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### Platelet aggregation in complex vessel geometries

*An in silico study on cellular blood flow mechanics*

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# Chapter 6

## Conclusion and Outlook

### 6.1 Conclusion

The aim of this thesis was to study cellular fluid mechanics of whole blood in microscale flow environments of increasing complexity, to improve the understanding of the initial aggregation of platelets in thrombus formation. To achieve this, cell-resolved blood flow simulations are deployed using the HemoCell framework and their results are evaluated for implications on the mechanical triggers of hemostasis and thrombosis. The thesis investigates three different flow scenarios, building on each other in order of increasing geometrical complexity and spatial scale. These simulation evaluation studies are complemented with microfluidic experiments, supplied by a collaborating wet lab, to substantiate their findings. Finally, a first attempt is made to combine the findings of the previous chapters into a coarse-grained platelet binding model as an extension to the HemoCell framework.

Chapter 2 explores the effects of channel curvature on cellular blood flow. The results reveal significant differences in the emerging shear rate values and distributions between the inner and outer arc of the channel curve, while the cell distributions remain predominantly uninfluenced. The simulation predictions are compared to experimental platelet adhesion measurements in a matching geometry. The inner side of the curvature arc exhibits elevated platelet adhesion intensity, which correlates with sites of increased rate of elongation.

To build on this foundation, Chapter 3 investigates if the elongational flows discovered in the curved channel can be detected in the physiological environment of a damaged vessel and discusses possible implications for the wound healing process. Therefore, punctured vessel simulations are studied together with complementing microfluidic aggregation experiments. The performed simulations show, that a compressed region of high elongational flows and the local cell concentrations favor a faster hemostatic response for a smaller vessel puncture. Furthermore, a favorable thrombus growth direction opposing the flow direction, which is observed in parts of the microfluidic aggregation assay, can be explained by the cellular distributions in the puncture area of the simulations.

After investigating a hemostatic environment, the work of Chapter 4 shifts closer towards a clinical application and its thrombotic risk factors: here, a comparison between two carotid stent designs is presented. As in the previous chapters, a combined *in vitro* and *in silico* setup is utilized. To match the spatial scale of the carotid artery, the simulations are structured in a multiscale setup. The simulation results point towards an increased thrombotic risk for one of the two stent designs, based on the presence of high elongational flows among other factors. Finally, a significant influence of stent strut recirculation regions on local cell distributions is revealed by the simulations. This introduces a new approach for possible design optimizations of vascular stents in the future.

The studies performed in Chapters 2-4 show a gradual advancement in the combined application of cellular blood flow simulations and microfluidic experiments. With each study, the complexity of the investigated domain increases, while the respective setups converge: the diameters of the simulated geometries advance from a 25  $\mu\text{m}$  curved channel, to a 100  $\mu\text{m}$  arteriole up to a 6 mm major artery. At the same time, the spatial gap between *in silico* to *in vitro* setup is continuously reduced, from a 1:10 ratio in Chapter 2 to 1:2 in Chapter 3 until Chapter 4 reaches a 1:1 ratio with its multiscale setup.

Finally, Chapter 5 sets out to combine the observations of the previous chapter into a cellular platelet adhesion and aggregation model. It is based on a simplified constraint-dependent platelet binding process that coarse-grains

influential components of clot formation discussed in this work into a set of thresholds. The model is implemented as an adjustable and modular addition to the HemoCell framework. While the early case study showcases the functionality of the model and already hints towards the importance of hemodynamic flow conditions in initial aggregate formation, further calibration and validation of the model is required before it can be applied in realistic scenarios.

In general, the work of this thesis highlights the implications vessel shape and the geometrical characteristics of blood-contact devices have for local flow properties, cellular distributions and ultimately the mechanical triggers of both physiological and pathological platelet aggregation processes. During the investigations, a special focus is placed on the importance of elongational flow and its role in platelet aggregation through interaction with von Willebrand factor (VWF) molecules.

## 6.2 Outlook

While this thesis is a modest contribution towards understanding the complexities of platelet aggregation as the initial step of thrombosis, the combination of cell-resolved *in silico* information with *in vitro* results in novel flow geometries did reveal the importance of several biomechanical processes. The emergence and dynamics of these processes should be investigated further in the future.

Each previous chapter refers to elongational flows and possible implications on initial platelet aggregation via the unfolding of VWF and subsequent mediation in platelet binding. Improving the understanding of elongational flows as a trigger of the mechano-sensitive platelet aggregation process in both hemostasis and thrombosis could help improve the efficacy of anti-thrombotic therapies through the development of mechano-selective agents.

Work by Sing and Alexander-Katz and Kania *et al.* studies the phenomenon of VWF unfolding under elongational flow in numerical analyses, but results are not in concordance and comprehensive experimental validation is missing [23, 202]. The current research is still lacking depth and requires further understanding. Subsequent studies on the topic require a uniform definition of elongational flow that is adopted across the field. Furthermore, thorough

experimental validation of elongational flow unfolding VWF molecules is needed. Since the effects of elongational flows are difficult to capture *in vivo* or in classic microfluidic setups, such as parallel plate flow chambers, a different experimental setup is required: cross-slot microfluidic devices, commonly deployed in the field of polymer rheology, have the ability to create pure elongational flows at the center of their channel [87, 203, 204]. Another method to control the rate of elongation within a fluid is droplet deposition [205]. In combination with adhesive surface coatings and VWF-blocking agents, a parametric study can be performed utilizing a cross-slot device to clearly define threshold values of VWF unfolding (and subsequent platelet adhesion) under elongational flow.

The findings of Chapter 2 can be strengthened by validating the dependence of the observed thrombi coverage differences on VWF mediation by performing further blocking agent experiments. Additionally, matching the scales between the simulations and microfluidic experiments would increase transferability between their respective observations here, and also in Chapter 3. As shown later in Chapter 4, this could require a multiscale solution.

In Chapter 3, the discovered effects of the puncture on cellular flow conditions downstream in the vessel indicate that wound healing abilities might be affected as well and give reason for further investigation on how vessel injury affects downstream conditions in the future. A combined *in silico* and *in vitro* setup similar to the one presented in Chapter 3.2 could be utilized for this future study.

The increased thrombotic risk for one of the two stent designs identified in the simulations of Chapter 4 requires additional experiments to validate the findings. The collagen coating used in the experiments only corresponds to a single component of the atherosclerotic plaque found in *in vivo* conditions. A different adhesive surface, which reflects the real environment more closely, could be tested in follow-up experiments. Finally, a wide-range parametric study could assess geometrical stent parameters to optimize the overall stent design towards a lower ST risk.

The platelet binding model of Chapter 5 is presented in a preliminary state and necessitates additional work before its application. The addition

of a temporary binding process (see Appendix D) can improve accuracy of the model; however it will require more detailed calibration and validation to be applied together with matching experimental setups. The original model concept idea by van Rooij considered the implementation of advection-diffusion fields to include the combined effects of pro- and anticoagulants [112]. Together with different activation states of platelets, these additions to the model could widen its focus from an initial aggregate model to a more complete thrombosis and hemostasis model. Once sufficiently validated, the model can be applied to the domains of Chapter 2-4 to further verify and extend their current findings. Finally, scale bridging techniques, such as time splitting or amplification could be applied in a multiscale model approach to extend the feasible range of time and spatial scales of the simulations [37].

While computational resources are advancing into an era of exascale computing, the scale of application for cell-resolved blood flow simulations is growing as well. Nonetheless, moving towards a heterogeneous multiscale model of blood flow is a sensible advancement, that will benefit from the increasing computational capacity [75, 76]. The cardiovascular system itself and local processes within it, such as thrombus formation, can be structured in a multiscale manner. Therefore, it is reasonable to simulate them as such to increase model accuracy and scope. Such heterogeneous multiscale models of blood will help advance the understanding of blood flow and clotting behaviour and can aid in the development of anti-thrombotic therapies [39, 55]. Multiscale coupling covering large spatial and temporal scales across multiple internal processes and organs can ultimately advance towards a virtual physiological human that can function as a complete digital twin to deliver optimal patient-specific treatment and improve clinical diagnostics and preventive measures [206, 207].