DISPOSABLE BIOANALYTICAL MICRODEVICE FOR MONITORING THE EFFECT OF ANTI-PLATELET DRUGS

L. Basabe-Desmonts[1,2], S. Ramstrom[2], A. Lopez-Alonso[2], M. Somers[1], A.J. Ricco[1], D. Kenny [2]

[1] *Biomedical Diagnostics Institute (BDI), Dublin City University, Dublin, IRELAND*

[2] *Biomedical Diagnostics Institute Programme, Molecular & Cellular Therapeutics, Royal College of Surgeons in Ireland (RCSI), Dublin, IRELAND*

ABSTRACT

We report a disposable self-powered integrated microfluidic chip that enables a rapid and simple platelet-function assay from small samples of whole blood. The chip integrates a single-cell adhesion assay with a microfluidic platform; it enables accurate quantification of platelet adhesion, and it controls whole blood flow rate, shear stress, volume of sample, and assay time.

KEYWORDS: Cardiovascular, whole blood, platelets, self-powered microfluidics, disposable, point of care.

INTRODUCTION

Cardiovascular disease is the major cause of morbidity and mortality globally. Antiplatelet drugs reduce cardiovascular events by decreasing the risk of thrombosis but they also increase the risk of bleeding. There is a compelling need to develop diagnostic assays of platelet function that could be used at the point of care and influence clinical management to avoid adverse events. Most common assays to evaluate anti-platelet drug effects, including aggregometry, bleeding time, and flow cytometry, are time consuming and require specialized personnel and equipment; available point-of-care systems require large sample volumes and/or complex cartridges or electronics [1].

Platelet adhesion is the primary function of platelets. We have recently reported a simple method to study platelet adhesion using interfacial platelet cytometry (iPC) [2]. iPC is a new tool to measure platelet function under near-physiological conditions; iPC platform technology enables the single-step separation of platelets from whole blood by capturing platelets on a transparent substrate patterned with dots of platelet-specific proteins such as fibrinogen, von Willebrand factor (VWF), and collagen (Figure 1). In order to produce a disposable device suitable for point-of-care platelet function testing, we now report the integration of the iPC platform with a self-powered microfluidic device.

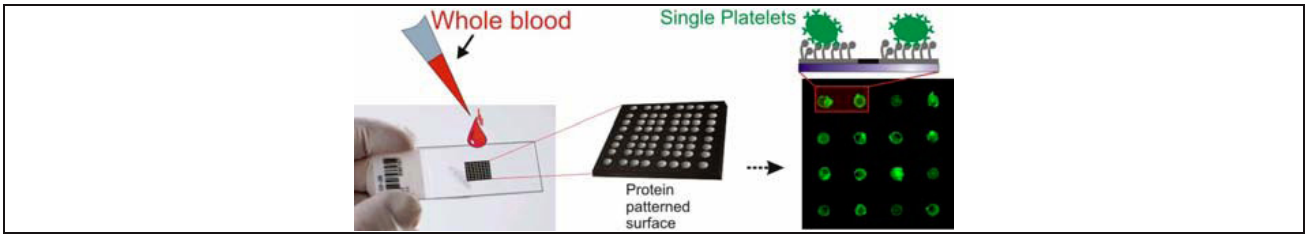

Figure 1: Schematic depiction of the incubation of whole blood on an iPC substrate patterned with a platelet-specific protein. Single platelets from whole blood adhere to the protein spots in a single step; the remainder of the blood is then rinsed away and the platelets are fluorescently labeled. Arrays of single platelets are fabricated on disposable transparent substrates, enabling the direct observation of isolated single platelets and a straightforward and accurate method for quantifying platelet adhesion

EXPERIMENTAL

Patterned protein dots on the iPC device surface are designed to control platelet-platelet contacts and to facilitate the counting of adhered platelets. By varying the size of the dots, the number of platelets (one or more) to be captured on each protein dot is controlled. An iPC designed to create single-platelet arrays (each protein dot sized to hold a single platelet) provides a straightforward method to quantify adhesion (occupied dot) and non-adhesion (non-occupied dot) events, the percentage of occupied dots assaying platelet adhesion properties (Figure 2).

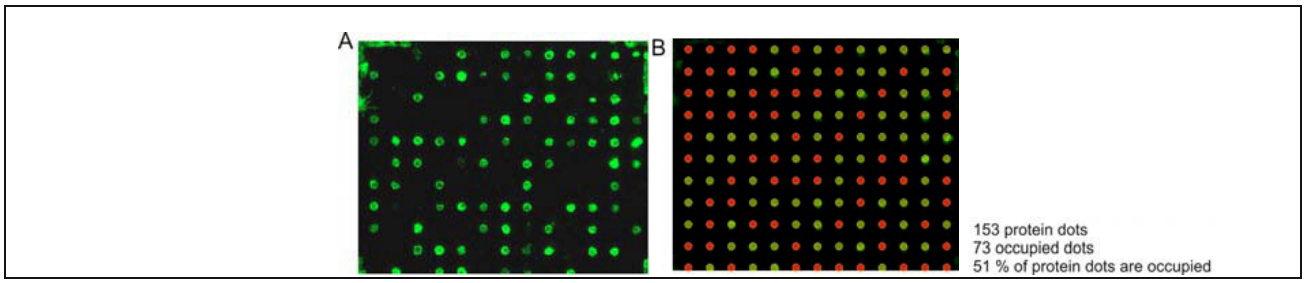

Figure 2: Quantification of platelet adhesion. A) Fluorescence microscopy image of platelets adhering to a 6-μm dot-size fibrinogen array. Platelets are stained using fluorescently labeled antibodies. B) Overlay of protein dot array and image of platelets adhering to the fibrinogen surface. Empty fibrinogen dots are colored red while occupied dots are colored green. Percentage of occupied dots indicates platelet adhesion level.

The microfluidic device was comprised of a glass bottom plate supporting the protein pattern and a PDMS slab containing the microchannels (Figure 3). A power-free pumping method was used to enable whole-blood flow through the chip over the patterned protein surface [3]. To create the pumping force, the PDMS slab was degassed under vacuum. Re-absorption of air by the degassed bulk PDMS when exposed to atmospheric pressure drives fluid flow into the chip.

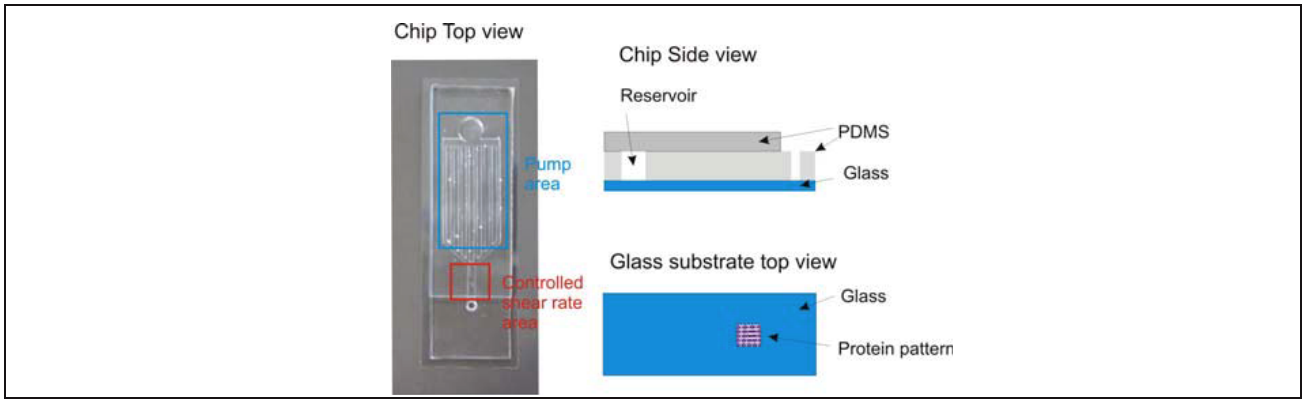

Figure 3: Picture and cartoons showing details of the self-powered microfluidic platform for quantification of platelet function.

RESULTS AND DISCUSSION

Various anti-platelet drugs now target GPIIb/IIIa, the fibrinogen receptor in platelets. GPIIb/IIIa antagonists, such as the drug abciximab, are routinely used to prevent thrombotic ischemic cardiovascular complications during percutaneous coronary interventions. The GPIIb/IIIa receptor mediates platelet adhesion to immobilized fibrinogen on surfaces. We have demonstrated the use of the iPC platform to assess the inhibition of platelet function by abxicimab. Quantification of platelet adhesion (by automated counting) on a fibrinogen iPC correlates well with the inhibition of platelet function by the drug (Figure 4).

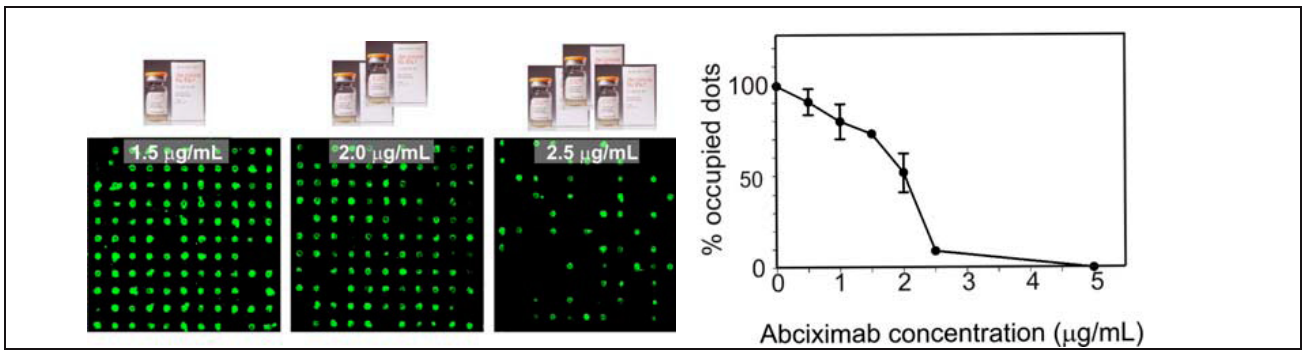

*Figure 4: **Left**, pictures showing a decrease in platelet adhesion quantified on a fibrinogen iPC as a consequence of the blockage of the platelets' fibrinogen receptor by the drug abciximab. **Right**, drug dose response curve measured on the fibrinogen iPC.*

Platelet adhesion assays require control of multiple parameters: flow rate, shear force at the protein surface (to which platelets are sensitive), volume of sample assayed, and assay time. All these parameters were controlled by the design of

the chip. Specifically, the cross sectional area provided shear control; the height-to-width aspect ratio and the volumetric capacity of the chip enabled the flow rate control. Increasing the volumetric capacity increased the flow rate (Figure 5).

The re-absorption of gas and vapor into the previously degassed chip drove the flow of blood into the device upon (single-step) sample loading. Platelet adhesion assays were performed by allowing blood to flow over the protein-patterned area on the bottom plate of the device during a defined period of time; subsequently, the surface cleared of all fluid by the removal of the blood, which continued to flow towards the ends of the channels, enabling optical readout without sample interference.

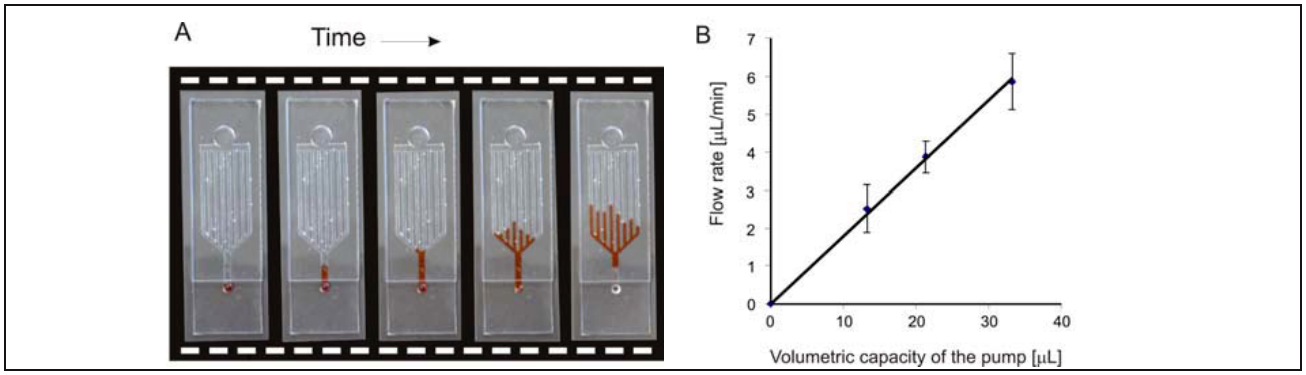

Figure 5: A) Photos during degas-driven flow in the chip. B) Graphical representation of the flow rate dependency upon the volumetric capacity of the pump.

CONCLUSIONS

We have developed a self-powered microfluidic device that enables control of flow rate, shear rate, and assay time. The current microfluidic platform has volume capacity exceeding 50 µL; the volume is controllable by changing the microfluidic channel network design. The device enables assay time control by adjusting sample volume and flow rate. Platelet adhesion assays based on the iPC assay approach are easily integrated with this low-cost disposable device. Platelet function assays can be performed by allowing blood to flow over the protein-patterned area on the bottom plate of the device over a defined time period; subsequently, the surface clears of all blood by its continued flow through the channel network, enabling optical readout of adhered platelets without sample interference. These results pave the way for the development of new disposable platelet-function assays for point-of-care testing, which we are confident will influence clinical decision making and lead to the development of personalized therapies.

ACKNOWLEDGEMENTS

The material in this article is based upon works supported by the Science Foundation Ireland under grant no. 05/CE3/B754.

REFERENCES

[1] A.D. Michelson, "Antiplatelet Therapies for the Treatment of Cardiovascular Disease," *Nature Reviews Drug Discovery*, vol. 9**,** pp. 154-169, 2010.

[2] L. Basabe-Desmonts, S. Ramstrom, G. Meade, S. O'Neill, A. Riaz, L.P. Lee, A.J. Ricco, and D. Kenny, "Single-Step Separation of Platelets from Whole Blood Coupled with Digital Quantification by Interfacial Platelet Cytometry (iPC)," *Langmuir*, vol. 26, DOI: 10.1021/la9039682, 2010.

[3] T. Ito, A. Inoue, K. Sato, K. Hosokawa, and M. Maeda, "Autonomous Polymer Loading and Sample Injection for Microchip Electrophoresis," *Anal. Chem.*, vol. 77, pp. 4759-4764, 2005.

CONTACT

*L. Basabe-Desmonts, tel: +353 (0)1700-6443; Lourdes.b.desmonts@dcu.ie