MECHANICAL CIRCULATORY SUPPORT THROMBOSIS MODELLING ON-A-CHIP DELINEATES MATERIAL AND HEMODYNAMIC EFFECTS

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Introduction

The safety and efficacy of mechanical circulatory support (MCS), including extracorporeal membrane oxygenation (ECMO) and ventricular assist devices (VAD), is complicated by blood clot formation (thrombosis) (Fig.1A), which cause fatal complications including device occlusion and failure, patient embolism and stroke [1,2]. Anticoagulants administered to prevent thrombosis cause bleeding risks to patients [1,2]. Thrombosis is induced by the combined effects of foreign artificial material surfaces and pathological hemodynamic conditions of MCS [3]. Thus, to understand biological mechanisms of MCS thrombosis and evaluate the thrombogenicity of MCS design, *in vitro* models need to incorporate both factors at clinically relevant conditions. We demonstrate an *in vitro* model of MCS thrombosis achieving 1) physiologically relevant human and patient whole blood samples, 2) customisable clinical material and flow combinations, 3) detailed real-time visualisation, and 4) higher-throughput screening than traditional models.

Methods

ECMO patient circuits from Royal Prince Alfred Hospital were decannulated, flushed and inspected for thrombus. Computational fluid dynamic modelling was performed on ECMO tubing-connector models at 2 – 6 L min^{-1} to determine thrombosis-relevant flow regimes. Microfluidic models mimicking such conditions were made with ECMO materials polyvinylchloride (PVC) or polycarbonate (PC), within which confocal microscopy recorded real-time thrombosis and platelet activation.

Results

ECMO tubing-connector junctions were common sources of adhered thrombus (Fig.1A). *In situ* modelling correlated these regions to wall shear rates from 500 s^{-1} to 5000 s^{-1}, and velocity gradients. *In vitro* microfluidic experiments showed that shear regimes less than 1000 s^{-1} significantly increased platelet adhesion compared to shear rates above 2000 s^{-1}. In expanding or constricting microfluidics mimicking ECMO tubing-connector velocity gradients, platelet aggregate growth, occlusion and embolism were visualised in real-time (Fig.1B), accurately replicating established clinical phenomena.

We importantly demonstrated the models' ability to experimentally delineate the influence of material properties and flow conditions in activating thrombosis. This was achieved by real-time spatial quantification of platelet markers e.g. P-selectin, indicating thrombosis activation. We showed increased P-selectin on the platelets adhered to PVC compared to PC (Fig.1C). For the first time, we showed selective P-selectin expression on the leading-edge of individual adhered platelets under shear at 3000 s^{-1}, but not 1000 s^{-1} (Fig.1D).

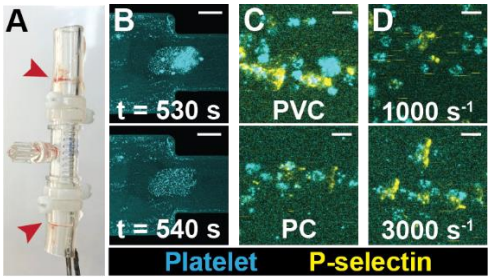

Figure 1: (A) ECMO tubing-connector thrombi. (B) Platelet thrombus embolism in real-time (C) Increased platelet activation on PVC vs. PC. (D) Spatial P-selectin is shear dependent. Scale bar (B)=50µm, (C&D)=5µm.

Discussion

Our model of whole blood, MCS thrombosis achieved material and flow customisability, clinical relevance, and low blood volume for improved throughput. Real-time monitoring provided the advantage of visualising phenomena including occlusion and embolism, and comparing temporal platelet activation under different materials and flow, to improve our understanding of the biological mechanisms underlying MCS thrombosis.

These results will be useful for guiding future MCS design. For example, our result suggests that low operation flow rates, flow velocity gradients, and the use of PVC over PC should be avoided to reduce activating thrombosis in ECMO, occlusion and embolism. Additionally, future MCS material development could use this model to rapidly screen the hemocompatibility of novel materials under operationally relevant flow conditions, as the model only required microlitres of human blood. Together the detailed biological mechanistic information provided by our model, combined with its customisability, provides a powerful tool for future evaluation of MCS design safety.

References

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