 different start times t_0 and 31 start positions y_0, resulting in a total of 4,030 cell paths. The start positions (and trajectories) are half as many as for the higher resolution data.

In order to display the results in a clear and compact form, trajectories are clustered in 3 groups $\{Y_1, Y_2, Y_3\}$ according to the start location y_0 (e.g. Y_1 includes trajectories initiated in the lower part of the frame). The averaging of BDI within each group is solely intended to reduce the amount of data points to display and highlight the trends. It is not essential to the signal chain and for this reason it was not included in the flowchart reported in Fig. 4. The

group-averaged values of blood damage index are plotted in Fig. 12 and 13 as a function of the start time t_0.

The results for platelet lysis are plotted in Fig. 12 for both high and low resolution. Blood damage is underestimated by the low-resolution measurements. The largest difference occurs in the group Y_2, which is the closest to the leaflet tip: here during the acceleration stage the BDI drops up to 55.3%, with an average reduction of 36.8%. The reduction of predicted damage reflects the fact that for the lower resolution measurements we observe generally lower levels of shear stress.

In the results of the platelet activation model, plotted in Fig. 13, the reduction of BDI with lower resolution data is still evident, though not as remarkable as seen in Fig. 12. During early acceleration ($t_0 < 50$ ms) the loading pattern exhibits high shear stresses and the BDI for platelet activation, calculated at full resolution, peaks to about 3.5×10^{-7}. Calculations with PIV data at lower resolution yield an average reduction of the BDI of 27.1% for the group Y_2. In the time interval 120 ms $< t_0 < 200$ ms the shear stresses are lower but the longer exposure times produce even higher BDI values, peaking at about 5×10^{-7}. In this case the BDI reduction is 14.6%.

4. Discussion

An ideal experiment to assess the link between blood damage and mechanical loading would require simultaneous measurement of both quantities. Evaluation of fluid forces is based on optical techniques, which in that case would have to face two orders of problems: optical access to an opaque medium like whole blood or platelet-rich plasma; resolution limits due to the scale of flow-cell interactions (estimated between 10^1 and 10^2 µm). Furthermore platelet activation can only be measured off-line, which would make it difficult to relate the measured quantity with a specific site in the flow or a specific instant in the heart cycle. Therefore at

the present stage platelet activation and shear stress have to be assessed in separate experiments.

In this investigation we exploited the concept of fluid-dynamic similarity to design a scaled-up experiment allowing standard PIV to capture flow features at the highest spatial (60 µm) and temporal (570 µs) resolution achieved so far simultaneously. This resolution (equivalent to 8 red blood cell diameters, or 20 times red blood cell thickness) is at or near the limit at which blood may reasonably be modelled as a homogeneous fluid. For comparison, recent PIV measurements with physiological scale valve are at resolution of {135 µm, 67,000 µs}[27] and {1200 µm, 333 µs}[26]. The current resolution limit, achieved using a scaled-up valve, is {120 µm, 570 µs}[36].

4.1. Lagrangian tracking

We used Lagrangian tracking to compute cell trajectories and loading histories from PIV experiments. The accuracy of Lagrangian tracking is closely connected to the resolution (both in space and time) of the velocity field. For this reason it has been used extensively in combination with numerical data, with examples in the field of prosthetic heart valves[23,28]. There are very few examples of Lagrangian tracking in cardiovascular flow based on experimental, rather than computational, data. One study[42] deals with the flow through a 5:1 scaled-up model of a stenosed coronary artery. However, in that investigation, the accuracy of Lagrangian tracking is restricted by the temporal resolution of the PIV data (the frame rate is 15 Hz), since the largest displacement between two consecutive measurement times (the largest velocity is about 0.4 m/s) is about twice as large (27 mm) as the stenosis diameter (15 mm). Therefore the temporal gap between time points is filled with streamlines calculated by interpolation from the available flow fields. For comparison, in the present study the

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maximum displacement between two measurement times is about 2% of the device length scale.

Lagrangian tracking shows that in the wake of the leaflet cells can linger for a time (up to 100 ms before peak systole and up to 250 ms afterwards) significantly longer than the time they need to travel across the lateral orifice. For cells travelling close to the leaflets, path-averaged shear stresses are as high as 3 Pa, with instantaneous peaks up to 10 Pa. Notably, these values represent the true local instantaneous stress in the fluid, and are distinct from the Reynolds stress often used to characterise stress in turbulent flow when data are not adequate to resolve fine structure[27,43].

These results suggest that in this region cells are exposed for hundreds of ms to shear stresses sustainedly larger than the normal physiological range (between 0.5 Pa and 1 Pa). The shear stresses we measured for cells travelling in the lateral orifice jet, farther from the leaflet tip, fall within the normal physiological range.

4.2. Blood damage

Cell response to the measured mechanical loading was estimated using predictive tools established from the measurement of platelet response to controlled shearing in somewhat simplified flow conditions. We considered two mathematical model formulations[20,21], designed to produce physically sound predictions of blood damage level. The comparison of the two mathematical models revealed that, in spite of the quite dissimilar formulation, they produce the same results, at least in the limit of $\Delta t \to 0$. We used the formulation proposed by Goubergrits[21] because it is less sensitive to the time step. We also considered two sets of empirical parameters for the model, meant to assess the level of platelet lysis[15] and activation[22].

Our results show that the largest values of platelet lysis, being more strongly influenced by the intensity of the stress, are localised near the leaflet during the acceleration stage, where the highest stresses are detected. Here the average *BDI* is 2.5×10^{-5}. Goubergrits and Affeld[19] suggested that the predicted level of platelet lysis be compared to the blood damage accumulated during a single passage through the whole circulation system. Assuming that the lifespan of platelets is 14 days and that blood completes one passage in about 1 minute, the reference damage accumulated in one passage[19] is about 5×10^{-5}. That means that the brief exposure to high shear downstream of the valve contributes an additional 50% damage for each passage.

For platelet activation, the integration of the model of Nobili et al.[22] along cell trajectories shows that the largest damage is predicted after the systolic peak, during the deceleration stage. Observation of the loading histories indicated that at this stage stress levels are lower than during acceleration, but still larger than physiological values, at least for trajectories initiated before $t_0 = 200$ ms. During the deceleration stage the exposure time increases significantly, as cells remain longer in the region where non-physiological shear stresses are detected. According to the model parameters, platelet activation has a stronger dependence on exposure time (exponent $a = 1.3198$) than shear stress ($b = 0.6256$). Unlike cell lysis, platelet activation is maximised by long exposure to even moderate shear (after systole peak) rather than brief exposure to high shear (before systole peak). With respect to the role of the start location y_0, our results indicate that the average activation levels for trajectories initiated close to the tip of the leaflet in the time interval [120, 200] ms is 15 times larger than in the bottom frame for $t_0 < 200$ ms.

4.3. Length scales

In a previous publication[36] the valve flow was characterised with PIV measurements at a spatial resolution of 120 µm. These measurements revealed vortical structures in the leaflet wake, with energy distributed across a wide range of frequencies, according to spectral analysis.

In the present article, the same flow is described at a spatial resolution increased by a factor of 2, and this improvement has been exploited to implement Lagrangian tracking of virtual blood cells and estimate the potential of the flow for blood damage. However, to assess the role of small-scale flow structure in blood damage, flow-field images have also been processed at coarser resolution. Processing at lower resolution yields lower estimates of shear stress, with consequent underestimation of platelet damage and activation (particularly in conditions where the blood damage model is most sensitive to stress intensity). This finding indicates quantitatively that significant blood-damaging flow structures of length scale 120 µm or smaller exist in MHV wake flow, based on the Nyquist criterion applied to the effective spatial resolution (60 µm) of these measurements, and suggests the existence of flow structures across a broad spectrum of length scales. These results confirm the need to pursue highly resolved measurements of the flow field, since a coarser resolution overlooks the smaller active scales, which give a substantial contribution to the mechanical damage and activation of platelets.

4.4. Limitations

The data collected in this paper does not allow direct identification of the smallest active length scales of the flow, which may be smaller than the state-of-the-art resolution achieved in this investigation. The existence of under-resolved spatial scales is suggested by the

sensitivity of shear stress results both to interrogation window size and Gaussian smoothing of velocity data.

Smoothing is motivated by the sensitivity of calculated shear stress to noise intrinsic in the experimental velocity measurements. It was employed on the smallest possible stencil (3×3 pixels) to minimise impact on the effective spatial resolution. Comparison between smoothed and non-smoothed velocity data in the frame closest to the leaflet tip shows that mean shear stresses along each trajectory is 63% higher, on average, when using non-smoothed data. Such variation can be a marker of under-resolved length scales, as well as velocity measurement noise.

Predicted cell lysis and platelet activation are also larger for non-smoothed data, showing average increments of 340% and 41% respectively. However the qualitative results of the damage models, and the conclusions drawn in this article, are not significantly affected by smoothing, as shown by the comparison between the platelet lysis predicted from smoothed data (Fig. 12, high-resolution plot) and the lysis predicted from non-smoothed data, reported in Fig. 14.

Since the valve leaflets are fixed in the present model, flow phenomena due to valve opening and closure are not reproduced. Observation of leaflet kinematics indicates that valve opening and closure actually occur in a very short time[31], so leaflets are fully open during most of systole. The additional contribution of leaflet dynamics will be fully appraised in future investigation that will benefit from the processing tools and the reference results presented in this paper.

The use of a straight tube downstream of the aortic valve in our model omits the geometry of the aortic sinus, but mimics the geometry realised when aortic valve replacement is associated with aortic root reconstruction using a straight conduit graft. It has been

demonstrated that the flow separation and vortex shedding downstream of the valve measured in a straight duct[36] is similar in structure to the case when aortic sinuses are considered[5]. This is valid at least for unsteady flow with leaflets locked in fully open position. Further investigation should address the role of the aortic sinuses when leaflets are free to move.

The effects of out-of-plane velocity have not been included in the analysis. Consequently, it is likely that the results underestimate loading on blood cells. However, measurements were conducted on a plane of symmetry where the mean out-of-plane flow is zero, and the two-component approach is therefore expected to give a meaningful approximation to the true situation.

5. Conclusions

Enhanced measurement resolution was demonstrated to be crucial for evaluating the hemodynamic performance of mechanical valves. In spite of the current limits of measurement technology, we showed that scaling up blood flow experiments allows for computation of mechanical loads on blood cells in a Lagrangian fashion. We demonstrated that flow structures are active at scales smaller than 120 µm, and therefore only high-resolution measurements can provide correct assessment of platelet damage and activation.

Our measurements are suitable for integration of empirical models taken from the open literature. These models turned out to give similar results, whereas the sets of parameters available, tuned for different types of damage, resulted in dissimilar predictions, showing that platelet activation is mostly promoted in the deceleration stage after systole, where loading histories are characterised by longer exposure times to moderate non-physiological shear stress.

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the start time t_0 and start location y_0. The position of the leaflet's tip is sketched on the left side and the aortic waveform of the flow rate is plotted in the top part of the figure. Frame labels (T, M, B) are used according to Fig. 3.

12 Effect of resolution: *BDI* calculated using the platelet lysis model for high (left) and low (right) resolution. Data are separated in 3 groups $\{Y_1, Y_2, Y_3\}$ according to the start location y_0.

13 Effect of resolution: *BDI* calculated using the platelet activation model for high (left) and low (right) resolution. Data are separated in 3 groups $\{Y_1, Y_2, Y_3\}$ according to the start location y_0.

14. Blood damage index predicted using the platelet lysis model, calculated from non-smoothed velocity data. Data are separated in 3 groups $\{Y_1, Y_2, Y_3\}$ according to the start location y_0.