MICROFLUIDIC PLATELET ANALYSIS PLATFORM BASED ON IMPEDANCE SPECTROSCOPY

Mikael Evander[1, 2], Antonio J. Ricco[2], John Morser[3, 4], Gregory T. A. Kovacs[2, 3], Lawrence L.K. Leung[3, 4], Laurent Giovangrandi[2].

[1] Dept. of Measurement Technology and Industrial Electrical Engineering, Lund University, Sweden
[2] Dept. of Electrical Engineering, Stanford University, USA
[3] Dept. of Medicine, Stanford University School of Medicine, USA
[4] Veterans Affairs Palo Alto Health Care System, Palo Alto, USA

ABSTRACT

We present a microfluidic platform for platelet analysis based on multi-frequency electrical impedance spectroscopy (EIS). Two-phase dielectric focusing is used to enable sensitive detection while maintaining low shear forces to avoid platelet activation. Discrimination between erythrocytes and platelets was achieved by measuring the in-phase amplitude at a single frequency. Discrimination between resting and activated platelets was demonstrated using multi-frequency impedance measurement in combination with discriminant analysis. The percentage of activated platelets before and after a passage through the chip was measured using flow cytometry analysis and showed that no significant activation of the platelets occurred in the chip. This technique has the potential to be developed into a point-of-care assay for platelet monitoring requiring minimal preparation of whole blood samples.

KEYWORDS

Platelets, impedance spectroscopy, two-phase flow.

INTRODUCTION

A higher-than-normal number of activated platelets in the blood stream can be a sign of several serious diseases as well as the result of medical procedures that process the blood outside of the body, such as dialysis. There is therefore an interest in developing an analysis platform that is able to assess the platelet activation state in real time.

Since shear stresses or contact with negatively charges surfaces, such as glass, can alter the activation state of platelets, a gentle analysis method is needed that preferably is label-free and capable of differentiating between different cell types and platelet activation states. Electrochemical impedance spectroscopy meets these criteria and has been demonstrated for single cell experiments as well as differentiation of different cell types. Impedance spectroscopy is, however, typically used with small channel dimensions to maximize the impedance signal for single cells. Due to the high shear stress in these channels, this approach is, however, not suitable for platelet studies. Through the use of two-phase, dielectric focusing, high signal-to-background signals can be achieved while maintaining the low shear forces of a larger channel.

In this paper, we present a platelet analysis platform based on impedance spectroscopy that combined with two-phase dielectric focusing enables monitoring of platelet activation states in a flow-through format.

EXPERIMENT

The microfluidic chip consisted of two glass wafers with a 50-µm thick, 350-µm wide fluidic channel fabricated from a double-sided pressure-sensitive adhesive (Adhesives Research, Glen Rock, PA). Dielectrophoresis and impedance measurements were performed using 20-µm wide platinum electrodes (Figures 1 and 2). The detection sensitivity of a narrow fluidic channel was achieved without the associated high shear forces via two-phase dielectric focusing [1, 2]. The ionically conductive platelet suspension was infused through the sample/center inlet while non-conductive mineral oil was infused through two side inlets, resulting in a conductive center core 30 µm in width.

The platelets were centered in the channel using negative dielectrophoresis (930 kHz, 5 V_{rms}) to avoid activation from either glass surface contact or high shear forces close to the channel walls. Platelet suspensions were prepared from intravenously collected blood from healthy volunteers. After centrifugation and washing, platelets were resuspended before activating one aliquot with 20 µM thrombin receptor-activating peptide (TRAP); a second aliquot was not intentionally activated ("resting" platelets). The number of activated platelets in each sample was measured using activation-specific labeling and flow cytometry; note that our EIS approach requires no such labeling step. A resting platelet sample was also analyzed before and after passage through the chip to show that this did not alter their activation level.

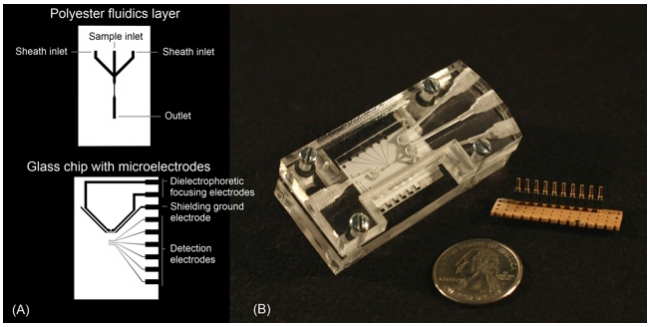

Figure 1. (A) fluidic design of laser-ablated polyester film and electrode layout on glass chips. (B) photograph of device in an acrylic fixture (US quarter dollar for scale). Spring-loaded headers connect the amplifier to the chip.

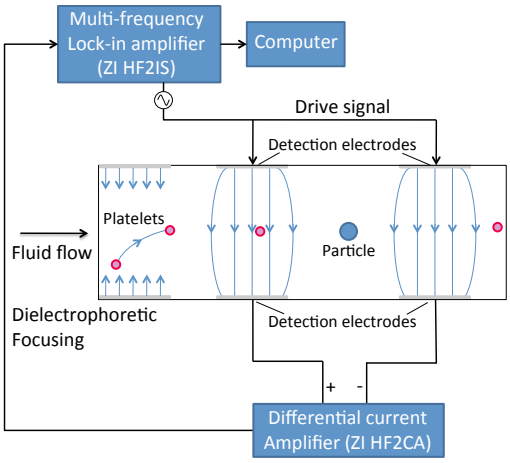

Figure 2. Block diagram showing dielectrophoresis and detection electrodes and instrumentation for EIS measurements.

Impedance measurements were performed using a Zürich Instrument HF2IS impedance spectroscope together with an HF2CA current amplifier at four simultaneous frequencies (284 kHz, 1.20 MHz, 2.39 MHz and 4.02 MHz, each with a 1 V_{rms} amplitude). The in-phase and out-of-phase components were acquired and analyzed in MATLAB®. Wavelet-based algorithms were used to detect particles and platelets and extract peak amplitudes. From the extracted in-phase and out-of-phase peak amplitudes, the magnitude, opacity and *tan(ϕ)* (out-of-phase amplitude divided by in-phase amplitude) were calculated for each frequency. All parameters were further analyzed using multivariate discriminant analysis with a statistical software package (JMP®, SAS, Cary, NC) to differentiate and classify the platelets.

RESULTS

By analyzing the in-phase amplitude at a single frequency, 5 and 10 µm microparticles could be discriminated from each other as well as erythrocytes and platelets (Figure 3).

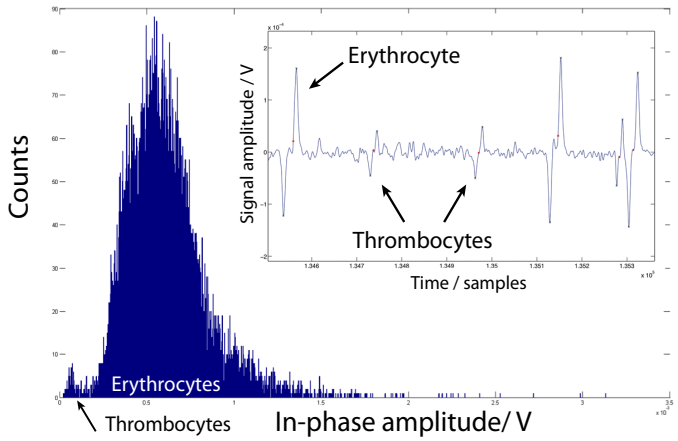

Figure 3. Histogram of in-phase amplitudes for a mixed sample of erythrocytes and platelets. The inset shows the impedance peaks for platelets and erythrocytes.

To discriminate between resting and TRAP-treated (activated) platelets, multiple frequencies were used in combination with discriminant analysis. Figure 4 shows a classification scatter plot as obtained by the discriminant analysis where a clear separation between resting and activated platelets can be seen.

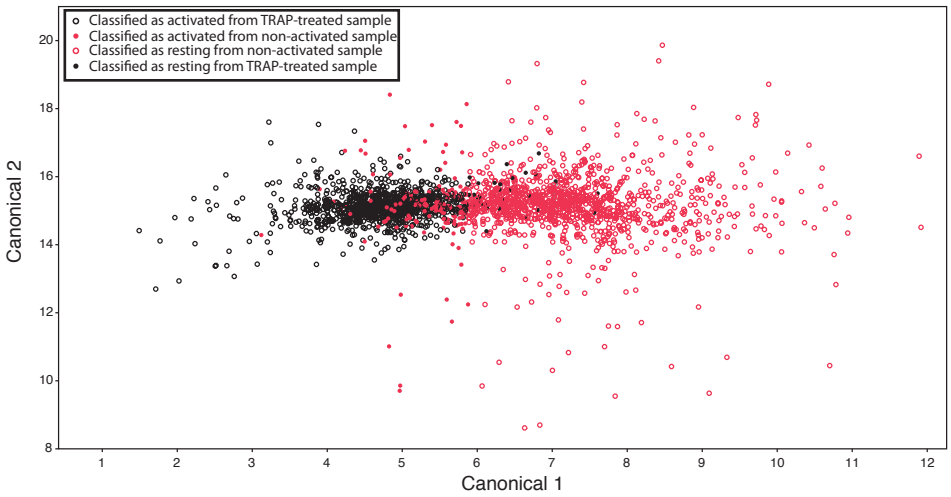

Figure 4. Scatter plot from discriminant analysis showing classification of platelets in resting and TRAP-treated samples.

The percentage of activated platelets in the resting and activated samples from EIS classification and flow cytometry analysis shows good correlation with the sample type (Figure 5). No significant change was measured in the percentage of activated platelets in a resting sample before and after passage through the chip (6.1% vs. 5.9%, respectively).

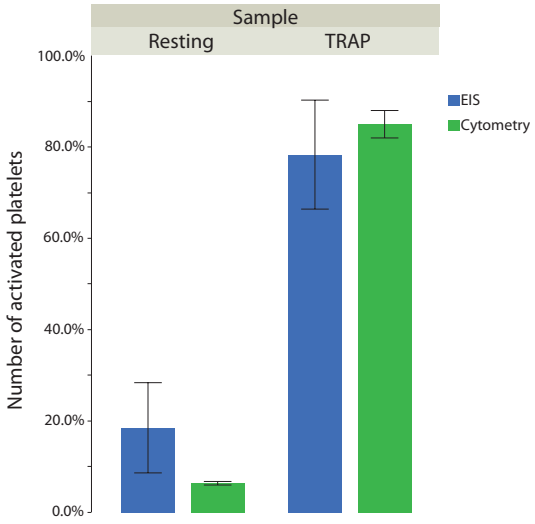

Figure 5. Percentage of activated platelets in resting and TRAP-treated samples as measured by electrical impedance spectroscopy and flow cytometry. Error bars correspond to ± 1 standard deviation for averages of 12 measurements for EIS data and two measurements for flow cytometry data.

CONCLUSIONS

The results presented here demonstrate a successful classification of the activation state of platelets using EIS and provide a critical proof of concept for a point-of-care assay free of the labeling and other complexities of conventional optical flow cytometry instrumentation.

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CONTACT

Mikael Evander, mikael.evander@elmat.lth.se