The modulatory roles of collagen and endothelial cells on platelet function

Impact

Thrombosis represents the major pathological condition underlying common cardiovascular disorders (including ischemic heart disease, stroke and venous thromboembolism), comprising a major global disease burden, causing 1 in 4 deaths worldwide [1]. Antiplatelet therapy constitutes the current standard of care for the secondary prevention of arterial thrombosis [2]. However, patients taking antiplatelet therapy have an overall increased risk of bleeding and, despite treatment, can further develop ischaemic events [2]. Hence, improved therapies that specifically prevent thrombosis without affecting haemostasis are still needed. Similarly, it is important to develop new technologies to study the complex pathophysiological mechanisms underlying thrombosis and haemostasis onset, and to test the effects of novel drugs. For these purposes, microfluidic assays for studying the interactions between blood and vascular components under flow conditions, represent valuable tools [3, 4]. This thesis provided several new additions in this direction, as detailed below.

In Chapter 3, we validated a multiparameter microfluidic approach to test the antithrombotic effects of the Syk inhibitor PRT-060318 in human blood. The drug inhibited the signalling downstream of the platelet receptor GPVI, which is the main signalling receptor for collagen. As stated above, a major drawback of conventional antiplatelet medication in the prevention of thrombosis is the recurrence of bleeding. Current knowledge suggests that targeting GPVI or tyrosine kinases of the downstream signalling thereof [5] may provide an antithrombotic effect with only minimal consequences for normal haemostasis. The antithrombotic effects of the drug PRT-060318 have been tested, so far, mostly in mice [5]. In Chapter 3, the use of the microfluidic model of thrombosis allowed us to test this drug in vitro using blood samples from healthy subjects under physiologic conditions of whole blood perfused over different collagens or collagen-mimicking peptides, thus confirming an overall thrombo-protective effect in humans. Although representing promising anti-thrombotic therapeutic tools, Syk inhibitors show potential off-target effects which should not be ignored. In addition, this chapter highlights how diverse collagens differently induce thrombus formation, with as examples the fibrillar type I and type III human collagens, inducing the formation of smaller thrombi with lower parameters of platelet activation, when compared to the type I-enriched highly fibrillar collagen Horm, which is standardly used in platelet function tests. Therefore, fibrillar collagens which differ by type, preparation, tissue and species of origin, induce platelet activation patterns corresponding to the availability of specific receptor binding motifs. This is relevant for the (diagnostic) examination of collagen-mediated platelet responses that often rely on the use of Horm collagen. The use of synthetic peptides of defined composition of receptor-selective peptides will therefore

help to further enhance the current knowledge on activated signalling pathways, highlighting possible overlap downstream of receptor activation.

Microfluidic technologies provide important alternative tools to the use of animals, in line with the 3Rs approach to “Reduce, Refine and Replace” animal use in research. Accordingly, the above-mentioned microfluidic model was adapted in Chapter 4 and further optimised in Chapter 5 to also integrate vascular endothelial cells and the procoagulant tissue factor. The work resulted in the development of an integrative vessel-on-a-chip model to assess the endothelial control of thrombus formation and coagulation activation in real time, using only small amounts of blood. The flow studies revealed novel anticoagulant functions of the negatively charged luminal endothelial glycocalyx. It appeared that the shedding of the heparan sulphates in the luminal glycocalyx resulted in increased fibrin generation and overall accelerated kinetics of fibrin generation. Glycocalyx shedding can occur in pathological conditions like post-ischemic organ damage, sepsis and inflammation, renal disease, diabetic vasculopathy and atherosclerosis [6]. This can lead to the accumulation of proteoglycans in lesions that are more resistant and prone to endothelial erosion, which is associated with the formation of platelet-rich thrombi [7, 8]. In Chapter 6 we show that heparan sulphate-proteoglycans (HSPGs) in the endothelial basal lamina also display more indirect regulatory mechanisms affecting platelet-collagen interactions, which pointed to the presence of polarised properties of the endothelial glycocalyx. Our finding of the formation of contracted and more stable collagen-induced thrombi in the presence of HSPGs is a potentially relevant mechanism in the pathogenesis of atherothrombosis.

Chapter 5 describes the use of quantitative mass spectrometry (MS)-based phospho-proteomics analysis of healthy human platelets, revealing novel complex networks of biological pathways that are targeted by still unresolved endothelial cell products and by the platelet-activatory stimuli collagen and thrombin. Herein, phospho-proteomics confirmed to be a powerful tool to obtain large-scale information of post-translational modifications in a single big experiment [9]. In addition, as evidenced in this thesis, recent technological developments including the introduction of automated and more sensitive instruments, have made the MS-based phospho-proteomics valuable techniques to measure post-translational modifications accurately and reproducibly, in small sized samples with great potential for clinically evaluation [10, 11].

Towards clinical applications, in Chapter 7, we have successfully used the multi-parameter microfluidic approach of thrombus formation to evaluate the platelet phenotypes of patients with inherited platelet function disorders. Herein, the microfluidic

technology fulfilled existing diagnostic gaps to detect platelet-related bleeding disorders, thus showing a complementary addition to standard diagnostic tests.

Taken together, the results of this thesis contribute at multiple levels to both basic and applied research. Crucial in this sense has been the adoption of novel technologies, that allowed us to make progress towards the full understanding of the complex mechanisms of platelet function in health and disease, of which both the scientific and the clinical communities can benefit.

The study of haemostatic and thrombotic processes finds its limitations in the under-representation of fluid dynamics which, as pointed out throughout this thesis, contribute significantly to our understanding of platelet functions. In this regard, our microfluidic tests have proven to be essential tools for basic research to understand the mechanisms of thrombotic disorders. It appears increasingly clear that such tests play a role in the screening of new drugs in a preclinical setting, helping to accelerate the development of more effective therapies in the treatment of thrombosis. The same tests also show a great potential in the clinical lab, where these can add to standard platelet functional tests for the differential diagnosis of congenital platelet-related bleeding disorders. These still represent a diagnostic and therapeutic challenge that demand for more research. The studies in this thesis are therefore relevant to researchers, clinicians, as well as patients communities and society.

One last reflection I like to make on the engagement of society and the general public in this area. Leveraging the experience of the pandemic, it is clear how powerful scientific communication, also in the field of platelet function and disease, has become in an era where information is easily accessible to everybody. As a scientific community, we must be aware of the great potential of a correct scientific communication to increase the impact of our research and to prevent the manipulation of information and public mistrust. Research conferences are effective means to reach out researchers, clinicians, pharma companies and policy makers. Yet it is necessary to invest more efforts to extend our communication from an academic audience to patients communities and to schools, where the future scientists are being educated. The European TAPAS H2020 consortium, which provided financial support for this thesis, has helped to reach those goals with its virtual enterprise by promoting public engagement activities that I had the opportunity to contribute to.

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