FOUR-STAGE MECHANISTIC MODEL OF DYNAMIC PLATELET AGGREGATION IN A MICROFLUIDIC CHIP

Miguel E Combariza[1], Francisco J Tovar-Lopez[1], Warwick S Nesbitt[3], Xinghuo Yu[2], and Arnan Mitchell[1]

[1]Microplatforms Research Group, [2]Platform Technologies Research Institute, School of Electrical and Computer Engineering, RMIT University, Melbourne, Australia; [3]The Australian Centre for Blood Diseases, Alfred Medical Research and Educational Precinct, Monash University, Melbourne, Australia

ABSTRACT

Understanding the dynamics of platelet aggregation is fundamental to the formulation of antithrombotic treatments that can reduce morbidity rates associated with cardiovascular diseases. A recent study, supported by a microfluidics platform emulating a thrombogenic stenosis in an environment independent of chemical pathways and under non-recirculating conditions, has revealed the primary role of hemodynamics to initiate platelet aggregation. We identify four distinct stages in the thrombus growth present in such a platform, and formulate an explanatory physical model of the relationship between the hemodynamics and thrombus growth. This model provides insight into the mechanistic variables regulating platelet aggregation.

KEYWORDS: stenotic flow, hemodynamics, microfluidics, non-recirculating, mechanistic model, thrombus

INTRODUCTION

A key area in cardiovascular research is the study of platelet aggregation at sites of vascular injury. This phenomenon is vital to stop bleeding at those sites, and responsible for subsequent repairs. Nonetheless, an exaggerated response of this process can generate thrombi, which can result in cardiovascular disease states, and is associated with acute coronary syndromes. Despite extensive research carried out on the study of cardiovascular diseases, these remain one of the highest causes of death worldwide. Although chemical events leading the formation of aggregates of platelets in suspension are well understood, a recent study demonstrated the inefficiency of current anti-thrombotic treatments under stenotic flow [1]. This challenged the common assumption that platelet aggregation is mainly driven by chemical agonists, and proposed that shear microgradients are the main driving factor. This breakthrough has opened a new approach to the study of cardiovascular diseases using microengineered technologies [2]. Ultimately, it is important to model this response to have a better understanding of this biophysical process.

Most mathematical models available in the literature focus on the activation of platelets due to the traditional agents (endothelial matrix, ADP, TXA2, thrombin) and partly describe the effect of the blood flow dynamics in the process. Recent sophisticated models recognize the importance that flow dynamics play in early events of aggregation (mainly physical) and in the transport of chemicals from and into the porous thrombus throughout the process [3]. However, these models do not yet include the effects of shear microgradients identified in [1,2].

The development of models incorporating this newly discovered mechanism of aggregation is impeded by difficulties in accurately studying thrombosis under stenotic flow. Typically, experiments are conducted within constant shear rate environments. For example, a previous study of thrombogenic stenosis was carried out at the millimeter scale [4], containing a recirculation zone downstream of the stenosis. This recirculation zone does not occur in the platform [1,2] and is not required for the initiation and sustenance of aggregation. Hence, the model developed by [4] is only partially complete and further analysis is warranted in order to refine models of this sort.

This paper proposes a mechanistic model of thrombus formation within a microfluidics platform emulating a thrombogenic stenosis in an environment independent of chemical pathways and under non-recirculating conditions [2]. This work identifies four distinct stages observed experimentally in the formation of thrombus and formulates an explanatory physical model. We also explore whether additional insight into these stages of formation can be gained from examination of both the size of the thrombus and also its density as indicated by fluorescent intensity. Once characterised rigorously, this model will be useful to gain more insight in the mechanistic variables regulating platelet aggregation.

METHODS

Blood was perfused through a microfluidic chip containing a microcontraction using a syringe driver as illustrated in Fig 1 a). Anticoagulated whole blood was introduced to a 200µL reservoir, connected to a microfluidic channel. The negative pressure of the syringe drew the blood from the reservoir into the channel and then through a microfabricated filter to remove undesired inclusions. The blood was then funnelled into a smaller scale channel and drawn, at high velocity, through a microcontraction of 80% in the microchannel, mimicking a stenotic scenario in a blood vessel. At the end of the microchannel, a bore exhaust was connected to a syringe driver to drive the flow of blood using negative pressure, and collect the waste as shown in Fig 1b). The chip was fabricated using a cast of PDMS on a KMPR mould employing standard photolithographic processes [2].

In order to isolate the mechanical effects of blood flow from the well-known chemical pathways of platelet activation (ADP, TXA_2 and thrombin), blood, from consenting human donors, was previously treated with pharmacological inhibitors of the canonical platelet amplification loops (amplification loop blockade -ALB). Lipophilic membrane dye $DiOC_6$ was used to label platelets and an imaging system, comprising of an invert microscope and a CCD camera, monitored the epi-fluorescence illumination of the aggregate of platelets over time, at one frame per second, as shown in Fig 1 c). The size of the main aggregate was estimated frame-by-frame in Matlab R2010a by thresholding the brightness of video, segmenting the main thrombus and calculating the area of pixels above a minimal value. Similarly, the density of platelets in the forming thrombus was estimated by calculating the average intensity of the pixels inside the edge of the thrombus.

RESULTS AND DISCUSSION

Several experimental trials were performed using a constant flow rate (shear rate of $1800s^{-1}$) and blood from a healthy donor. Results showed a consistent trend in the aggregation in which we identified four distinct stages in the growth of the thrombus as illustrated in Fig 2. An initial recruitment of platelets occurred after a lag time of approximately 100s (Stage I). Then, the thrombus presented acute growth, lasting approximately 200s, (Stage II), followed by a plateau (Stage III) of about the same duration. Finally (Stage IV), the size of the thrombus presented regular undulations accompanied by the ejection of micro-aggregates. Figure 2 a) shows snapshots of the video signal showing the shape that the thrombus takes over time. A correlation between the area plots in Fig 2 b) and the video signal found that the end of the growth in Stage II occurs when the thrombus reaches the bottom of the channel (Fig 2 a) at 5:30). It also showed that while the size of the thrombus appears to remain almost constant during Stage III, a measurement of its average intensity, as shown in Fig 3 a) and b) respectively, indicated an increase in the platelet density over time. The rate of growth in Stage II appears to be parabolic as the thrombus grows radially (vertically and laterally), and in Stage IV appears to be linear as the thrombus only grows in the lateral dimension.

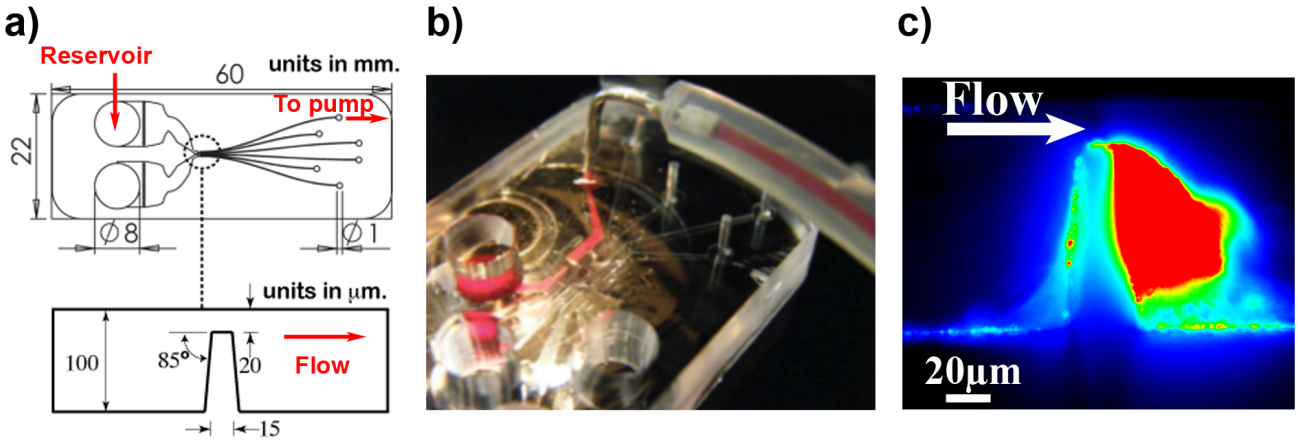

Figure 1. Microfluidic chip for blood flow studies a) Schematic (above) and microcontraction (below) b) Photograph of blood perfusion through one microchannel c) . Representative epi-fluorescence image of blood perfusion

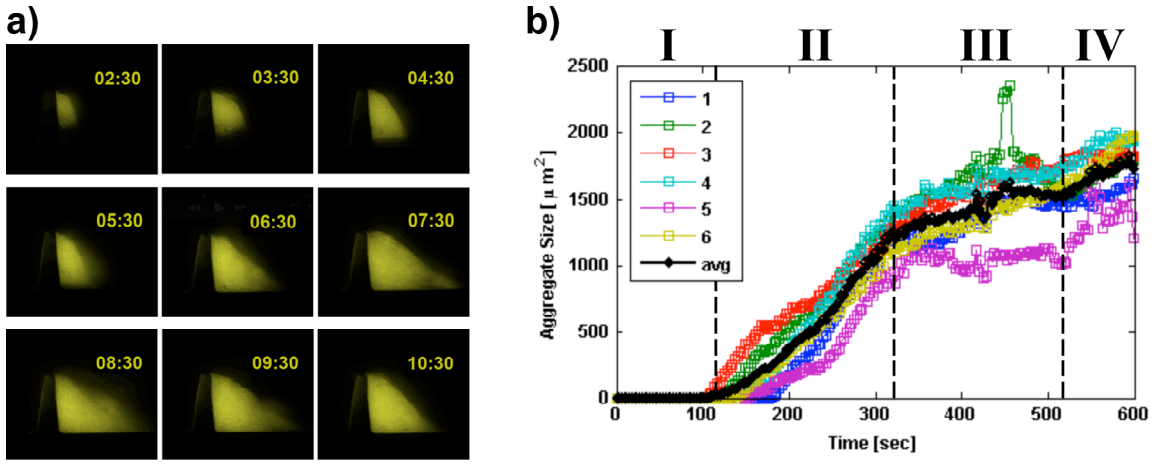

Figure 2. Blood perfusion experiments a) Image sequence showing the shape of forming thrombus over time b) Aggregation traces representing the relative size of the thrombus and four stages of thrombus formation

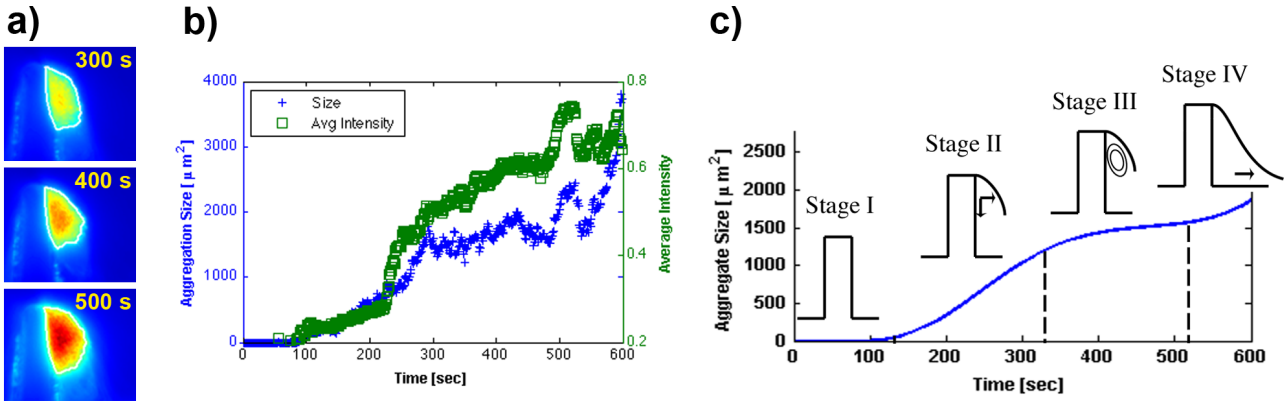

Figure 3. a) Snapshots of thrombus fluorescence during Stage III. b) Plot of average intensity and relative size of thrombus over time. c) Four-stage mechanistic model of dynamic platelet aggregation: monolayer formation, acute growth, densification, and embolisation.

Our physical model (Fig 3 c)) suggests that the initial recruitment of platelets to form an initial monolayer is driven by kinetic absorption of Von Willebrand Factor in the microchannel, influenced by shear, and it has a low density of platelets. Then, we observe a rapid aggregation starting to form downstream of the apex of the microcontraction, characterised by vertical and lateral growth. This takes place in the low shear region near the wall and appears to slow down as the aggregate perimeter reaches flow streamlines of higher shear (at the end of Stage II). At this point the thrombus growth seems to plateau, however, platelet recruitment continues with the hydrodynamics forcing the platelets to group together into a denser thrombus. At the end of Stage III, the thrombus begins to grow again, and we suggest that once a critical density is reached, the advective flow within the thrombus is minimal, and so, the chemical environment of the main thrombus can diffuse and sustain aggregation, allowing the formation and ejection of micro-aggregates –embolisation past the initial thrombus (Stage IV). Since the current imaging configuration focuses on a singular plane of the thrombus, it is possible that increase in the average intensity is due to addition in the total number of platelets accrued in other planes. Further experimentation using confocal microscopy could support the conclusion on thrombus densification.

Understanding the dynamics of the stages of platelet aggregation is crucial to the development of accurate mathematical models that can predict under which conditions the embolisation of the thrombus can result in vessel occlusion. Our interest lies in the development of control systems that can stabilise the thrombus at a particular stage through the modulation of the shear rate of the platform. If successful, such control will allow study of dynamic platelet aggregation under stable conditions, providing new insight into aggregation mechanisms. Eventually, these tools will aid the development of safer and more efficient anti-thrombotic treatments that accommodate and exploit the new mechanisms of platelet aggregation being studied.

CONCLUSION

The proposed mechanistic model describes how hemodynamics influences the initiation of platelet aggregation and thrombus growth, observed experimentally. It is possible to gain insight into thrombus development by monitoring both the size of the thrombus, as well as its density as indicated by fluorescent intensity. While the former appears to be constant at times, the latter indicates an increase in the density of platelets. Physiological significance of these findings is still under discussion. These results will facilitate the incorporation of shear microgradients into mathematical models of platelet aggregation, and eventually provide insight into the mechanistic variables regulating this phenomenon.

REFERENCES

[1] W. Nesbitt, E. Westein, F. Tovar-Lopez, E. Tolouei, A. Mitchell, J. Fu, J. Carberry, A. Fouras, and S. Jackson, *"A shear gradient-dependent platelet aggregation mechanism drives thrombus formation,"* Nature Medicine, vol. 15, pp. 665-673, (2009).

[2] F. Tovar-Lopez, G. Rosengarten, E. Westein, K. Khoshmanesh, S. Jackson, A. Mitchell, and W. Nesbitt, *"A microfluidics device to monitor platelet aggregation dynamics in response to strain rate micro-gradients in flowing blood,"* Lab on a Chip, vol. 10, pp. 291-302, (2010)

[3] K. Leiderman and A. L. Fogelson, *"Grow with the flow: a spatial-temporal model of platelet deposition and blood coagulation under flow,"* Mathematical Medicine and Biology, vol. 28, pp. 47-84, (2011)

[4] D. M. Wootton, C. P. Markou, S. R. Hanson, and D. N. Ku, *"A mechanistic model of acute platelet accumulation in thrombogenic stenoses,"* Annals of Biomedical Engineering, vol. 29, pp. 321-329, (2001)

CONTACT

Miguel Combariza +61-3-9925 3616 or m.combarizapacheco@student.rmit.edu.au