VACUUM FLOW FOCUSING MICROFLUIDICS TO STUDY BLOOD CELL DYNAMICS UNDER SHEAR GRADIENT AGGREGATION MECHANISM

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ABSTRACT

This paper reports on the development of a microfluidic platform to study mass transport behaviour of blood cells at micro-scale stenosis where local strain-rate micro-gradients trigger platelet aggregation. We present the design, fabrication and blood testing of a microfluidic device that incorporates symmetric or asymmetric flow focusing using a single pressure driver to study the hemodynamic variables promoting platelet aggregation at defined micro-contraction geometries. The thickness of the different streams can be controlled by changing the hydraulic resistance of the inlet feeder channels and by controlling the hydrostatic pressure of the reservoirs. This platform will facilitate the study of the role of mass transport phenomena in platelet thrombus formation and will lead to a greater understanding of the mechanism(s) underlying shear-gradient dependent discoid platelet aggregation in the context of cardiovascular diseases such as acute coronary syndromes and ischemic stroke.

KEYWORDS: flow focusing, multilayer soft-lithography, platelet aggregation, advective transport, shear gradients.

INTRODUCTION

The emergence of micro-technologies and in particular microfluidics has enabled unprecedented control of the experimental conditions for studying the role of haemodynamics in platelet aggregation at the micro-scale[1]. Application of these new microfluidics approaches, in combination with micro-imaging techniques applied to platelet function analysis has begun to challenge aspects of the existing models describing the early events that drive platelet aggregation. Using these methods recent observations we have demonstrated that modification of local haemodynamics through stenosis can trigger platelet aggregation in the absence of soluble platelet agonist signaling (ADP, TXA2 & thrombin), through the shear micro-gradient driven aggregation of discoid platelets.[1, 2] Based on these findings we hypothesize that platelet aggregation in a stenosis may be affected by platelet stress-history and advective (mass) transport of platelets to thrombogenic surfaces. We have developed a microfluidics platform that enables the control of blood flow streams to study the role of platelet transport at micro-scale contractions and investigate blood cell behavior under conditions of complex flow through stenosis. Control of blood streams is achieved using a Hydrodynamic Flow Focusing (HFF) technique that relies on the absence of turbulence and very low mixing rates present at the micro-scale, to confine a number of defined streams within the same micro-channel.

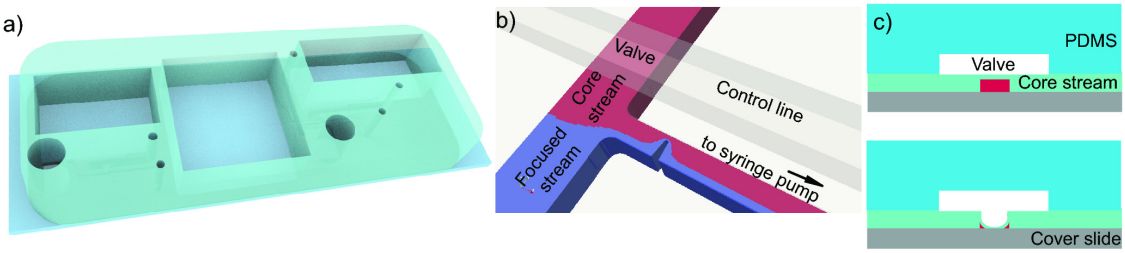

Figure 1: Design of the microfluidics device. a)3D representation of a PDMS device including HFF for two and three streams. b) Schematic of the implemented valve to assist the purging of the device. c) Schematic of the valve operation. Top: no pressure in the line. Bottom: pressure in the line, which blocks the core stream and allows to purge the focused stream. A partial closure is enough to operate the focused stream and remove any air bubbles.

HFF has been previously used in a number of applications, including; fluid mixing, measurement of hydraulic resistance, micro-Particle Image Velocimetry, particle focusing, and to decrease the effective sample volume required for lab on chip based applications. To date, methods employing HFF have relied on positive pressure driven delivery of the sample via syringe pump; a method that has a number of practical limitations in the context of blood handling and experimentation. In this manuscript we present a HFF platform that utilized a negative pressure driven (single pressure driver) blood delivery system. We present the HFF platform design, fabrication, blood testing to validate

stream generations and control. Application of the device to examine the dynamics of human whole blood demonstrates platelet transport to the within the stenosis expansion.

METHODS

Figure 1 presents the overall device design and layout. The microfluidics device was designed to allow an easy manipulation of blood minimizing contact with glass or plastic materials and long dead volumes (all of which can lead to pre-activation of blood cells and modification of blood samples) by using a reservoir where blood sample is simply loaded close to the micro-contraction. The micro-contraction of the device was designed to mimic the hemodynamics conditions of a stenosed artery on mice.[2, 3] Blood flow rate was induced by using negative pressure (suction) with a single syringe connected to the outlet port. Different thicknesses of the focused streams are achieved by changing the resistance of the inlet feeder channels and the hydrostatic pressure of the reservoirs. A manually operated pneumatic valve was incorporated into the chip using multi-layer soft lithography[4] to allow an easy purging of the fluid lines. Figure 2 presents the fabrication steps of the device.

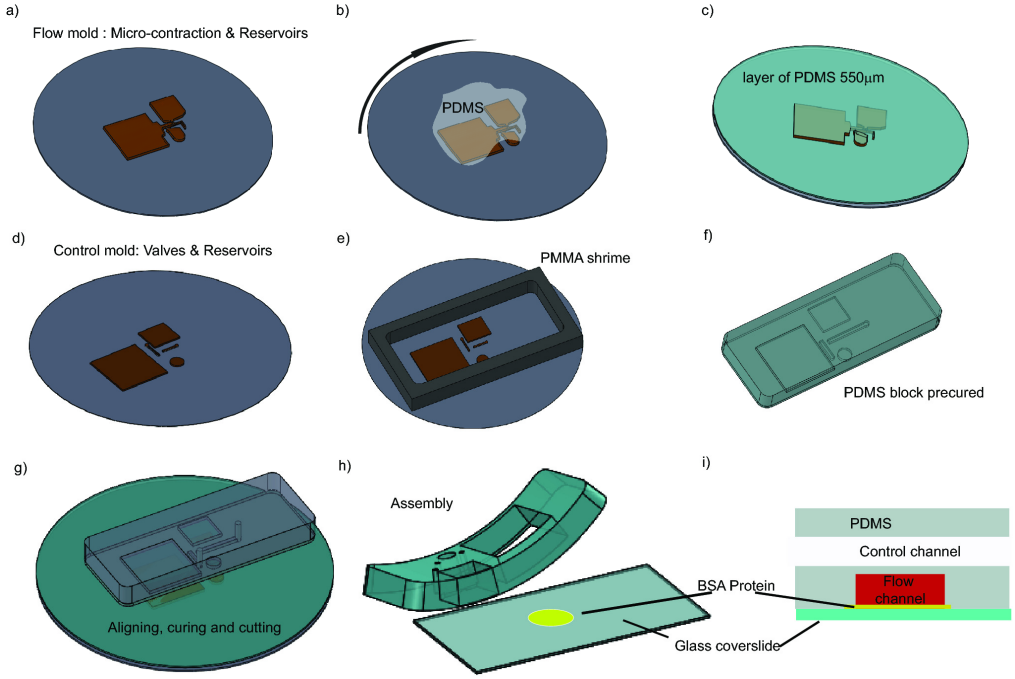

Figure 2. Fabrication of the device. The microfluidics device was fabricated using two molds photo-lithographically patterned. The first mold contained the flow layer and the second mold the micro-valves and reservoirs. a) The first mold was produced using a high resolution chrome mask to achieve well defined features and straight side walls. b-c) The first mold was spin coated with PDMS (Sylgard) to achieve a film of ~250μm thickness. d) The second mold was fabricated using a low resolution pdf mask. e) A Perspex (PMMA) enclosure of 6 mm of thickness was attached to the second mold to allow formation of a PDMS block of 6mm. f) PDMS was peeled off and the outlet ports of the micro-valves were fabricated. Simultaneously the PDMS layer on the first mold was partially cured g) The main PDMS block was aligned and both parts which were partially cured were sealed to each-other by completely curing them . h) A 60x22 mm borosilicate #1 microscope cover-glass was treated with BSA (Bovine Serum Albumin) in order to avoid specific adhesion of platelets to the cover-slide. i) Finally, the fabricated PDMS chip was surface adhered to the BSA treated microscope cover-glass which formed the bottom wall of the micro-channel and allowed for both transmitted light and epi-fluorescence imaging at the micro-contraction geometries.

RESULTS AND DISCUSSION

Proof of concept studies were carried out to demonstrate the utility of the HFF device to define the blood streams contributing to platelet aggregate formation under conditions of a well characterized strain rate micro-gradient[3]. In order to isolate the mechanical effects of blood flow from biochemically driven platelet activation, all experiments were performed in the presence of pharmacological inhibitors of the canonical platelet amplification loops[3]. Figure 3a-d show DIC (Differential interference contrast microscopy) and epi-fluorescent images of whole blood perfusion experiments over a 10 minute time-course in the symmetric iteration of the HFF device that produces two 75 μm /25μm asymmetrically focused streams, upstream contraction. Figures 3a-b show that platelet aggregation is formed within this device where DiOC6 labeled blood was confined to the bottom 75 μm stream and strong fluorescent platelet incorporation into the developing aggregate was found. In reciprocal experiments where DiOC6 labeled blood was confined to the top 25 μm stream, significantly, no fluorescence incorporation into the developing platelet aggregate was observed for this case (Fig. 5d). This data suggests that only platelets within streamlines biased to the micro-contraction

geometry side of the micro-channel take part in platelet aggregate formation. Figure 3c-d correspond to the reciprocal experiments to a-b, and demonstrate that platelet within the top 75% of streamlines make no significant contribution to aggregate formation. It should be noted however that after 3 minutes of perfusion some level of fluorescent platelet adhesion was observed, with platelets forming a thin mono-layer immediately downstream of the micro-contraction geometry due to the generation of a flow recirculation region adjacent to the platelet aggregate that develops as a function of aggregate size. Some degree of recirculation and/or disturbed flow was evident in the DIC imaging experiments (data not shown).

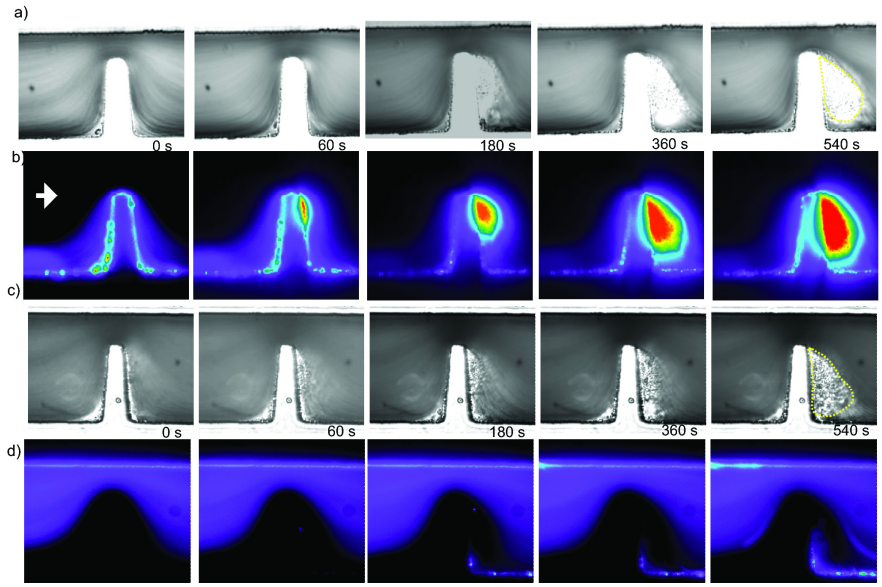

Figure 3: Application of asymmetric HFF to platelet aggregation at micro-scale stenosis. a) DIC images of blood perfusion experiments through 10' of monitoring on a device that produces two non-symmetrically focused streams (75 μm and 25 μm). The blood stream at the bottom wall was stamped with a fluorescent dye. An aggregate formed downstream the contraction using two streams can be observed. b) Epi-fluorescent images of the same experiment as (a)), showing that an aggregate was formed downstream the contraction. The white arrow indicates the flow direction. c) DIC images of the same experiment as a) but the fluorescent stream was located at the top wall. d) Epi-fluorescent image of the same experiment as (c)), showing that no aggregate was formed downstream the contraction, implying that most of the platelets that contribute to the aggregate come from a region of 25 μm upstream contraction.

CONCLUSION

The platform presented allowed us to explore mass transport phenomena contributing to aggregate formation under shear micro-gradient conditions. This device offers a number of advantages for the investigation of hemodynamic and blood cell behavior, including: i. The ability to controllably constrain multiple streamlines with a single syringe pump; ii. The ability to monitor streamline dynamics and blood cells behavior in real-time using epifluorescence and transmitted light micro-imaging approaches and; iii. The flexibility to introduce multiple sample combinations (plasma, dyes, particles or blood) in the same chip using a series of sample reservoirs by purging the device just once at the start of the experiments. Overall the HFF platform presented represents a valuable tool in our efforts to further understand the fluid dynamic variables at play in promoting shear micro-gradient platelet aggregation.

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