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Robotic microfluidic imaging of blood stimulation- towards high-throughput portable measurement of haemostasis

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SUMMARY

Measuring blood and platelet function is vital for the development and use of drugs that combat cardiovascular disease, such as anti-platelet drugs and other medicines that reduce the risk of thrombosis. We propose combining mass-produced microfluidic devices with open-source robotic instrumentation to enable development of affordable and portable, yet high-throughput and high-performance haematological testing. A time- and distance-resolved fluid flow analysis by Raspberry Pi imaging integrated with controlled sample addition and illumination, enables simultaneous tracking of capillary rise in 120 individual capillaries within 5 minutes. We showed that time-resolved microcapillary rise imaging permits blood function measurement by measuring thrombin-triggered activation of global haemostasis. Thrombin stimulation slowed vertical fluid velocity, consistent with a dynamic increase in viscosity. Microfluidic systems expand haematological testing towards high-efficiency, multi-parameter blood analysis necessary for understanding and improving cardiovascular health.

INTRODUCTION

Haemostasis and thrombosis play a major role in cardiovascular health and disease. Measurement of these processes remains critical to health and wellbeing; both for optimising cardiovascular disease treatments in acute and chronic clinical settings, and in research progressing our understanding the cardiovascular system. However, the current landscape presents a significant gap between easy-to-use portable tests that provide limited data and laboratory tests that are inaccessible and require expensive equipment (Michelson et al. 2006). This is made harder by the need to test blood immediately, since storage affects platelet function and coagulation pathways. Typically, such blood function assays need to be performed in a laboratory within 4h of sample being taken, and samples cannot be frozen, refrigerated, or archived in biobanks (Favaloro 2019). Better tests are therefore essential both for clinical trials to find new drugs, and for diagnostics that will allow us to make best use of existing and future drugs that affect thrombosis and haemostasis.

MATERIALS AND METHODS

Fluoropolymer microcapillary film (MCF) of 200 µm diameter (Lamina Dielectric Ltd, Billingshurst, UK)

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was coated internally by incubation with 10 mg/mL molecular weight polyvinyl alcohol as previously described (Reis and Li Puma 2015). To establish proof-of-concept for measuring the dynamics of coagulation during capillary flow, 100 mm long test strips were loaded by freeze-drying with 0, 5, 15, 50 and 150 U/mL Thrombin from bovine plasma (product T4648) in water. Blood samples were collected from healthy donors using citrate blood collection tubes (BD Vacutainer®) with 3.2% buffered sodium citrate solution.

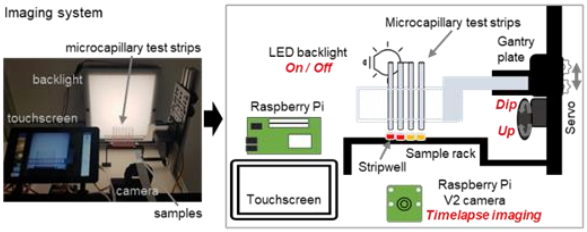

Fig. 1. *'Dip stick' microfluidic devices using open-source Raspberry Pi robotic imaging system.*

A low-cost (approximately £300), open-source, customizable Robotic Microfluidic System (RMS), (350 mm x 415 mm x 288 mm) (Figure 1) combines dip-and-test microfluidic devices. Full material list, build instructions, and software codes are published in Zenodo (DOI: 10.5281/zenodo.6617301).

RESULTS AND DISCUSSION

Stimulating whole blood with thrombin within the capillaries led to a rapid and concentration-dependent reduction in the height of capillary rise at all timepoints, compared to unstimulated blood (Figure 2a). Viscosity is expected to increase over time after thrombin dissolution, resulting in a gradient of coagulation with the highest viscosity observed at the top, where the blood has been exposed to stimuli for the longest duration (Figure 2b).

We clearly observed decreased fluid velocity consistent with a rapid and dynamic increase in viscosity following stimulation, seen clearest when instantaneous dH/dt was plotted against reciprocal height 1/H(t), as expected since coagulation of blood during haemostasis should lead to obstructed flow (Figure 3). Response to thrombin demonstrating that coagulation can be activated within the capillary during capillary rise, and that instantaneous velocity is sensitive to rapid changes in coagulation.

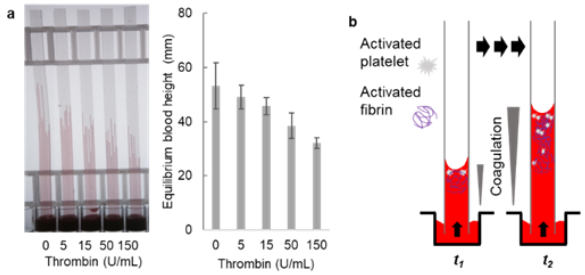

Fig. 2. *Stimulation of blood samples with thrombin.*

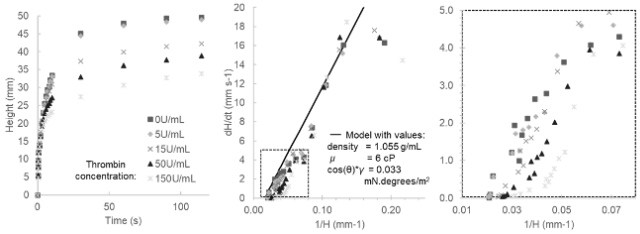

Fig. 3. *Flow dynamics and equilibrium capillary rise changed with thrombin concentration.*

CONCLUSIONS

Our findings contribute to the development of high-throughput, multi-parameter microfluidic blood analysis systems, paving the way to global point-of-care haemostasis testing.

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