Published in final edited form as:

Ann Biomed Eng. 2008 July ; 36(7): 1130–1141. doi:10.1007/s10439-008-9494-z.

Micro-Flow Visualization of Red Blood Cell-Enhanced Platelet Concentration at Sudden Expansion

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Abstract

Microscopic steps and crevices are inevitable features within prosthetic blood-contacting devices. This study aimed to elucidate the thrombogenicity of the associated microscopic flow features by studying the transport of fluorescent platelet-sized particles in a suspension of red blood cells (RBCs) flowing through a 100 μm :200 μm sudden expansion. Micro-flow visualization revealed a strong influence of hematocrit upon the path of RBCs and spatial concentration of particles. At all flow rates studied ($\text{Re} = 8.3\text{--}41.7$) and hematocrit 20% and lower, RBC streamlines were found to detach from the microchannel wall creating an RBC-depleted zone inside the step that was much larger than the cells themselves. However, the observed distribution of particles was relatively homogeneous. By contrast, the RBC streamlines of samples with hematocrit equal to or greater than 30% more closely followed the contour of the microchannel, yet exhibited enhanced concentration of particles within the corner. The corresponding size of the cell depletion layer was comparable with the size of the cells. This study implies that local platelet concentration in blood within the physiological range of hematocrit can be elevated within the flow separation region of a sudden expansion and implicates the role of RBCs in causing this effect.

Keywords

Platelet margination; Red blood cell; Complex microflow; Flow expansion; Artificial organs; Thrombosis

INTRODUCTION

Thrombosis is a potentially fatal condition that leads to millions of severe disabilities, heart attack, and stroke.³³ It is also a common complication associated with mechanical circulatory support.^{7,17,22,39,40} Flow features associated with thrombosis, such as boundary layer separation, recirculation, and stagnation can be found at geometric irregularities such as bifurcations, stenoses, and aneurysms.^{4,11,25,30,34} In the context of design of blood-wetted

devices, it is known that steps and crevices in the flow path serve as a nidus for thrombus development.³¹ An illustrative example is the design of miniature blood pumps for small children (see Fig. 1). Internal crevices and steps are an inevitable consequence of the assembly of such a device from multiple parts. In practice, great effort is made to minimize these features by precision machining and polishing, however it is virtually impossible to eliminate them altogether. The challenge therefore is to determine the tolerable limits of these features by studying how they contribute to thrombus development.

The general process of thrombosis in blood-wetted devices includes platelet adhesion on artificial surfaces, which is dependent upon the transport of platelets from the bulk flow to the surface.^{24,35,38} Over the past few years, several mathematical models were developed to predict platelet adhesion in complex flow.^{12,35,37} The results were obtained by simulating convective-diffusion transport of various species associated with the coagulation cascade, such as thrombin and platelets. Most of these models were only applied to platelet-rich plasma, and the red blood cell (RBC) effects were omitted. However, RBCs have been observed to promote a near-wall excess of platelets in tube flow.^{3,15,16,21,42} This phenomenon is also likely to occur in more complex flows under certain conditions.

Previous experiments by Karino and Goldsmith who studied flow of suspended RBCs in microscopic concentric expansions ($151\text{ }\mu\text{m}$: $504\text{ }\mu\text{m}$) reported the existence of an RBC depletion zone in the corner region for $Ht = 15\text{--}30\%$.²⁵ Subsequent studies by the same group observed enhanced platelet aggregation and adhesion at the channel expansions.^{26,27} These observations imply the impact of hematocrit on the platelet transport behavior, although these studies did not explicitly observe the distribution of platelet concentration in the flow field.

The relevance of RBC-enhanced platelet deposition is further illustrated by simulation studies in a sudden expansion reported by Sorensen *et al.*³⁶ and Jordan *et al.*²⁴ The influence of RBCs was modeled as a hematocrit-dependent platelet diffusivity. These simulations exhibited a positive dependence of deposition on hematocrit. However, these models were unable to accurately predict the axial distribution of platelet adhesion observed by Karino and Goldsmith.^{26,27} The authors surmised that this inconsistency may be due to the assumption of a uniform distribution of RBCs in the flow field. They suggested that the predictions could be improved by incorporating a model of RBC depletion and platelet margination as a function of shear rate and hematocrit. They further recommend future experiments to quantify the effect of the vortex on local cell concentrations.

The latter experimental and theoretical studies emphasize the importance of both the distribution of RBCs and their effects on platelet transport in a sudden expansion. This was the motivation for the current study: to investigate these phenomena through micro-flow visualization. Resuspended bovine RBCs ($Ht = 5\text{--}60\%$) and platelet-sized particles were visualized while flowing through a microchannel containing a $100\text{ }\mu\text{m}$: $200\text{ }\mu\text{m}$ sudden expansion. The spatial distributions of both RBCs and platelet-sized particles with various hematocrits and shear rates explored the effect of localized RBC concentration and RBC streamlines on the spatial distribution of platelet-sized particles.

METHODS

Experimental Setup

A layout of the experimental setup is shown in Fig. 2. A suspension of RBCs and small fluorescent particles (described below) was propelled by a syringe pump (KDS 100, KD Scientific Inc., Holliston, MA) at controlled flow rates (range: 6–30 mL h⁻¹) through a polydimethylsiloxane (PDMS) microchannel with rectangular cross section, having a height of 100 μm (z -direction) and width (y -direction) containing a 100 μm :200 μm expansion. The length of the region of interest (x -direction) was 200 μm (see Fig. 3). The corresponding range of Reynolds number was from 8.3 to 41.7, based on a characteristic length of 100 μm . The samples were collected in a small reservoir which was vented to atmospheric pressure. The microchannel was visualized through an inverted microscope (IX70, Olympus Inc., Melville, NY) with a 20 $\times$ objective (LCPlanFL, 20 $\times$, NA = 0.40, Olympus Inc., Melville, NY). Two types of illumination were employed. For visualization of the platelet-sized fluorescent particles, an Nd:YAG laser (Solo PIV I-15, New Wave Research, Inc, Fremont, CA) was used with fluorescent filter cube to block out the projection of RBCs. This enabled accurate quantification of the particle concentration field. A halogen backlight was employed for bright field visualization of both RBCs and fluorescent particles. This was used to record the trajectory of RBCs as well as observe the cell depletion layer. A CCD camera (PCO Sensicam QE double exposure, The Cooke Corp., Romulus, MI) attached to the side port of the microscope was synchronized to the laser to acquire a series of digital images (688 $\times$ 520 pixels) in the region of interest. The focal plane of the images was centered 5 μm from the bottom surface of micro-channel. The depth of field was 6 μm (see Fig. 3).

Sample Preparation

Fresh bovine blood was collected by venopuncture of healthy calves and was immediately centrifuged at 1320*g* for 10 min. The plasma and buffy coat containing white blood cells and platelets were then removed, and the RBCs were washed three times in phosphate buffer solution (PBS) (Sigma-Aldrich Corp., St. Louis, MO). Bovine albumin (Sigma-Aldrich Corp., St. Louis, MO) was added at a concentration of 1% to stabilize cell morphology. Five levels of hematocrit, 5%, 20%, 30%, 40%, and 60% were obtained by resuspension of the RBCs in PBS.

Platelet-sized neutrally buoyant fluorescent particles (R0200, Duke Scientific Corporation, Palo Alto, CA) with a mean diameter of 2 μm and a light excitation peak of 542 nm and emission peak of 612 nm were added to the solution. The concentration of the particles was adjusted to approximately $2.5 \times 10^8 \text{ mL}^{-1}$, which is within the physiological range of platelet concentration in bovine blood (1×10^8 – $4 \times 10^8 \text{ mL}^{-1}$). The preparation was mixed for 30 min in a plastic centrifuge tube on a rocker plate prior to start of experiments.

Image Processing

Laser-illuminated images were post-processed with a user-designed Matlab program (Mathworks, Natick, MA). The procedure is similar to the one previously reported by the authors.⁴² Briefly, the images were cropped to isolate the region of the microchannel sudden

expansion (from $x = -50 \mu\text{m}$ to $x = 150 \mu\text{m}$, see Fig. 4a). Subsequent processing entailed background homogenization to equilibrate uneven illumination and contrast-enhancement by histogram stretching. The minimal and maximal intensity values in this process were carefully selected according to the original intensity histogram. The particles in the image were identified by thresholding followed by erosion to eliminate spurious noise (see Fig. 4b). Particles were counted by a custom-written Matlab program described previously⁴² and averaged over 100 images. The intensity threshold was adjusted to minimize the error between the automatically generated concentration and the manually counted concentration in several representative images. The locations of fluorescent particles in the binary images were recorded.

Data Analysis

To obtain a 2-D concentration profile, images were partitioned into square grids of $20 \mu\text{m} \times 20 \mu\text{m}$ (Fig. 4b), $8 \mu\text{m} \times 8 \mu\text{m}$ or $4 \mu\text{m} \times 4 \mu\text{m}$ (not shown). The concentration profile normalized by the bulk concentration (relative concentration) was calculated as

$$C_r(x, y) = \frac{N_p(x, y)/[A(x, y) \times d]}{C_B} \quad (1)$$

where $N_p(x, y)$ is the number of particles within the grid element centered at position (x, y) in the current experimental coordinate system—indicated in Fig. 4b. $A(x, y)$ denotes the area within the grid element occupied by blood. For grid elements straddling the boundary, the proportional area within the flow region was computed using a binary mask with the contour of the microchannel. d is the depth of field ($= 6 \mu\text{m}$), shown previously in Fig. 3. C_B is the bulk concentration of particles (count/unit volume).

To illustrate the variation of relative concentration within a particular image, the concentration elevation (CE) was defined as:

$$\text{CE}(x, y)[\%] = \frac{C_r(x, y) - \bar{C}_r}{\bar{C}_r} \quad (2)$$

where $\bar{C}_r$ is the average relative concentration over the entire cropped image. Student's t -test was used to determine differences in concentration in the experiments. A value of $p < 0.05$ was assumed to indicate statistical significance.

The localized RBC concentration and cell-depleted zone were observed using bright field imaging at $20\times$ and $100\times$ magnification. The RBC concentration profile was estimated by assuming an inverse relationship with transmitted light intensity, and denoted as RBC relative light attenuation (RLA). The brightest pixel in the field (RLA = 0) of view was assumed as zero hematocrit and the darkest pixel (RLA = 100%) was associated with the bulk hematocrit. These quantitative data were averaged over 20 images.

RESULTS

Particle Concentration Profile

Figures 5a and 5c show representative images of platelet-sized particle distribution in samples with 20% and 40% hematocrit (Ht) respectively at a flow rate of 15 mL h^{-1} . A locally concentrated area of plate-sized particles was clearly observed at the corner of the expansion in the sample with 40% Ht (see Fig. 5c). The particles were found to distribute more evenly in the sample with 20% Ht, however, without a perceptible concentrated region (see Fig. 5a). Computer-processed concentration profiles are shown in Figs. 5b and 5d, based on a grid size of $4 \mu\text{m} \times 4 \mu\text{m}$. Both samples show an average relative concentration of approximately 3.1 within the experimental focusing plane. The relative concentration increased to nearly 10.0 in the corner for the sample with higher Ht. This concentration peak was not found in the sample with lower Ht.

Figure 6 shows the concentration elevation (CE) in the four sub-regions ($50 \mu\text{m} \times 50 \mu\text{m}$) depicted in Fig. 3 for different hematocrits. The particle CE in Region A (expansion) ranged between -27% and 36% , inversely dependent on hematocrit. The concentration in Region B (corner) was, however, directly related to hematocrit and ranged from -16% to 50% . The most dramatic elevation of particle concentration was found in the corner region for samples with Ht $\geq 30\%$.

Axial Dependence of Particle Concentration Elevation

Figure 7 shows the concentration elevation profile for $20 \mu\text{m} \times 20 \mu\text{m}$ sub-regions as a function of axial position measured from the point of expansion ($x = 0$, Fig. 3). The concentration elevation within the $20 \mu\text{m}$ layer adjacent to the lateral wall (near-wall CE) is provided in Fig. 7a for samples with 20% and 40% Ht at the flow rate of 15 mL h^{-1} . While the Ht 20% sample showed a relatively low CE, which was nearly constant along the length of the wall, a strong axial concentration gradient was found for the Ht 40% sample. At $x = 0$, the concentration was 121% higher than the average. The peak CE (approximately 170%) was observed at $x = 20 \mu\text{m}$, where after the concentration declined smoothly downstream, back to baseline at approximately $x = 120 \mu\text{m}$. The same phenomenon was observed for all three flow rates studied (Fig. 7b); however, the maximum concentration decreased slightly with increasing flow rate.

Figures 7c and 7d show the concentration elevation profile along the centerline of the channel using the same scale as Fig. 7a. Overall, the axial concentration gradient was much lower than near the wall; however, decreased gradually from 6% to -14% for the 20% Ht sample and from -10% to -55% for the 40% Ht sample at 15 mL h^{-1} (see Fig. 7c). The concentration gradient increased with increasing Ht. The same phenomenon was also found at higher and lower flow rates (30 mL h^{-1} and 6 mL h^{-1} , respectively) (see Fig. 7d).

Lateral Dependence of Particle Concentration Elevation

Figures 8a and 8b show the lateral profile of particle CE at four different axial positions ($x = -20, 40, 80, 140 \mu\text{m}$). The concentration gradient in the lateral direction was found to be more pronounced for the Ht 40% sample at all axial positions (Fig. 8b). There was

negligible margination observed in the 20% sample (Fig. 8a). The 40% Ht sample, exhibited a moderate degree of near-wall excess of particles in the throat of the channel ($x = -20 \mu\text{m}$, $\text{CE} = 67\%$ vs. -25% at the centerline). The greatest margination ($\text{CE} = 132\%$) was found $20 \mu\text{m}$ downstream of the sudden expansion near the wall. At $140 \mu\text{m}$ downstream there was negligible lateral variation in particle concentration (see Fig. 8b).

RBC Streamlines and Cell Depletion Zones

Bright field images revealed a clear depletion zone observed in the 20% Ht samples, demarcated by a distinct streamline boundary that originates at the corner and reattached downstream (see Figs. 9a, b). Within this zone, cells were observed to recirculate; and outside the zone, cells were confluent with the central jet. The increase in hematocrit caused the streamlines to better conform to the boundary, hence decreasing the size of the cell-depletion zone (see Fig. 9c). The cell-depletion layer was not perceptible at $20\times$ magnification but was observed in the images at $100\times$ magnification (see Fig. 10). The size of this layer was found to be on the scale of several cells.

The approximate lengths of cell depletion zone (normalized to the step width H) for both 20% and 5% Ht samples are plotted in Fig. 11 for various flow rates. The results were compared to previous experimental observation of separation bubble length in single-phase fluids,^{6,13} particulate suspensions,³² and diluted RBC suspensions.²⁵ The current result for 5% Ht sample agreed well with the previous experimental observation of Karino and Goldsmith²⁵ in diluted RBC suspensions but exceeded the length observed in single phase fluids.^{6,13} The 5% Ht sample consistently exhibited a larger cell depletion zone than the Ht 20% sample. This is contradictory with the recent experimental observation of particulate suspensions performed at very low Reynolds numbers (0.01–2.5).³²

Concentration Profile of RBCs and Particles in the Corner

The lateral concentration profile of platelet-sized particles $20 \mu\text{m}$ downstream of the sudden expansion are plotted in Fig. 12 for both 20% Ht and 40% Ht samples at a flow rate of 15 mL h^{-1} . The RBC concentration as derived from relative light attenuation (RLA) is also illustrated for comparison. For the 20% Ht sample, a large reduction of RBC concentration was observed within an approximately $25 \mu\text{m}$ wide region adjacent to the wall, indicated by a 60% depression in RLA. However, the particle concentration was evenly distributed. For the 40% Ht sample, there was no perceptible change of RBC light attenuation, implying a constant RBC concentration, but the particle concentration was locally elevated in the corner region.

DISCUSSION

Computational fluid dynamic analyses are being increasingly adopted for analyzing the hemodynamic conditions within blood-wetted medical devices such as prosthetic heart valves,^{5,14,18,19,41} blood pumps,^{8–10} and vascular stents.^{28,29} However, the interpretation of these simulation results in terms of thrombogenicity is not trivial. It is known experimentally that thrombosis in blood-wetted medical devices often occurs in complex flow regions featuring flow separation, recirculation, and stagnation. This has motivated development of

numerical models to simulate the fluid dynamic processes governing transport and deposition of platelets to prosthetic surfaces.^{12,24,35} Unfortunately, these models do not consistently predict the platelet deposition rate observed experimentally. A likely explanation is that these models do not account for the enhancement of platelet transport toward the solid boundary caused by dynamic interactions with RBCs. This study was therefore undertaken to explore this hypothesis, inspired by the well-known observations of near-wall depletion of RBCs²⁰ and platelet excess¹⁶ in small blood vessels.

A recent study confirmed the existence of a platelet margination in straight micro-channels under high-shear conditions found in prosthetic devices.⁴² Unlike the flow paths within biological blood vessels which are relatively smooth, prosthetic devices often possess surface irregularities, such as steps and crevices, which may further disturb the flow. The current experiments aimed to elucidate the effect of one such disturbance, a 50 μm backwards-facing micro-step, upon the transport of platelets-sized particles in blood.

The current observations demonstrated the existence of cellular inhomogeneity in the expansion region of the step which was dependent on shear rate and hematocrit (from $\text{Ht} = 5\%$ to 60%). This result is consistent with the experimental results of Karino and Goldsmith who observed a red-cell-depletion layer within the downstream corner of a $151\ \mu\text{m}:504\ \mu\text{m}$ circular expansion ($\text{Ht} = 20\%$).²⁵ Although this group also observed enhanced platelet aggregation and adhesion at the channel expansions,^{26,27} they did not explicitly observe the spatial distribution of platelets.

The current experiments revealed a strong influence of the red cell phase on the spatial distribution of platelet-sized particles. For example, the concentration of platelet-size particles was found to be locally elevated at the corner for hematocrit greater than 30% but essentially equal to baseline for the samples with lower hematocrit. Conversely, the 5% Ht sample exhibited an elevation of platelet concentration near the core of the flow field. This supports earlier experimental observations by Karino and Goldsmith²⁷ who likewise found a positive dependence of platelet deposition on hematocrit. Figure 6 suggests a critical value of hematocrit at which particles begin to accumulate in the corner. This value was 30% in the current experiments, but is likely dependent on other factors, such as shear stress, Reynolds number, size of the step, rigidity of RBCs, concentration, and shape of particles.

The flow rate had much less influence on the platelet concentration distribution compared to hematocrit. An increase of flow by 500% ($6\text{--}30\ \text{mL h}^{-1}$, corresponding to wall shear rate of approximately $5938\text{--}29692\ \text{s}^{-1}$ within the throat of the channel, based on closed-form solution) caused a 20% increase in concentration in the 20% Ht sample as compared to 20% decrease in the 40% Ht sample (Fig. 7b).

A commensurate dependence of the size of the RBC depletion zone on hematocrit was observed. Flow separation at the corner of the step was clearly observed in the lower Ht samples ($\text{Ht} = 20\%$) but was virtually indistinguishable (at $20\times$ magnification) for the case of $\text{Ht} = 40\%$. However, RBC depletion did not necessarily imply platelet excess. This was true for the $\text{Ht} = 40\%$ case, but the platelets of 20% Ht sample were uniformly distributed in spite of the large RBC-depleted zone (see Fig. 12). This would suggest that the phenomenon

of platelet exclusion is dependent either on the size of the depletion zone (with respect to the size of an RBC) or the concentration of RBCs in the core flow. The former hypothesis implies that the effectiveness of RBCs to exclude platelets has a limited spatial range. In the latter case, it implies that RBCs work collaboratively to exclude platelets. In other words, exclusion may be dependent on RBC-platelet distance and/or the RBC-RBC distance. Additional experimentation, and numerical simulation, would be valuable to further elucidate these mechanisms.

The normalized length of the cell-depletion bubble (L/H) was compared to previous experimental observation of separation bubble size in single phase fluids,^{6,13} particulate suspensions,³² and diluted RBC suspensions²⁵ (Fig. 11). The current result for Ht 5% sample agreed well with the previous experimental observation of Karino and Goldsmith²⁵ with diluted RBC suspensions. However, previously reported experimental data for bubble length in a single-phase fluid was smaller than the observed length in dilute RBC suspensions. This may be due to the difference in geometry, specifically the aspect ratio of the experimental channels used in these single-phase experiments. However, recent study by Moraczewski *et al.*,³² performed at very low Reynolds numbers (0.01–2.5), observed the separation length to decrease with decreasing particle concentration whereas the present study demonstrated an inverse relationship between Ht and length of the depletion zone. It should be pointed out that the former study revealed a very strong dependence on the concentration of suspended particles (e.g., 0.48 vs. 0.50). Furthermore, the relative size of particles was much greater than the current study, increasing the Stokes number and therefore the slip velocity. It may be inferred that other factors, such as particle deformability, density, and shape may also affect the size of the depletion zone, and hence would be a worthwhile topic for future studies.

Two mechanisms have previously been hypothesized to explain the influence of RBCs on the lateral transport of platelets. One is *exclusion*, whereby RBCs migrating to the centerline of the tube force platelets outwards, toward the wall^{15,21,24}; another is *shear-induced mixing*, whereby rotation of RBCs increase the regional diffusivity of platelets in their vicinity, thereby creating a convection gradient.^{1,2} In the current study, it was not possible to clearly observe RBC rotation, however, the results clearly demonstrate that the accumulation of platelets in the recirculation zone of the step requires the presence of RBCs. This is a necessary condition but not a sufficient condition inasmuch as the 20% Ht sample did not cause any platelet excess in the corner. Although the current experiments were not able to explicitly track the path lines of cells or particles, there was evidence that the origin of the excess particles found in the corner may be traced to the upstream boundary layer. Indeed, the microscopic images revealed a near-wall particle excess prior to the expansion (see Fig. 13). The associated stream tube is contiguous with the corner region. Therefore rheological factors that influence the upstream boundary layer would also affect the concentration of particles in the corner region.

Previous numerical simulations of platelet deposition in sudden expansion have attempted to account for the presence of RBCs through the inclusion of a hematocrit-dependent diffusivity.^{23,24,36} Although it is difficult to compare the current results quantitatively with these simulations inasmuch as they report surface deposition density (platelet count/area)

rather than platelet concentration. Nevertheless, it is reasonable to assume that deposition density is proportional to deposition rate, which in turn is proportional to concentration, whereby relative comparisons can be made. The current experiments indicate an elevation of concentration (CE) of approximately 200% (for 40% Ht), and 50% (for 20% Ht). These ratios may be compared to the upstream vs. downstream deposition density measured by Karino and Goldsmith to be approximately 50% and computed by Jordan *et al.* to be approximately 200% irrespective of Ht. This may help explain the error encountered in these numerical models, but does not necessarily imply a solution.

These micro-flow visualization experiments entailed various assumptions and sources of error that could influence the results and associated conclusions. The high density and opacity of RBCs created challenges for visualizing the platelet-sized particles. The intensity of the particles was affected by both obstruction by RBCs and the depth with respect to the focal plane. This could have affected the automated counting of particles. This error was mitigated by comparing manual and automatically generated particle counts in five randomly selected images and iteratively adjusting image processing parameters to reduce error below 10%. Since manual counting is a subjective measure, it may itself have introduced a source of systematic error. The count may also be affected by overlapped particles or hidden particles. The image processing program is incapable of identifying two or more overlapping particles. A more sophisticated automatic counting method is being sought and will be used in the future to eliminate this error. The opacity of RBCs in the solution also limited the depth at which particles could be visualized, as indicated in Fig. 3. Consequently, the observations reported here are likely affected by the boundary layer beneath the coverslip, and may not be representative of the particle distribution at the centerline of the channel, where margination is likely to be more pronounced. This limitation could be addressed in a future study by using RBC ghost cells.

The shape of the platelet-sized particles could also have been a factor. If indeed lift and drag are responsible for relative motion of platelets, the use of flat particles to replace the currently used spherical particles would be advisable. An alternate choice to be considered in future experiments will be fluorescently labeled platelets.

Finally, the choice of sub-region size of the image processing, $20\ \mu\text{m} \cdot 20\ \mu\text{m}$, was selected to achieve a compromise between precision of particle count and spatial resolution. However, this relatively large resolution as compared to the $2\ \mu\text{m}$ size of a particle may have prevented observation of fine detail of the near-wall concentration profile.

In spite of the experimental limitations, these results accentuate the importance of explicitly distinguishing the platelet phase from the red cell phase of blood in a microscopic sudden expansion. This observation suggests that a multi-phase constitutive model is needed to accurately simulate platelet transport, and hence predict thrombosis in microscopic steps and crevices.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. Sachin Velankar for his help in design of the microchannel. This study was supported in part by grants from the Department of Defense, US Army through Pittsburgh Tissue Engineering Initiative.

REFERENCES

1. Aarts PA, Bolhuis PA, Sakariassen KS, Heethaar RM, and Sixma JJ Red blood cell size is important for adherence of blood platelets to artery subendothelium. *Blood* 62:214–217, 1983. [PubMed: 6860793]
2. Aarts PA, Heethaar RM, and Sixma JJ Red blood cell deformability influences platelets – vessel wall interaction in flowing blood. *Blood* 64:1228–1233, 1984. [PubMed: 6498337]
3. Aarts PA, van den Broek SA, Prins GW, Kuiken GD, Sixma JJ, and Heethaar RM Blood platelets are concentrated near the wall and red blood cells, in the center in flowing blood. *Arteriosclerosis* 8:819–824, 1988. [PubMed: 3196226]
4. Ahn T, Shin E, Merhi Y, Thai P, and Bilodeau L Influence of stent expansion states on platelet deposition in an extracorporeal porcine arteriovenous shunt model using a multichannel perfusion chamber. *J. Korean Med. Sci* 16:31–38, 2001. [PubMed: 11289398]
5. Alemu Y, and Bluestein D Flow-induced platelet activation and damage accumulation in a mechanical heart valve: numerical studies. *Artif. Organs* 31:677–688, 2007. [PubMed: 17725695]
6. Armaly BF, Durst F, Pereira JCF, and Schonung B Experimental and theoretical investigation of backward-facing step flow. *J. Fluid Mech* 172:473–496, 1983.
7. Arslan S, Gundogdu F, and Bozkurt E Images in cardiology. Permanent pacemaker lead thrombosis leading to recurrent pulmonary embolism. *Heart* 92:597, 2006. [PubMed: 16614270]
8. Bachmann C, Hugo G, Rosenberg G, Deutsch S, Fontaine A, and Tarbell JM Fluid dynamics of a pediatric ventricular assist device. *Artif. Organs* 24:362–372, 2000. [PubMed: 10848677]
9. Burgreen GW, Antaki JF, Wu J, Le Blanc P, and Butler KC A computational and experimental comparison of two outlet stators for the Nimbus LVAD. Left ventricular assist device. *ASAIO J* 45:328–333, 1999. [PubMed: 10445740]
10. Burgreen GW, Loree HM, II, Bourque K, Dague C, Poirier VL, Farrar D, Hampton E, Wu ZJ, Gemp TM, and Schob R Computational fluid dynamics analysis of a maglev centrifugal left ventricular assist device. *Artif. Organs* 28:874–880, 2004. [PubMed: 15384992]
11. Crewe KH, and Feuerstein IA Platelet adhesion to fibrinogen-coated glass at an abrupt tubular expansion viewed with fluorescent video-microscopy. *Biorheology* 23:443–452, 1986. [PubMed: 3651571]
12. David T, Thomas S, and Walker PG Platelet deposition in stagnation point flow: an analytical and computational simulation. *Med. Eng. Phys* 23:299–312, 2001. [PubMed: 11435144]
13. Denham MK, and Patrick MA Laminar flow over a downstream-facing step in a two-dimensional flow channel. *Trans. Inst. Chem. Eng* 52:361–367, 1974.
14. Dumont K, Vierendeels J, Kaminsky R, van Nooten G, Verdonck P, and Bluestein D Comparison of the hemodynamic and thrombogenic performance of two bileaflet mechanical heart valves using a CFD/FSI model. *J. Biomech. Eng* 129:558–565, 2007. [PubMed: 17655477]
15. Eckstein EC, and Belgacem F Model of platelet transport in flowing blood with drift and diffusion terms. *Biophys. J* 60:53–69, 1991. [PubMed: 1883945]
16. Eckstein EC, Tilles AW, and Millero FJ, III. Conditions for the occurrence of large near-wall excesses of small particles during blood flow. *Microvasc. Res* 36:31–39, 1988. [PubMed: 3185301]
17. Fabijanic D, Giunio L, Vujicic M, Vukovic I, Ermacora R, and Knezevic N Right atrial pacemaker lead thrombosis incidentally detected by transesophageal echocardiography. *Coll. Antropol* 29:159–161, 2005.
18. Ge L, Jones SC, Sotiropoulos F, Healy TM, and Yoganathan AP Numerical simulation of flow in mechanical heart valves: grid resolution and the assumption of flow symmetry. *J. Biomech. Eng* 125:709–718, 2003. [PubMed: 14618930]

19. Ge L, Leo HL, Sotiropoulos F, and Yoganathan AP Flow in a mechanical bileaflet heart valve at laminar and near-peak systole flow rates: CFD simulations and experiments. *J. Biomech. Eng* 127:782–797, 2005. [PubMed: 16248308]
20. Goldsmith HL The microrheology of human erythrocyte suspensions. In: *Theoretical and Applied Mechanics Proceedings of the 13th IUTAM Congress New York: Springer, 1972.*
21. Goldsmith HL, and Turitto VT Rheological aspects of thrombosis and haemostasis: basic principles and applications. *ICTH-Report – Subcommittee on Rheology of the International Committee on Thrombosis and Haemostasis. Thromb. Haemost* 55:415–435, 1986. [PubMed: 3750272]
22. Goldstein DJ Coagulation and thrombotic complications associated with ventricular assist devices. *Clin. Adv. Hematol. Oncol* 3:766–768, 2005. [PubMed: 16258485]
23. Goodman PD, Barlow ET, Crapo PM, Mohammad SF, and Solen KA Computational model of device-induced thrombosis and thromboembolism. *Ann. Biomed. Eng* 33:780–797, 2005. [PubMed: 16078618]
24. Jordan A, David T, Homer-Vanniasinkam S, Graham A, and Walker P The effects of margination and red cell augmented platelet diffusivity on platelet adhesion in complex flow. *Biorheology* 41:641–653, 2004. [PubMed: 15477670]
25. Karino T, and Goldsmith HL Flow behaviour of blood cells and rigid spheres in an annular vortex. *Philos. Trans. R. Soc. Lond. B. Biol. Sci* 279:413–445, 1977. [PubMed: 19795]
26. Karino T, and Goldsmith HL Aggregation of human platelets in an annular vortex distal to a tubular expansion. *Microvasc. Res* 17:217–237, 1979. [PubMed: 459937]
27. Karino T, and Goldsmith HL Adhesion of human platelets to collagen on the walls distal to a tubular expansion. *Microvasc. Res* 17:238–262, 1979. [PubMed: 459938]
28. LaDisa JF, Jr., Guler I, Olson LE, Hettrick DA, Kersten JR, Wartier DC, and Pagel PS Three-dimensional computational fluid dynamics modeling of alterations in coronary wall shear stress produced by stent implantation. *Ann. Biomed. Eng* 31:972–980, 2003. [PubMed: 12918912]
29. LaDisa JF, Jr., Olson LE, Guler I, Hettrick DA, Audi SH, Kersten JR, Wartier DC, and Pagel PS Stent design properties and deployment ratio influence indexes of wall shear stress: a three-dimensional computational fluid dynamics investigation within a normal artery. *J. Appl. Physiol* 97:424–430; discussion 416, 2004. [PubMed: 14766776]
30. Lassila R, Badimon JJ, Vallabhajosula S, and Badimon L Dynamic monitoring of platelet deposition on severely damaged vessel wall in flowing blood. Effects of different stenoses on thrombus growth. *Arteriosclerosis* 10:306–315, 1990. [PubMed: 2317165]
31. Leonard E Principles of cardiovascular design. *Cardiovasc. Pathol* 2:3s–10s, 1993.
32. Moraczewski T, Tang H, and Shapley NC Flow of a concentrated suspension through an abrupt axisymmetric expansion measured by nuclear magnetic resonance imaging. *J. Rheol* 49:1409–1428, 2005.
33. Rosamond W, Flegal K, Friday G, Furie K, Go A, Greenlund K, Haase N, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell CJ, Roger V, Rumsfeld J, Sorlie P, Steinberger J, Thom T, Wasserthiel-Smoller S, and Hong Y Heart disease and stroke statistics – 2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 115:e69–171, 2007. [PubMed: 17194875]
34. Sato M, and Ohshima N Hemodynamics at stenoses formed by growing platelet thrombi in mesenteric micro-vasculature of rat. *Microvasc. Res* 31:66–76, 1986. [PubMed: 3959916]
35. Sorensen EN, Burgreen GW, Wagner WR, and Antaki JF Computational simulation of platelet deposition and activation: I. Model development and properties. *Ann. Biomed. Eng* 27:436–448, 1999. [PubMed: 10468228]
36. Sorensen EN, Burgreen GW, Wagner WR, and Antaki JF Simulation of platelet deposition in disturbed flow. In: *Second Joint EMBS-BMES Conference, Houston, TX, 2002.*
37. Wootton DM, Markou CP, Hanson SR, and Ku DN A mechanistic model of acute platelet accumulation in thrombogenic stenoses. *Ann. Biomed. Eng* 29:321–329, 2001. [PubMed: 11339329]

38. Xu C, and Wootton DM Platelet near-wall excess in porcine whole blood in artery-sized tubes under steady and pulsatile flow conditions. *Biorheology* 41:113–125, 2004. [PubMed: 15090680]
39. Yamanaka H, Rosenberg G, Weiss WJ, Snyder AJ, Zapanta CM, and Siedlecki CA Multiscale analysis of surface thrombosis in vivo in a left ventricular assist system. *ASAIO J* 51:567–577, 2005. [PubMed: 16322720]
40. Yamanaka H, Rosenberg G, Weiss WJ, Snyder AJ, Zapanta CM, and Siedlecki CA Short-term in vivo studies of surface thrombosis in a left ventricular assist system. *ASAIO J* 52:257–265, 2006. [PubMed: 16760713]
41. Yin W, Alemu Y, Affeld K, Jesty J, and Bluestein D Flow-induced platelet activation in bileaflet and monoleaflet mechanical heart valves. *Ann. Biomed. Eng* 32:1058–1066, 2004. [PubMed: 15446502]
42. Zhao R, Kameneva MV, and Antaki JF Investigation of platelet margination phenomena at elevated shear stress. *Biorheology* 44:161–177, 2007. [PubMed: 17851165]

FIGURE 1.

The design challenge of prosthetic blood-wetted devices. (a) Section of rotary blood pump; (b) ring thrombus formed at the transition between metal barb and polymer cannula; (c) exaggerated magnified view of a blood-wetted seams; (d) well-known flow separation downstream of a backward step. Courtesy of Dr. Zhongjun Wu.

FIGURE 2.
Experimental setup for micro-flow visualization with laser illumination.

FIGURE 3.

Schematic of 100–200 μm microchannel expansion indicating the location of $50\ \mu\text{m} \times 50\ \mu\text{m}$ sub-regions of interest (A–D). The local coordinate system is also illustrated. The microchannel is 100 μm in height. The focal plane is approximately $5 \pm 1\ \mu\text{m}$ in depth from bottom surface of microchannel. The objective depth of field is 6 μm . The step width H is also indicated in the figure.

FIGURE 4.

Microscopic image of 2 μm particles interspersed with red blood cells within microchannel. (Filtered to eliminate red cells from image.) (a) Cropped raw image showing the sudden expansion; (b) processed image, partitioned into 20 $\mu\text{m} \times 20 \mu\text{m}$ square grid sub-regions to quantify the concentration distribution. Mask applied to isolate the interior of the channel.

FIGURE 5.

Particle concentration distribution in 1:2 sudden expansion. (a) Representative image of particle distribution, $Ht = 20\%$, $Q = 15 \text{ mL h}^{-1}$; (b) contour plot of concentration profile based on 100 frames, $Ht = 20\%$, $Q = 15 \text{ mL h}^{-1}$, 50×50 grids, spatial resolution: $4 \mu\text{m} \times 4 \mu\text{m}$; (c) representative image of particle distribution, $Ht = 40\%$, $Q = 15 \text{ mL h}^{-1}$; (d) contour plot of concentration profile indicating elevated concentration at the corner, $Ht = 40\%$, $Q = 15 \text{ mL h}^{-1}$, 50×50 grids, spatial resolution: $4 \mu\text{m} \times 4 \mu\text{m}$. Black dot represents the origin of the local coordinate system.

FIGURE 6.

Concentration elevation (CE) of platelet-sized particles in four sub-regions ($50 \mu\text{m} \times 50 \mu\text{m}$) of the micro-channel, shown in Fig. 3. Region A: expansion, Region B: recirculation zone, Regions C and D: downstream areas. Flow rate = 15 mL h^{-1} . Error bars indicate the 95% confidence interval, $n = 100\text{--}300$. Particle CE for 30% Ht sample is much greater than the one for 20% Ht sample in the corner region ($p < 0.05$).

FIGURE 7.

Concentration elevation profile along axial direction for the samples with Ht of 20% and 40%. CE shows a greater gradient for the higher Ht sample. (a) Near wall, $Q = 15 \text{ mL h}^{-1}$; (b) near wall, $Q = 6, 15, 30 \text{ mL h}^{-1}$; (c) centerline, comparing 20–40% Ht at $Q = 15 \text{ mL h}^{-1}$; (d) centerline, comparing effect of flow rate $Q = 6, 15, 30 \text{ mL h}^{-1}$ for 40% Ht. Values on x-axis indicate midpoint of the grid ($n = 300$). The near-wall region refers to a 20 micron layer adjacent to the lateral wall.

FIGURE 8.

Lateral concentration elevation profile from $x = -20 \mu\text{m}$ to $x = 140 \mu\text{m}$. $Q = 15 \text{ mL h}^{-1}$. (a) $H_t = 20\%$; (b) $H_t = 40\%$ ($n = 300$).

FIGURE 9.

RBC streamlines indicating cell-free layer (magnification = 20×). Concentration of RBCs indicated by opacity in image. (a) $H_t = 20\%$, $Q = 6 \text{ mL h}^{-1}$; (b) $H_t = 20\%$, $Q = 30 \text{ mL h}^{-1}$; (c) $H_t = 40\%$, $Q = 30 \text{ mL h}^{-1}$. Size of RBC free layer increases with flow rate. RBC streamlines detached from the wall at lower H_t and remained conformal at higher H_t ($n = 300$).

FIGURE 10.

RBCs in the corner of an expansion at high hematocrit ($Ht = 40\%$), magnification = $100\times$.

(a) $Q = 6 \text{ mL h}^{-1}$; (b) $Q = 30 \text{ mL h}^{-1}$. RBC free layer is not visible at $20\times$ but is observed at $100\times$ and reveals effect of flow rate.

FIGURE 11.

Normalized length of cell free layer for the samples with Ht of 5% and 20% under varied Re. The cell free layer increases with Re for both samples. Size of cell free layer is greater at 20% Ht vs. 5% Ht for all Re. The current result for Ht 5% sample agreed well with the previous experimental observation of Karino and Goldsmith²⁵ but greater than observations in single-phase fluids.^{6,13}

FIGURE 12.

Radial distribution of particle concentration and local hematocrit (represented by RBC RLA) in sudden expansion ($x = 20 \mu\text{m}$, half channel). CE denotes concentration elevation. $Q = 15 \text{ mL h}^{-1}$. (a) Ht 20% sample: depletion of RBCs in corner without increase of particle concentration. (b) Ht = 40% sample: elevation of particle concentration with uniform RBC concentration.

FIGURE 13.

Lateral concentration elevation profile inside the microchannel throat feeding the expansion ($x = -24 \mu\text{m}$, half channel, $Q = 15 \text{ mL h}^{-1}$). The near wall elevation of platelet-sized particles was found to be higher in the 40% Ht sample compared to the 20% Ht sample.