Discovering new pathways in thrombus formation

Valorization

In recent years, an increasing pressure has been put on researchers to provide not only scientific impact, but also social or economic impact of their scientific findings.[1] Given the different nature of science, it is not equally straightforward to have a clear sight of the beneficial outcome of scientific research for society. Whereas in applied research one focuses on pragmatic questions and aspires to develop applications that can be implemented directly in current practice, in basic research emphasis lies more in expanding and exploring the underlying mechanism(s) of processes of interest without primarily aiming to develop specific applications. However, these two types of research should ideally complement each other in order to span the entire trajectory from bedside to bench and vice versa.[2]

Platelets have an essential role in hemostatic system. Upon vascular damage, platelets are in the frontline and rapidly form a platelet plug in order to cessate bleeding. However, aberrant platelet function can cause imbalance of the hemostatic process, potentially leading to thrombosis or bleeding, and hence cardiovascular events.[3] Commonly, anti-platelet drugs are chosen to prevent cardiovascular events of the arteries, in conjunction with lipid-lowering medication and anti-hypertensive agents. Bleeding is the main negative side effect of anti-platelet drugs. Unfortunately, current platelet function tests in general lack sensitivity for predicting bleeding or thrombosis risks. Studies evaluating methods for monitoring platelet function upon anti-platelet treatment revealed that results from light transmission aggregometry (LTA), PFA-100, VerifyNow do not correlate well.[4,5] Furthermore, clinical studies have also shown that the correction of anti-platelet treatment based on results from platelet function analysis did not improve the clinical outcome in patients.[6] So far, it is unknown whether monitoring is actually useful for clinical practice.

In my thesis, I focused on unraveling the consequences of the effect of changes in platelet function, either due to genetic deficiency or pharmacological modulation, on platelet function and thrombus formation. Throughout the thesis, an *in vitro* microfluidic device was employed to study platelet function under shear conditions. The capability of this test to monitor platelet function has been shown before,[7] however it has not been extensively used with patient blood. In Chapter 4, this microfluidic device has been employed to characterize platelet function in platelets from immunodeficient patients. For the first time, we could confirm that the genetic alteration causing immunodeficiency affected platelet function, which could explain the increased bleeding risk. Improvement of platelet function could also be recognized by this method as in samples from the same patients after bone marrow transplantation an improved platelet function was detected

Besides studying the effect of genetic alterations on platelet thrombus formation, this microfluidic device can be also used for testing the effect of pharmacological agents, such as drug candidates with direct effect on platelets or with regards to monitoring the indirect effect of other medication on platelet function, as is described in Chapter 7 and 9. Chapter 9 aimed to explain the reduction in cardiovascular events upon Dipeptidyl peptidase-4 antagonist treatment by linagliptin and sitagliptin. By using this microfluidic device, we unraveled a negative priming effect on platelets as an indirect effect of this treatment. The latter illustrates the diverse range of applicability of *in vitro* microfluidic devices.

Furthermore, microfluidic devices are ideal to investigate the interplay between platelets and other cell types, for instance endothelial cells[8] or immune cells. In Chapter 5, the interaction between platelets and leukocytes have been extensively studied thereby focusing on the consequences of platelet inhibition on leukocyte activation and responses. Here, the inhibition of platelet secretion is appeared to reduce the leukocyte activation *in vitro*. Given the increasing interest in thrombo-inflammation, it is important aspect to be able to monitor such interactions between platelets and inflammatory/ inflamed cells.

A recent paper highlighted that *in vitro* thrombus formation measurements are good reflection of results obtained in experimental mouse models for collagen-dependent arterial thrombosis,[9] supporting the relevance of such an *in vitro* device and suggesting their possible involvement into the 3R approaches by reducing, refining and replacing animal experiments. In Chapter 6, we have compared the *in vitro* thrombus formation results assayed with blood from 33 different genetically modified mouse strains using a standardized image analysis protocol. The original microfluid experiments were performed over the last decade using the same protocol and device but in different laboratories. These comparisons unraveled that despite of the difference in time, the results are comparable. This is an important finding for future application and for developing a possible point-of-care device.

In this thesis, only one type of microfluidic device has been employed, but there are many commercial and costume-made microfluidic devices available for studying platelet functions under physiological and pathological conditions.[10] Currently, no uniform guidelines exist on how to employ microfluidic devices and how to report the obtained results. In theory, laboratories using the same devices, could perform the experiment differently, thereby hampering comparison of results. To overcome this problem, the Biorheology Subcommittee of the Scientific and Standardization Committee of the

ISTH initiated a multi-center study on flow assay protocols and designs in which the results of one common microfluidic device and one custom microfluidic device will be compared across several centers/laboratories. One of the goals of this multicenter study is to formulate a general guideline regarding the most crucial aspects of performing microfluidic experiments.[11] As already been demonstrated in Chapter 6, using the same protocol and device can provide highly comparable results regardless of the executor.

Given the broad applicability of the microfluidic devices, its usage as a point-of-care (POC) test to assess platelet function has been postulated for already a number of years and several devices have been tested.[12] For instance, the total thrombus formation analysis system (T-TAS) has appeared to be suitable for recognizing the effect of antiplatelet and anticoagulant treatment in patients with coronary artery disease, acute coronary syndrome or ischemic stroke.[12] However, there are issues that have to be solved regarding preanalytical variables and on how to perform the test in a standardized manner, but also on how to interpret the outcome of the test.[13] At present no uniform cut off values have been set for microfluidic assays with regard to platelet function. Given the natural variability in platelet function within the normal population, cut off values have to be set carefully. In addition, in order to improve and understand the results from patients with different disease states.[13]

Taken together, the results obtained from different patients, healthy volunteers and mice give a good overview of the usefulness of microfluidic devices in unravelling defective platelet function. Depending on the construction, it is possible to monitor both loss- and gain-of-function of the platelets leading to hemorrhage or thrombosis as it was proven for the Maastricht flow chamber in Chapter 6.

References

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