Impact
Cardiovascular diseases are still the leading cause of death globally and are often characterised by thrombotic complications. When a vessel wall is injured, platelets are the first responders that close the gap by the formation of a platelet plug, also indicated as a platelet thrombus. This process is accompanied by blood clotting and the formation of fibrin, which stabilises the platelet thrombus. Abnormal functioning of these processes can lead to bleeding or thrombotic complications. Unfortunately, current diagnostic platelet and coagulation tests used in the clinic often lack sensitivity for accurate bleeding or thrombotic risk prediction. Furthermore, they still lack integration of all processes involved in thrombus formation. Importantly, most clinical assays do not include the critical aspect of blood flow. The platelet function analyser (PFA)-100 so far is the only clinical device that includes flow to assess platelet function, but this assay is insensitive for the detection of mild platelet disorders.

In this thesis, I focused on the development and validation of new flow measurements, ultimately aiming to result in methods for the laboratory assessment of platelet- and coagulation-related diseases. In Chapter 3, we employed a novel technique that uses whole blood perfusion through a microfluidic channel in order to mimic the injured human blood vessel. First, we investigated the differences in thrombus formation between healthy individuals and the causes of this variation. A minor part of the variation between individuals could be attributed to differences in platelet count and mass, as well as specific genetic variation between people. Moreover, we confirmed a relation between the level of receptors on the platelet surface and the outcomes in our flow parameters. Since e.g. diabetic or septic patients have altered levels of these receptors, a flow method like the present one can be a promising tool to detect changes in thrombus formation in these patients. Blood from patients with a functional platelet defect also showed reduced thrombus formation by microfluidic measurements, indicating the usefulness for characterising patients with such a defect. The flow method was extended in Chapter
4, where thrombogenic components located in the vessel wall were combined in the microfluidic channel to simultaneously assess platelet activation and fibrin formation. By assessing the thrombus-forming potential of seven components that are exposed upon vascular damage, we were able to estimate the contribution of each component to platelet and fibrin activation under flow conditions. Blood samples from patients with different coagulation abnormalities were used to validate the (altered) thrombus and clot formation under flow. We could show that flow measurements indeed are suitable to phenotype patients which have increased (patients with a thrombotic tendency) or decreased (patients with a bleeding tendency) thrombus formation compared to healthy controls. This method was also used to phenotype patients with a heterozygous protein C or S deficiency in Chapter 5. Although these patients have an increased risk of venous thrombosis, decreased platelet aggregation and fibrin formation were found in flow measurements. Further analysis on the molecular mechanism behind this should reveal if and how this method can be implemented for phenotyping these specific coagulation defects.

In order to study the interaction of platelets and coagulation factors with other cell types, such as endothelial cells, further modifications need to be made to the flow channel system. Endothelial cells covering the inside of a healthy vessel wall prevent the activation of both platelets and coagulation. In Chapter 6, such interactions between platelets, coagulation factors and endothelial cells were studied in vitro. These flow studies revealed a consistent inhibition of platelet adhesion and fibrin formation by the presence of endothelial cells in the flow channel. We could show that in this model platelets still adhered and promoted fibrin formation at sites where no endothelium was present, thus mimicking the local damage upon vascular injury. The flow system thus comprised key elements present in the human vasculature. As a result, we propose that this newly developed ‘humanised’ vessel-on-a-chip model can be used to target the 3R approaches of
reducing, refining and replacing of animal experiments.

The combined results of this thesis are likely to be useful for clinical diagnostics in the future. With the use of tailored flow chamber systems, new opportunities are provided for the diagnosis of platelet and/or coagulation defects and risk prediction of thrombosis and bleeding. However, some limitations of these flow chambers still require improvement before they can be applied in the clinic. First of all, inter-donor variability is still high in all set-ups, making it difficult to set correct reference ranges for patient diagnosis. Furthermore, there is still a lack of a device that is easy to use and directly reports the outcome during the measurement. At the moment, highly trained personnel is required to execute the measurements, perform the image analysis and interpret the outcome data. An easier point-of-care device, preferably including all pathways on platelets, coagulation and vascular cells together, should therefore be established. Until now, our flow measurements have only been used for phenotyping of patients with a known platelet or coagulation defect. For future purposes, it is of high importance to develop these type of methods in a way that they are suitable for prediction of bleeding or thrombosis risks. In this way, they can be used for disease prevention before clinical manifestations occur. To mimic the physiological situation more closely, the performance of flow methods at 37°C should be considered and tested. New uniform guidelines should be provided and evaluated over different laboratories to standardise the method. Importantly, larger clinical studies of thrombus formation under flow are necessary to proof their value for diagnostic and predictive purposes.

To further develop these measurements and get such flow methods ready for clinical diagnosis, it is important to involve commercial parties that develop a prototype device and contribute to large scale testing and validation. Although advances still need to be made, the results of this thesis emphasise that whole blood flow measurements can be promising tools to better predict bleeding or thrombotic complications. Accordingly, flow-
dependent thrombus measurements in the various modalities used in this thesis provide new possibilities aiming to improve personalised therapy management, hopefully with lower clinical events.

References