Computational modeling of angiogenesis : from matrix invasion to lumen formation

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Summary

A network of blood vessels is formed throughout our bodies during embryogenesis. Postnatally, blood vessels grow new sprouts towards oxygen deprived regions, such as wounds or growing tumors, a process called angiogenesis. A better understanding of angiogenesis and vascular network formation can aid the development of medical therapies and improve the engineering of vascularized tissues. Computational modeling helps to find and understand the mechanisms that drive blood vessel formation. In this thesis, we propose computational models for several steps in the complex process of angiogenesis: matrix invasion, sprouting dynamics, and lumen formation. Chapter 1 describes the biology of angiogenesis and introduces the main computational modeling approaches that are used in the field.

Chapter 2 introduces a computational model of angiogenic-like invasion of endothelial cells into fibrin matrices. Koolwijk *et al.* (1996) have developed an experimental assay of sprouting in fibrin matrices, which is the temporal matrix scaffold formed during wound healing. Weijers *et al.* (2010) showed that the composition of fibrin in this assay impacts the level of angiogenesis; there is more ingrowth on high molecular weight (HMW) than on low molecular weight (LMW) fibrinogen. In Chapter 2, we studied which mechanisms underlie the reduced angiogenic ingrowth on LMW compared to HMW with a cell-based computational model that represents the *in vitro* setup. Based on the model results, we propose that a local feedback mechanism selects cells in the monolayer for matrix invasion and subsequently continues sprouting: plasmin-mediated fibrinolysis by an invading cell releases transforming growth factor $\beta 1$ (TGF$\beta 1$) from the fibrin matrix and TGF$\beta 1$ subsequently stimulates the ability of that cell to perform fibrinolysis. This model also reproduces a reduced ingrowth on LMW compared to HMW, when we included the experimental observation that LMW contains less fibrin-bound TGF$\beta 1$ than HMW.

Chapter 3 develops a model of dynamic sprouting and intercellular signaling to study tip cell overtaking. During angiogenesis, endothelial cells differentiate into tip cells and stalk cells through lateral inhibition mediated by Delta-Notch signaling. Tip cells are equipped with long filopodia to sense the local environment and guide the stalk cells along the sprout. It has long

been thought that once a differentiation pattern was established, the tip cell situated at the sprout tip will stay the leader of the sprout for the entire sprouting process. However, more recently it was shown that cells continuously compete for the sprout tip position, a process called tip cell overtaking. The biological function of tip cell overtaking is unclear. We asked whether tip cell overtaking is merely a side effect of sprouting or whether it is regulated through a vascular endothelial growth factor (VEGF)-Dll4-Notch signaling network, and thus might be functional. For this purpose, we studied two existing computational models of angiogenic sprouting, allowing us to study the effect of different sprouting dynamics on tip cell overtaking. In our models, cells spontaneously move back and forth along the sprout as a side effect of the sprouting mechanisms, as was also experimentally observed. This suggests that tip cell overtaking and sprouting dynamics may be interdependent and, therefore, should be studied and interpreted in combination. However, in experiments with mosaic endothelial spheroids, it was found that wild type cells have a competitive advantage over *Vegfr2* haploid cells for the tip cell position, suggesting that VEGF-Dll4-Notch signaling might regulate tip cell overtaking. In agreement with these experiments, in one of the two models the wild type cells also end up at the tip position more frequently than *Vegfr2* haploids due to VEGF-Dll4-Notch signaling, simply because the wild type cells more often differentiate into tip cells do to the large differences in *Vegfr2* levels. Combining these results, we propose that tip cell overtaking is a non-functional side effect of sprouting and that the function of VEGF-Dll4-Notch signaling might not be to regulate which cell ends up at the tip, but to assure that the cell that randomly ends up at the tip position acquires the tip cell phenotype.

Chapter 4 introduces a model of a next step in blood vessel formation: lumen formation. Once new blood vessels are formed, they hollow to allow blood perfusion. The mechanisms of lumen formation have been debated for centuries. Experimental research has led to two main hypotheses: vacuolation and cell-cell repulsion. During vacuolation, vacuoles are suggested to form by the fusion of pinocytotic vesicles that fuse into large vacuoles. These vacuoles form lumens intracellularly by spanning the entire cell and fusing to the cell membrane on both sides of the cell, or extracellularly by the secretion of vacuoles between cells. During cell-cell repulsion, cell membranes of adjacent cells are suggested to repulse each other to form an extracellular lumen between the cells. Both hypotheses are funded with strong experimental evidence, leaving the debate unresolved. In Chapter 4, we address this debate with a computational model of lumen formation that can represent both hypotheses. Continuous lumens can be formed in the model through a branched blood vessel by vacuolation as well as by cell-cell repulsion. However, lumen formation is far more robust for the values of the parameters

of the model when the two hypotheses are combined, suggesting that the two hypotheses work synergistically. One may question synergy of the two hypotheses as experimentalists mostly found evidence for one or the other hypothesis. It is important to realize that lumen formation by vacuolation is mostly studied in small intersegmental vessels (ISV) of zebra fish, whereas cell-cell repulsion is mostly studied in aortae of mice. In the model, when lumen formation by synergy of the two hypotheses is performed in a one-cell thick vessel, the resulting lumen formation visually resembles vacuolation, whereas it visually resembles cell-cell repulsion when it is performed in a multi-cell thick vessel. In conclusion, the computational model of lumen formation suggests that vacuolation and cell-cell repulsion work synergistically and that the discrepancy between observations of different experimental groups might be explained by the vessel sizes they are studying.

Chapter 5 proposes global sensitivity analysis as a tool to study and falsify morphogenesis models, using a model of vascular morphogenesis as a case study. The exact mechanisms that drive vasculogenesis *in vivo* are not yet clear. Our group has generated multiple cell-based models that support different mechanistic hypotheses. The proposed mechanisms for blood vessel formation may all be functional *in vivo*, but at different times in the process or under different environmental circumstances, or perhaps work simultaneously to reinforce one other. However, some mechanisms may merely be functional for blood vessel formation *in silico*. Despite many attempts for biological validation, we cannot give a final answer to this question. In Chapter 5, we suggest a global sensitivity analysis for such models as a new validation tool. In this chapter, we introduce a workflow to perform global sensitivity analysis on non-linear, multi-factorial models, using the cellular Potts-based model of contact-inhibited chemotaxis for network development as example. A global sensitivity analysis ranks the impact of parameters and their correlations on vascular network formation. Comparing the ranking of different models with knowledge derived from experimental data on the impact of the parameters can help to falsify models. Additionally, the sensitivity analysis results can be used to generate suggestions for validation experiments. The global sensitivity analysis for the contact inhibition model showed that *in silico* sprouting, measured by compactness, requires a combination of parameters that drive different mechanisms in the model. In contrast, the lacuna count of network depends only on the diffusion coefficient of the chemoattractant that is secreted by the endothelial cells. By a future study of each of the alternative models with this global sensitivity analysis approach, we hope to falsify some of the models and find the true operand mechanisms in vascular network development.

In summary, this thesis makes use of cell-based computational modeling to gain insight in different steps of angiogenesis and vasculogenesis, address-

ing questions that often originate from experimental observations. As a modeling philosophy, we study how mechanistic properties on the lower scale affect patterning of the higher scale, e.g. from cell shape to vascular networks, from proteolytic enzyme interactions to matrix invasion, and from fusion of subcellular vacuoles to lumens. In this thesis, we have shown that this modeling philosophy can help us to understand the counter-intuitive and unexpected phenomena in biology.