BLOOD CELL DIFFUSION VISUALIZATION USING MULTIPLE HYDRODYNAMIC FOCUSING

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ABSTRACT
This paper reports on the development of a microfluidic platform to study mass transport mechanisms of blood cells and fluid visualization of fluid streamlines on micro-contractions. Micro fabricated contractions can mimic the haemodynamics of pathological vessels to observe thrombi formation independent of chemical pathways1. Under these conditions thrombus formation is affected by a number of variables including blood cell stress history, cellular diffusion and cellular interaction. By using a multiple hydrodynamic flow focusing approach, these variables can be explored systematically. This platform will help to study the role of mass transport phenomena in platelet thrombosis at microscale.

KEYWORDS: flow focusing, flow sheaths, blood cell diffusion, thrombosis, mass transport.

INTRODUCTION
Hydrodynamic flow focusing is a technique that relies on the absence of turbulence and very low mixing rates present at the microscale consisting in confine more than one fluid stream within the same channel. Using this technique it is possible to achieve a number of defined streams into a microchannel. The applications of hydrodynamic flow focusing vary widely, for example it has been applied to selectively deposit protein into a channel, to measure the viscosity of fluids in a rheometer, or using different viscosity flows as a way to construct opto fluidic waveguides. In fluid mechanics, hydrodynamic flow focusing has been applied to micro-Particle Image Velocimetry (micro-PIV) as a selective seeding technique to improved the velocimetry measurements of the volume illumination set up. In all these applications, the fluid flow has been induced using positive pressure through a syringe pump, which implies that the samples must be loaded in the syringe. For flow assays using blood, minimizing the contact with glass or plastic materials and long dead volumes is essential, therefore it is preferable to use negative pressure since the blood sample can be loaded directly into a reservoir while fluid flow is induced by a syringe pump in refilling mode. In the present investigation, flow focusing is applied to investigate blood cell transport on micro-contractions to study platelet aggregation. Therefore none of the platforms described before can be implemented for our purposes to apply flow focusing on blood flow assays.

Platelet aggregation and thrombus development are initiated and regulated by the synergistic interactions of both blood borne chemical factors (ADP, TXA2 & thrombin) and mechanical factors (haemodynamics). While the roles of chemical factors have been elucidated in some detail, the impact of haemodynamics on platelet function is poorly understood. A number of studies have examined platelet functional responses under conditions of constant strain rate; however the effects of transient strain rate regimes that more closely models the conditions found in the vasculature has not been explored to any significant degree. Recent observations within our laboratories in mice and in microchannels2 demonstrate that localized changes in strain rate play a key role in initiating platelet aggregation independent of chemical factors. At sites of vessel stenosis flow may experience significant changes in velocity which can affect blood cell distribution, cell-cell interactions, and cell stress history3. In this study we present a microfluidic flow focusing platform incorporating a series of micro-contraction geometries that generate defined strain rate gradients in flowing blood, allowing the control of fluidic streams. We present the design, characterization and experimental results of a microfluidics platform incorporating a flow focusing approach to examine mass transport under defined shear gradient conditions. Due to the final target of the platform is to be used with blood, the flow focusing device design operates with negative pressure by using a single syringe to drive the flow (Fig. 1).

THEORY
In order to model the flow focusing effect present at microscale for different fluid streams, the mass transport equation for several species was solved numerically. For the current case, no chemical reaction is present and the only phenomena present for the chemical species is the transport of mass and momentum. The governing equation of the mass transport for different species can be expressed as:

\[
\vec{V} \cdot \nabla C = D \nabla^2 C
\]

where \(\vec{V}\) represents the velocity vector, \(C\) represents the concentration of species, \(D\) represents the diffusion constant.
The velocity field and its derivatives were calculated solving the mass conservation equation using FLUENT 6.0 (Fluent USA, Lebanon, NH) based on a finite volume scheme. A 3D approach was applied and for the solution a double precision segregated algorithm was used. The flow was modelled as laminar, steady and incompressible. Two chemical species are used as fluids, with a density of \( \rho = 998.2 \text{ [kg/m}^3\text{]} \), a viscosity of \( \mu = 0.00345 \text{ [Pa s]} \) and a diffusion constant was assumed as \( \text{D} = 1.0 \times 10^{-15} \text{ [m}^2\text{/s]} \). A mesh independence analysis was performed using a two dimensional model to investigate the mesh density needed across the channel.

**EXPERIMENTAL**

The flow focusing platform was composed of two chips: a first chip containing microcontractions to monitor platelet aggregation under the microscope and a second chip containing the reservoirs (Fig. 1.1) and a microfabricated valve which assists to remove bubbles and purge the system before running the experiment. Both chips were fabricated using polydimethylsiloxane (PDMS Sylgard) from a KMPR 1025 (MicroChem Corp.) mould patterned using standard photolithography techniques. The channels were characterized using water-based dyes. The fluids were loaded directly into the reservoirs and once the system was purged and all bubbles removed, the flow was driven by a syringe pump using the single outlet. Whole blood in the presence of the pharmacological inhibitors of aggregation (Amplification Loop Blockade [ALB]) was perfused into the channels and platelet response to dynamic stress was observed using fluorescence blood streams. The width and ratio of streams was set by the number of inlets available from the reservoir chip to the microcontraction chip.
RESULTS AND DISCUSSION

Figure 1.1 shows the fabricated flow focusing platform driven by a single syringe pump. Figure 1.2 shows the mesh independence analysis from the CFD model. A two-dimensional model was used and the number of nodes across the channel was increased as the mixing rate at a probe downstream was calculated (See Fig 1.3b). For the current investigation a minimum of 100 nodes are needed to solve spatially the mass fraction rate of a 100µm wide channel. Figure 1.3 shows the thickness of the fluid streams can be controlled by the number of inlets. Figure 1.3a shows the platform operating with two inlets, Figure 1.3b with four inlets (three uncoloured streams and one coloured stream), Figure 1.3c shows the platform operating with ten inlets (five uncoloured streams and five coloured stream). Figure 1.4 shows the flow focusing platform applied to visualize flow recirculation, for both, experiments with water-based dyes and numerical simulation. A flow separation and recirculation region it can be observed downstream the microcontraction at a Reynolds number of 7, showing good agreement between the experiments and the CFD. This result highlights the great capability of the platform as a tool for fluid visualization, since in order to observe the flow recirculation region a more sophisticated imaging and illumination setup is usually required (like a micro-PIV & image processing).

Once the platform was tested with water-based dyes, it was used to investigate blood cell transport on thrombosis. In our previous publication1 we demonstrated that shear micro gradients can generate a localized thrombi (Fig 2a). However since blood is a non-homogeneous fluid it is of interest to study cell transport in the microcontraction. For example, cell collision is likely to occur, altering the mass transport at the contraction and disrupting the laminar condition, as the size of the contraction (20µm) and the size of the blood cells (platelets 2µm, red cells 10µm) are on the same order of magnitude. On the same manner little is known about what region of the flow contributes to form the thrombi. Figure 2b shows a blood flow assay where a fluorescent blood stream at 25µm from the bottom wall flows into a microcontraction. Non-fluorescent blood was also infused into the contraction elsewhere. As it can be observed in Figure 2b, a Differential Interference Contrast microscopy image (DIC) shows that a thrombus is formed downstream the contraction, but none of the fluorescent cells contributed to form the thrombus. Figure 2c shows a similar experiment but the fluorescent blood stream is located at the top wall. As it can be observed in the DIC image, a platelet aggregate was formed downstream the contraction and a few fluorescent cells appeared into the aggregate. This investigation shows that most of the platelets forming the aggregate come from a region close to 25µm from the bottom wall, and due to cell collision there are some platelets than can actually translocate streamlines in the contraction.

CONCLUSION

The flow focusing platform presented allows an easy visualization of fluid streamlines using water based dyes which is useful to characterize micro flows at different Reynolds number. On the other hand it enable to perform blood flow experiments to study mass transport at the condition of shear microgradients. This platform will be used to perform characterization of non-homogeneous fluids to study streamlines translocation.

REFERENCES


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