Microfluidic system for a label-free, real-time functional assessment of thrombotic risk

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Abstract—**High incidence of thrombotic diseases worldwide, together with the variability of patients' response to antiplatelet drugs, makes the management of antithrombotic regimes of paramount importance. Platelet function testing is the most promising tool in addressing this clinical need. Here we present the first prototype of a microfluidic system for rapid, label-free, real-time functional assessment of the thrombotic risk of patients undergoing antiplatelet treatment. Our platform allows to monitor pressure drop variations on a collagen-coated microchannel under a range of blood flow conditions, and to relate these measurements to thrombus formation and ultimately to platelet functionality. The preliminary testing campaign presented in this work demonstrated the feasibility of our approach and allowed us to determine the most suitable working range of the current system.**

Keywords—**Thrombotic risk, label-free, real-time monitoring, microfluidics.**

I. Introduction

ISCHEMIC cardio- and cerebrovascular events are the leading cause of morbidity and mortality in high-income countries, mainly caused by thrombotic or thromboembolic events. Platelets represent the main target for therapeutic treatments of thrombosis via antiplatelet medications. Yet, patients' responses to antiplatelet therapy significantly vary, with individuals showing high residual platelet activity, with higher susceptibility to thrombotic events [1]. To personalize thrombosis management and possibly predict pathological events, platelet function testing (PFT) has been suggested as a promising tool able to monitor the haemostatic impact of antiplatelet agents in real-time [2]. Most of the commercially available systems, though, are lab-based cumbersome instruments which hardly replicate physiological flow conditions [3]. Moreover, they usually allow a minimum degree of freedom, offering pre-set cuvettes and testing conditions.

To address this clinical need, we aim at developing a label-free microfluidic platform for identification of patients at risk of thrombosis. The platform performs experiments under flow conditions and can span a wide range of shear stresses. Unlike traditional immunoassays which require chemicals/antibodies, our label-free approach is low cost and more scalable for large scale patient screening, potentially becoming a point-of-care (POC) system. It also satisfies other critical requirements such as the use of a small blood volume, low-to-none blood handling and real-time analysis.

The present work presents the results obtained with the preliminary tests.

II. Materials and Methods

A. Blood sampling

The recruited healthy subjects (N=14) abstained from drugs known to affect platelet function for at least 10 days before enrolment. The study was carried out at ASST Santi Paolo e Carlo and approved by the institutional ethical committee; all subjects signed their informed consent. Blood samples were collected in the morning from an antecubital vein using a 21-gauge butterfly needle with minimal stasis. The first 3 mL of blood were collected into K-EDTA tubes (Becton Dickinson vacutainer, Australia) for a complete blood count; the following 10–15 mL were collected in 250 µg/mL INN-desirudin (Canyon Pharmaceuticals, United Kingdom), gently mixed, and allowed to rest at room temperature for 15 min before use. Platelets were marked with the green fluorescent dye DiOC6 (1 µM; ThermoFisher Scientific, Italy) for 15 min at room temperature.

B. Microfluidic device and experimental setup

Microfluidic devices featured six independent channels (1000 µm wide, 100 µm high and 3 cm long). The devices were fabricated in polydimethylsiloxane (PDMS, Sylgard™ 184, Dow Corning, USA) from silicon masters using standard soft lithography techniques (10:1 PDMS to curing agent ratio). Inlet and outlet fluidic ports were punched with a 1.5 mm diameter biopsy puncher. The PDMS layer was plasma-bonded to a glass coverslip (Figure 1, above).

Microfluidic channels were incubated at 4°C overnight with Horm fibrillar collagen type I (Mascia Brunelli, Italy) at two concentrations (10 and 100 ug/ml) [4], passivated with bovine serum albumin and finally rinsed with phosphate buffered saline (PBS).

The channels were connected to tubing for pumping system (Cellix, Ireland) and blood was perfused at two different shear rates, 950 and 1600 s^{-1}, simulating an arterial flow and a slightly pathological shear rate.

At this preliminary stage, we wanted to validate pressure measurements against the results obtained by image acquiring and processing using a platform from our previous works [3]. To this aim, all the experiments were performed under a fluorescence microscope (Axiovert A1 FL, Zeiss, Italy) equipped with a 16-bit camera (Crisel Instruments, Italy) (Figure 1, below). Images of the first portion of the collagen coating were acquired at 40x magnification every minute up to 4 min during blood perfusion through the microchannel. Mean and maximum fluorescence intensity (FI), together with

further parameters, were calculated, being FI an indicator of 3-dimensional thrombus growth in the channel lumen.

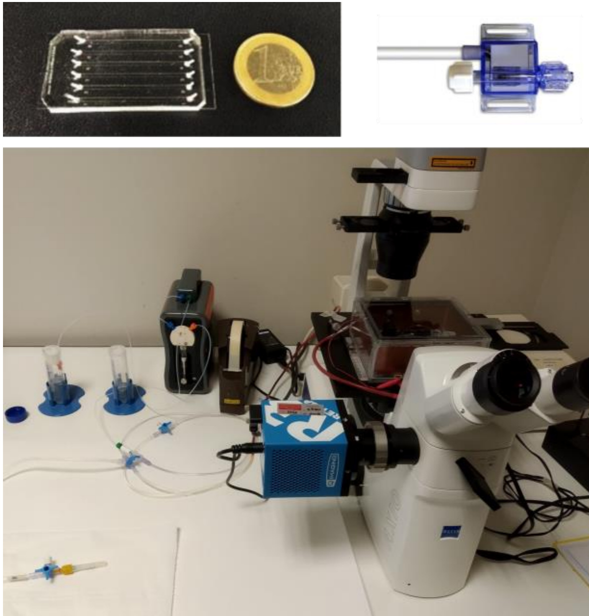

Fig. 1: Microfluidic chip and pressure transducer (above) and setup used for the preliminary testing campaign (below), comprising: an inverted fluorescence microscope equipped with a 16-bit camera, a micropump, a thermostatic chamber, the pressure transducer connected to a laptop and the microfluidic chip.

For pressure reading, an inline pressure transducer (PendoTech, USA) was placed along the circuit, to detect the pressure drop due to thrombi formation inside the channel. It was chosen for its working range, its affordability and washability. The transducer was connected to an amplifier and then to a laptop to record and visualise the pressure signal.

All the experiments were performed at 37°C thanks to a customised thermostatic chamber mounted on the microscope stage.

III. RESULTS AND DISCUSSION

Figure 2 shows pressure drop measurements (n=14) in microchannels coated with two different collagen concentrations, 10 and 100 ug/ml, and at two shear rates, 950 and 1600 s^{-1}. A two-way ANOVA test found statistically significant differences between the pressure readings at 1600 s^{-1} with a 100 ug/ml collagen concentration and all the other conditions (****p<0.0001).

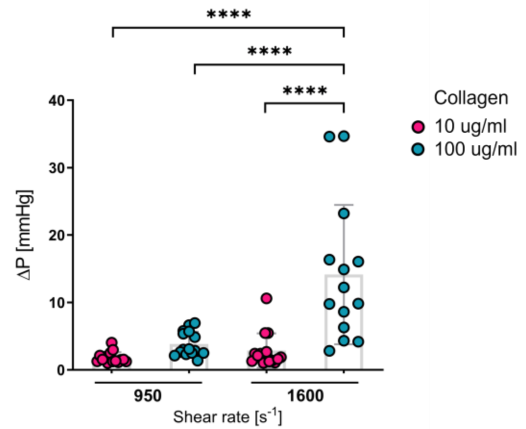

Fig. 2: Pressure drop due to thrombi formation inside the microchannel after 4 min experiments, classified for different shear rate and collagen concentration of the substrate. Bars represent mean ± standard deviation. N=14. Two-way ANOVA was applied. ****p<0.0001.

This indicates a transducer's sensitivity better working under more intense conditions. The higher collagen concentration appears to be a more suitable experimental condition, as indicated by the difference found between the data at the two different shear rates, conversely to what can be observed at 10 ug/ml. This is due to the bulkier thrombi forming on the 100 ug/ml substrate, as it works as a strong stimulus for platelets and exposes many bonding sites. The difference is more evident for increasing shear rate (Figure 3).

100 ug/ml is one of the most used concentrations in literature [5], [6], [7], while some groups employ even higher ones [8], [9].

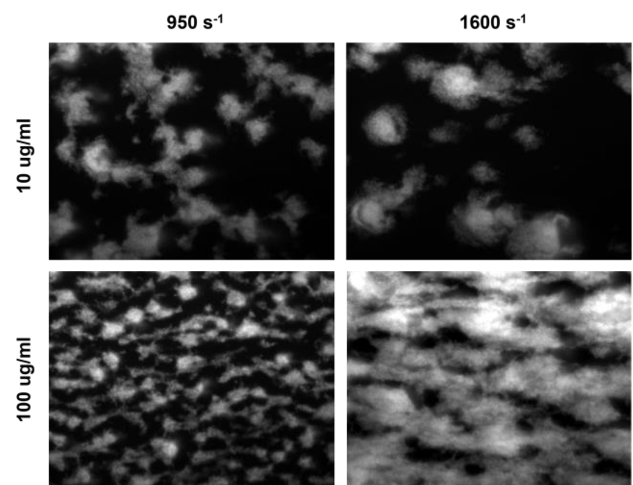

Fig. 3: Fluorescent images (magnification 40x) showing the typical thrombus formation over the two collagen substrates (10 and 100 ug/ml) at different shear rates (950 and 1600 s^{-1}).

The pressure drop results after 4 min of experiment, divided by collagen concentration, were compared with the mean and maximum fluorescence intensity computed via MATLAB from the corresponding images (Figure 4). The computed correlation demonstrated a fair agreement (R^2=0.81) between the two datasets, max FI and pressure variation, only at the higher collagen concentration, in accordance with our previous

observation. The encumbrance of the thrombi under this condition leads to partial occlusion of the channel, thus higher pressure drops.

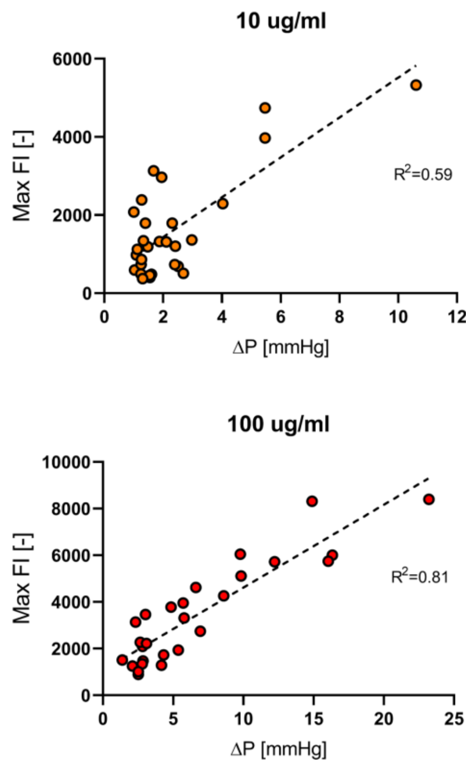

Fig. 4: Pressure drop after 4 min experiments plotted against the maximum fluorescence intensity for collagen concentration 10 and 100 ug/ml. Linear regression found R^2=0.59 and 0.81, respectively. N=14, n=28.

On the lower collagen concentration substrate, thrombi usually remain smaller and lower in height, and the employed transducer is not sensitive enough to detect low pressure variations.

Pressure kinetics was also analysed by monitoring pressure drop and fluorescence intensity throughout the experiments (4 min). Figure 5 shows a typical trend of the two parameters in an experiment carried out at 1600 s^{-1} on a 100 ug/ml collagen.

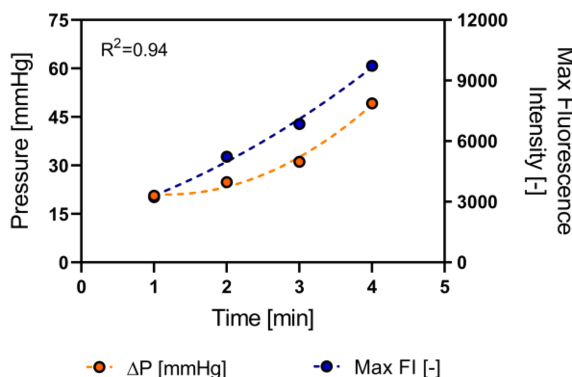

Fig. 5: Typical trend of pressure drop and max fluorescence intensity in time during an experiment conducted on collagen 100 ug/ml at 1600 s^{-1}. Dashed lines represent second order polynomial fitting. Pearson correlation coefficient R^2=0.94.

Under these conditions, platelets massively adhere to the collagen fibrils immediately after the start of the experiment and thrombi start to build up quickly. The pressure increases promptly in the first minute, then a parabolic trend can be noticed, while the channel lumen progresses towards occlusion (partial, within 4 min). A forming big thrombus is usually observed at the beginning of the coated area: it generates a hydraulic resistance which induces a concentrated pressure drop. This result shows how the pressure transducer is able to catch the kinetics of the process, especially after 2 min of experiment, displaying a trend similar to the one of the maximum FI (Pearson correlation coefficient R^2=0.94).

IV. Conclusion

Herein we presented the results of a preliminary testing campaign of a microfluidic platform for label-free, real-time functional assessment of the thrombotic risk, with the ultimate goal of a rapid monitoring of the therapeutic treatment. We demonstrated the feasibility of our approach by showing a good correlation between pressure drop and fluorescence images. These experiments allowed us to define the suitable working range of the current setup.

Future studies will investigate the correlation between pressure drop readings and occlusion rate of microchannels, obtained by confocal microscopy. Moreover, we will verify whether the pressure transducer is able to detect the detachment of thrombi from the substrate, to potentially study the patient population presenting aggregation defects.

A further improvement will be the addition of surface microelectrodes on the glass substrate, to detect platelet adhesion through electrical impedance variations.

This platform has the potential to become a POC PFT device, providing a useful tool for fast, real-time and label-free stratification of patients' thrombotic risk.

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