High-throughput simulation studies of angiogenesis - Reverse engineering the role of tip cells and pericytes in vascular development

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Summary

Angiogenesis is the process by which new blood vessels develop by splitting of or by sprouting from existing vessels. The sprouts formed in the latter mechanism, known as sprouting angiogenesis, branch out and connect with other sprouts to form a new network of blood vessels. This process involves both the endothelial cells, which make up the inner lining of a vessel, and the perivascular cells, which surround the vessel. The collective behavior of these cells results in the formation of sprouts and eventually vascular networks. The cells involved in angiogenesis differ in shape and behavior, which affects their collective behavior. Furthermore, the cells also affect one another via diffusive and membrane bound signaling molecules. In this thesis we aim to understand how the interaction between multiple cell-types exhibiting subtle differences in behavior change the resulting collective angiogenic sprouting.

To this end, we developed cell-based, computational models of angiogenesis, based on the cellular Potts model. The inputs of these models are the observed or hypothesized behavior of individual cells and the output is the resulting collective cell behavior: e.g., the formation of angiogenic sprouts or vascular networks. By assigning different behavior to a subset of the cells, these models can be used to study the interplay between cell types exhibiting different behavior. Because the exact parameter values are not always known, we need to perform simulations for a wide range of parameter values. For this, we developed a high-throughput simulation pipeline, which is presented in chapter 5, that automates setting up the simulation scripts, running the simulations on a computer cluster and analyzing the results. This pipeline allowed us to screen a wide range of new hypotheses concerning the differences between cell types, and thereby enabled us to develop new hypotheses that could be tested in the wet-lab.

In chapter 2, we studied the role of cell elongation in angiogenesis. Based on *in vitro* observations, previous simulation studies have proposed that cells form vascular networks because they are attracted to one another via an autocrine chemoattractant. With such a mechanism cells form aggregates, unless additional mechanisms make the cells organize into sprouts. One of these additional mechanisms is cell elongation. To understand how the elon-

gated cell shape contributes to the formation of network-like structures, we studied the aggregation of elongated cells in absence of the chemoattractant. We found that also without chemotaxis cells organize into networks, provided that the cells slightly adhere to one another. The elongated cells align side-to-side and thereby formed cell clusters. Individual, elongated cells rotate easily, but rotation becomes increasingly difficult as the cluster size increases. As the clusters grow in size, due to cell-cell adhesion, the dynamics slows down, until it essentially stalls. Even though the configuration still slowly evolves towards the equilibrium, consisting of a single cluster with groups of aligned cells, this equilibrium is not reached in practice; this phenomenon is known as "arrested dynamics". In the model with chemoattraction between the cells, the pattern continues to evolve, which suggest that in that model the network represents a true equilibrium.

With the insights into the role of cell elongation obtained in this way, in chapter 3 we studied the role of pericytes in angiogenesis. Pericytes are a type of perivascular cells that are observed in growing sprouts during ocular and tumor angiogenesis. To better understand how pericytes are involved in angiogenesis we attempted to reproduce *in vitro* observations of co-cultures of endothelial cells and pericytes with computational modeling. *In vitro*, endothelial cells and pericytes rapidly form networks. Unlike the stable networks formed in endothelial monocultures, the networks formed in the mixed cultures collapse into a cluster. Interestingly, new sprouts extend from these clusters after a couple of days. To test if and how reported chemotactic interactions between endothelial cells and pericytes could cause the *in vitro* patterns we simulated several model variants that differ in which cells secrete which chemoattractants. For this, we built a model with elongated endothelial cells and round pericytes that are each attracted to their own autocrine chemoattractant. Then, we generated model variants by adding additional chemoattractants for endothelial cells and varying which cell types secrete which chemoattractant. We found that networks develop in simulations where pericytes secrete a chemoattractant for endothelial cells and vice versa. Similar to the *in vitro* experiments, these network are unstable and quickly collapse into a cluster. To also reproduce sprouting, one model adjustment was needed: Not pericytes secrete a chemoattractant for endothelial cells, but endothelial cells that are in direct contact with pericytes secrete that chemoattractant. Together, the results presented in chapter 3 hypothesize crosstalk between endothelial cells and pericytes via chemoattractants. Whether these interactions indeed play a role in the collective behavior of endothelial cells and pericytes during angiogenesis is the topic of ongoing investigations.

The study presented in chapter 4 concerns differences between two subtypes of endothelial cells: the tip cells that lead the sprouts, and the stalk

cells that follow the tip cells and proliferate to facilitate sprout extension. Whereas tip and stalk cell differentiation is necessary for *in vivo* angiogenesis, computational models and cell culture models can recapitulate aspects of blood vessel formation in monocultures, without tip and stalk cell differentiation. To develop new ideas on the mechanisms by which tip cells could contribute to blood vessel formation we extended an existing computational model with tip and stalk cell differentiation, avoiding any a priori assumptions about the differences between tip and stalk cells. We then systematically changed the behavior of the tip cells, to identify model variants in which the computational tip cells' behavior matched that of real tip cells: They lead sprouts and impact the resulting blood vessel networks. Our model predicted that tip cells may be less attracted to the chemoattractant. Interestingly, this prediction matched the expression pattern of a known molecular signal, called Apelin. We tested our computational predictions in an actual cell culture model of angiogenic sprouting, which indeed turned out to be sensitive to interference with Apelin signaling.

In this thesis we aimed to understand how the interactions between cell-types exhibiting different behaviors affect angiogenic sprouting. In high-throughput simulation experiments we varied the chemotactic interacts between endothelial cells and pericytes and found that such interactions could reproduce the patterns these cells formed in *in vitro* cocultures. With a similar approach we searched for cell behavior for which computational tip cells resemble real tip cells. In this manner we found that the reduced sensitivity of the tip cells to Apelin may cause them to lead sprouts and affect the morphology of vascular networks. Altogether, in this thesis we reverse engineered possible roles for pericytes and tip cells in angiogenesis by performing high-throughput simulation experiments.