RAPID SEPARATION AND CAPTURE OF PLATELETS FROM WHOLE BLOOD

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ABSTRACT

We have designed and fabricated a physiologically inspired microfluidic device to capture and separate platelets from whole blood using selective adhesion molecules. It is comprised of a protein-patterned surface on glass, assembled with a PDMS channel. The dimensions of the protein patterns enable control of the number of platelets captured per spot; for the first time, ordered arrays of captured single or multiple platelets are easily created. A range of proteins can be patterned on the surface to control captured platelet activation and morphology, including antibodies that minimize capture-induced activation.

KEYWORDS: Single platelet, platelet adhesion, protein patterning, microcontact printing, PDMS microfluidics.

INTRODUCTION

Circulating platelets play a critical role in normal hemostasis: they aggregate at sites of vascular injury to initiate thrombus formation. However, platelets also play a central role in the pathogenesis of arterial thrombosis, which accounts for the clinical events associated with cardiovascular disease. The study of platelet adhesion to protein matrices such as von Willebrand factor, collagen, and Fibrinogen is crucial to understanding platelet function and regulation. Standard platelet adhesion assays require relatively large volumes of blood and variable methods of preparation that often induce platelet activation, precluding studies under physiological conditions. Microfluidic devices are now opening new possibilities for cell handling and analysis, allowing the study of single cells. Several designs have enabled on-chip continuous blood-cell separations by applying, for example, acoustic forces [1], biomimetic adhesion-protein-based collection and separation of cells [2], stream bifurcation [3], or deterministic lateral displacement [4]. These devices focus mainly on the separation of red and white blood cells, while the isolation of platelets has rarely been addressed. Furthermore, none of the above-mentioned methods isolates platelets in an inactivated state after the separation is complete.

We introduce here a physiologically inspired microfluidic device for integrated high-efficiency separation, capture, and study of platelets from whole blood. It enables the study under near-physiological, minimally perturbed conditions of (1)

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platelet adhesion to a variety of protein matrices; (2) the behavior of single or multiple immobilized platelets.

EXPERIMENTAL DETAILS

The whole-blood platelet capture device comprises selective protein patterns on a glass substrate mated to a polydimethylsiloxane (PDMS) microfluidic cover (Fig. 1). Microcontact printing was used to pattern specific platelet-adhesive proteins or antibodies onto the glass surface. After protein patterning the rest of the glass substrate is blocked with bovine serum albumin (BSA).

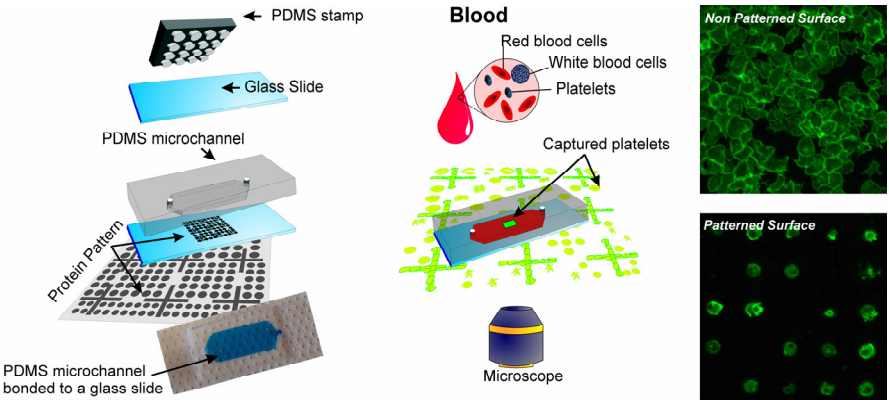

Figure 1. Fabrication of the microfluidic system for separation of platelets from whole blood via protein patterning on glass using microcontact printing and assembly of a PDMS microchannel-containing cover. A photograph of the device is shown at bottom left. On the right fluorescence images of platelets adhering to a non patterned and a patterned Fibrinogen surface.

RESULTS AND DISCUSSION

Exposure of the protein-patterned surface to whole blood results in the specific capture of platelets only on the spots covered by the adhesive protein Fibrinogen (Fig. 1). There is no significant capture of other blood components or cell types. Fluorescence microscopy images of platelets captured from whole blood on unpatterned and patterned Fibrinogen surfaces, which are compared in Figure 1, show that the unpatterned surface provides no means to control the distance and interactions between adhering platelets: they accumulate 'at random.' In sharp contrast, patterned protein shapes and sizes facilitate the control of the number of platelets captured per spot, strongly influencing the distance between them (Fig. 2). A key result is that the level of activation, morphology, and spreading of platelets differ according to the adhesive capture protein (Fig. 2): platelets adhering to Fibrinogen spread in a manner consistent with a highly activated state, but those adhering to CD42b antibodies are more similar to the non-activated state typical of physiological conditions. Additionally platelet adhesion rate on the patterned surface is highly dependent of incubation time, the protein present on the surface and the size of the protein spot (Fig. 3)

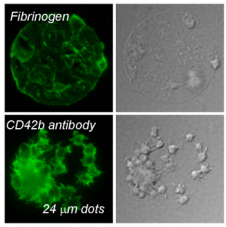

Figure 2. *Fluorescence images of captured platelets on 24 μm dots of Fibrinogen and CD42b antibody. The level of activation, morphology, and spreading of the platelets differs significantly between the two matrices*

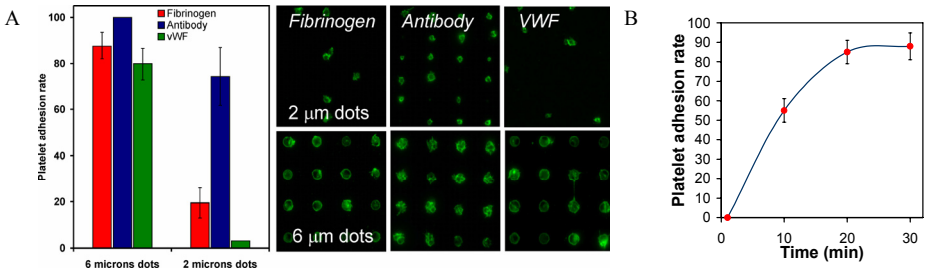

Figure 3. A)Platelet adhesion rate (percentage of protein spots occupied) on a Fibrinogen and CD42b antibody patterned surface containing arrays of 2 and 6 microns dots. B)Time dependency of the platelet adhesion rate from whole blood on a Fibrinogen patterned surface.

CONCLUSIONS

The capture of single platelets from whole blood onto adhesive protein and antibody-coated surfaces has been achieved for the first time using a microfluidic device comprised of a patterned protein surface on glass, assembled to a PDMS-based microchannel. This enables the study of platelet-platelet interactions at controlled surface density, as well as the study of single platelets on a range of adherent surfaces.

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REFERENCES

[1] F. Petersson, L. Aberg, A. M. Sward-Nilsson, T. Laurell, Analytical Chemistry, 2007, 79, 5117.
[2] Wesley C. Chang, Luke P. Lee and Dorian Liepmann, Lab on a Chip, 2005, 5 (1), 64-73.
[3] S. Yang, A. Undar, J. D. Zahn, Lab on a Chip 2006, 6, 871.
[4] N. Li, D. T. Kamei, C. M. Ho, 2007 2nd IEEE International Conference on Nano/Micro Engineered and Molecular Systems, Vols 1-3 2007, 692.