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6.1 Summarizing discussion

In this thesis we aimed to better understand why several types of cells with different behavior are involved in blood vessel formation. In particular, we studied how endothelial cells and pericytes interact during blood vessels formation (chapter 3), and we studied the role of tip and stalk cell differentiation of endothelial cells in angiogenesis (chapter 4). For this we used simple, cell-based, computational models. Such models can show how a multicellular tissue develops due to the collective behavior of single cells with cell-type specific behaviors and are therefore a good tool to study the role of specific cell types in angiogenesis. Whereas most previous modeling studies derived the modeled cell behaviors from experimental observations, we used our model to predict cell behavior that is sufficient for reproducing experimental observations. We chose this approach because it is often hard to infer the behavior of single cells from multicellular experiments in which the behavior of a cell is affected by the other cells and the cell’s environment. This modeling approach requires model simulations for large ranges of parameter values. For this, we created a protocol for large scale parameter sweeps with cell-based models (chapter 5). While this protocol was developed with the cellular Potts method and the modeling framework CompuCell3D in mind, the method can easily be adapted for other modeling methods and corresponding software that can run without user interaction.

Instead of building a new model for each of the topics we studied, we built upon two previous, computational angiogenesis models. In the first model, networks are formed by elongated cells that chemotact towards a chemoattractant secreted by all cells [25]. In the second model, round cells form networks due to contact-inhibited chemotaxis towards a chemoattractant that all cells secrete [28]. Before we can build upon these models, we must fully understand the mechanism that drives angiogenesis in each of the models.
In the contact inhibition model, angiogenesis is driven by two mechanisms. Initial sprouts form by the random movements of highly motile cells, or due to buckling caused by the inward force of chemotaxis on a volume conserved blob of cells. The chemoattractant gradient at the tips of these small sprouts is shallower than in between the sprouts and therefore new extensions are most likely at the sprout tips [28]. In contrast, for the model with elongated cells, it was not yet understood why these elongated cells form networks. Therefore, we studied how cell elongation can drive angiogenesis (chapter 2). For this we thoroughly analyzed the evolution of the networks and the behavior of individual cells using methods commonly used in liquid crystal theory. By analyzing the ordering of cells during pattern formation we showed that elongated cells tend to align along their long axis and this alignment is independent of chemotaxis. The clusters of cells that form due to alignment are limited in their rotation and this inhibits network collapse. These results suggest that cell elongation promotes the formation of branches and prevents merging of those branches.

After we analyzed how elongated cells contribute to angiogenesis we used elongated cells in a model of that includes two cell types: endothelial cells and pericytes (chapter 3). Pericytes are perivascular cells that wrap around small blood vessels [97]. They are thought to stabilize blood vessels [97], but various studies indicated that pericytes also play a role during angiogenesis [69–71]. When endothelial cells and pericytes are combined in a vasculogenesis assay, a network is formed that quickly collapses and from the resulting blob of cells sprouts reappear. To identify the endothelial-pericyte interactions may cause this pattern formation we built an angiogenesis model that includes the two cell types and tested for what endothelial-pericyte interactions the model reproduces the in vitro pattern formation. The behavior of endothelial cells and pericytes were based on vasculogenesis assays with only one cell types. In these assays endothelial cells formed networks that resembled those formed with elongated cells and therefore they are modeled as elongated cells that secrete their own chemoattractant. Pericytes formed networks that quickly collapsed into blobs and these patterns can be reproduced by modeling pericytes as round cells that secrete their own chemoattractant. Based on the endothelial-pericyte interactions described in the literature, we proposed two signaling scenarios: paracrine signaling and contact-dependent signaling. In the paracrine signaling scenario endothelial cells secrete a chemoattractant for pericytes and vice versa. In the contact-dependent signaling scenario endothelial cells secrete a chemoattractant for pericytes, and endothelial cells that contact pericytes secrete a chemoattractant for endothelial cells. Then, we systematically varied the chemoattractant properties for both scenarios, using the protocol from chapter 5. With both signaling scenarios the model reproduced network formation.
and network collapse, but only for the contact-dependent signaling scenario sprouting occurred after network collapse. These results suggest that endothelial cells and pericyte interact during angiogenesis via secreted chemoattractants.

Next, we studied the role of tip-stalk cell differentiation in angiogenesis to better understand why these two endothelial cell phenotypes are involved in angiogenesis. For this, we searched for cell behaviors that cause cells to lead and affect the resulting network morphology. We adapted the contact-inhibition model of angiogenesis to include both tip and stalk cells. Then, one by one, we varied the values of each parameter that controls tip cell behavior to find parameter values for which tip cells lead sprouts and affect angiogenesis. In this manner we found that when tip cells are less attracted to the chemoattractant secreted by all endothelial cells than stalk cells, these tip cells lead sprouts and affect angiogenesis. A study of published gene expression studies pointed towards Apelin as a candidate for this chemoattractant. Apelin is a chemoattractant for endothelial cells that is secreted by endothelial cells and the Apelin receptor, APJ, is only detected in stalk cells. Thus, Apelin is a chemoattractant for stalk cells and not for tip cells. We tested this hypothesis by inhibiting Apelin signaling in spheroid sprouting assays with either a wild-type population of endothelial cells, or a population of only stalk cells. In absence of tip cells, few sprouts develop in such sprouting assays. As we expected, Apelin inhibition reduced sprouting in spheroids with a wild-type endothelial cell population, while having little effect on spheroids with only stalk cells. These results suggest that Apelin could be a cause of the differential behavior of tip and stalk cells.

Altogether, we used cell-based models to study the role of cell mixing in angiogenesis. Specifically, we studied the interactions between endothelial cells and pericytes during angiogenesis, and the role of tip and stalk cell differentiation in angiogenesis. In both cases we used high-throughput simulations to explore the effects of cell behaviors or the properties of signaling molecules. In this manner we proposed that, during angiogenesis, endothelial cells and pericytes interact via diffusing molecules. Furthermore, we suggested that tip cell selection can stabilize network formation with tip cells, and we hypothesized that Apelin may be a cause of the differences in behavior of tip and stalk cells.

6.2 Future work

6.2.1 From abstract to realistic models

In this thesis we used relatively simple models to study the collective behavior of mixed cell types in angiogenesis. Such models provide a good tool to study the mechanisms by which several cell types affect angiogenesis. While
these model results can be compared with in vitro experiments on a qualitative level, as we did in chapters 3 and 4, comparing simulation results to experimental results at a quantitative level is often complicated. For example, when the proteins used for in vitro perturbations are not represented in the model, the in vitro perturbation must somehow be translated to a model parameter. This can only be done if the quantitative link between the protein and the model parameter is known, otherwise the model cannot be compared to experiments at a quantitative level. As a result, model validation is limited, as well as the predictive power of the models. Here we will discuss several improvements that would make the models presented in this thesis more realistic, and therefore more similar to in vitro and in vivo experiments.

In our models, cells behave according to a set of rules that remained unchanged during the simulation. However, in reality, the behavior of cells is the result of subcellular signaling in response to external signals. By including the subcellular regulation of cell behavior, the models can be directly related to in vitro experiments in which pathway components are upregulated, knocked out, or added ectopically. In the model with tip and stalk cells (chapter 4), cell fate depends on a simplified subcellular pathway but this pathway is not directly linked to cell behavior. Furthermore, in the model with elongated cells (chapter 2) and in the model with pericytes and endothelial cells (chapter 3) none of the cell behavior is connected to subcellular signaling. However, regulation of cell shape in time and space could affect the model outcome, but we did not consider this for cell elongation. In the literature two hypotheses are proposed for the cause of endothelial cell elongation. Several studies proposed that endothelial cells elongate in response to vascular endothelial growth factor (VEGF) [78, 79, 207]. If the VEGF concentration is homogeneous and constant over time, all cells will elongate and this would not affect the model outcome. However, when the VEGF concentration is heterogeneous, or changes over time, the model outcome could be affected. Other studies report that endothelial cells elongated in response to stress in the extracellular matrix (ECM) [208]. A recent computational model of angiogenesis in which the forces cells exert on the ECM induce strain-stiffening supports this hypothesis [126]. Elongation induced by stress in ECM implicates that cells elongate during angiogenesis. Overall, for both hypotheses cell elongation may occur during morphogenesis. Therefore, we should test if elongation during pattern formation affects the formed patterns.

As mentioned above the ECM may affect the shape of cells. Furthermore, the ECM can also affect the distribution of diffusive growth factors and signaling molecules by fixating them. A well-known example of a growth factor that binds to the ECM is VEGF-A [209]. VEGF-A has a heparin binding domain via which it can bind to heparin sulphate proteoglycans, which are part of the ECM [210]. Experiments with embryos that produce VEGF lacking the heparin
binding domain of VEGF showed defects in the developing vasculature [169], indicating that VEGF-ECM binding plays an important role in blood vessel formation. Furthermore, in vitro, the binding and unbinding of VEGF to the ECM determines the spatial VEGF pattern [52]. Köhn-Luque and coworkers [29, 52] built a model based on this hypothesis and showed that spatial pattern of VEGF via ECM binding can cause endothelial cells to form networks. In our study of pericyte-endothelial interactions we considered VEGF as one of the possible chemoattractants for endothelial cells, but we did not consider ECM binding. Therefore, a next step in this study would be to consider ECM binding of the chemoattractants. In this way we can further characterize what properties the chemoattractants should have to cause the patterns we observed in the in vitro experiments.

When considering mechanical feedback between cells and the ECM and the effect of the ECM on local gradients, we assumed that cells are moving on top of the ECM. In contrast, in 3D cells migrate through the ECM and this requires cells to degrade the ECM. In our study of endothelial tip and stalk cells we validated our 2D modeling results using a 3D sprouting assay. Because of this difference, we could only compare the results at a qualitative level. Furthermore, there were some discrepancies between the model and the experiments, which could be result of this difference. Therefore, to further validate the role of Apelin in tip and stalk cell behavior we should adapt the model presented in chapter 4 to include ECM tunneling and again compare the simulation results with the 3D sprouting assays. Creating a 3D model of angiogenesis with tip and stalk cells is possible, but such a model would be computationally expensive. Because of this, we propose a 2D approach in which cells have to degrade ECM before they can migrate. This approach has been presented previously in a 2D cellular Potts model that studied the role of haptotaxis and haptokinesis [197].

Up to here we discussed the role of the ECM and the regulation of cell behaviors that are included in our models. However, in all the models we omitted proliferation of endothelial cells based on the assumption that cells do not divide in in vitro assays. This assumption only holds for in vitro vasculogenesis where there is a supply of endothelial cells. In contrast, during in vivo sprouting angiogenesis, proliferation is necessary for sprout extension [56]. The cells that proliferate are the stalk cells just behind the tip cells, while the stalk cells farther away from the tip become quiescent cells that do not proliferate [15]. Exactly how this is regulated is not yet clear. Interestingly, both pericytes and Apelin have been implicated to play a role in the regulation of stalk cell proliferation [168]. Pericytes are known to stabilize blood vessels by inhibition proliferation [101]. Apelin, which is secreted in large amounts by tip cells [123, 142, 143], inhibits pericyte recruitment [168]. Thus, close to the sprout tip, there are no pericytes and the stalk cells proliferate. Farther away
from the tip, pericytes are associated to the sprout and proliferation is inhibited. This new hypothesis has been proposed based on experimental data [168], but has not yet been tested mechanistically. Our previous work on both pericytes and tip and stalk cells gives us the ability to test this hypothesis with a computational model.

6.2.2 Validation of angiogenesis models

As discussed in section 1.2.1 several models can explain the formation of vascular network formation. In these models network formation is driven by processes such as: contact-inhibited chemotaxis [28], mechanical interactions between cells and the ECM [21–24, 83, 126], attraction to elongated structures [30, 31], and chemotaxis to ECM bound VEGF [29, 52]. Several of these models can partially reproduce in vitro vasculogenesis. The cell-elongation model reproduces the temporal dynamics of pattern coarsening that is observed in vasculogenesis assays with HUVEC on Matrigel [25]. Furthermore, the models based on mechanical interactions between cells and the ECM reproduce the dependence of network formation on the stiffness of the ECM [22, 126]. Finally, in the angiogenesis model that considered VEGF-ECM association, the morphometrics of the networks fitted very well to the metrics of networks of quail embryos [29]. However, this fit only holds for a narrow range of parameters, as was shown by a parameter sensitivity analysis of the model [29]. Altogether, vascular network formation can be reproduced with models based on various hypotheses. Therefore, it remains unclear which hypothesis is true. It is likely that multiple mechanisms can play a role at the same time and that their importance depends on the stage of network development and the environment. For example, mechanical feedback only works when the substrate is sufficient stiff, while the several other mechanisms can produce networks independent of the substrate’s mechanical properties.

To determine which model is correct in which specific context of angiogenesis, we focus on the model parameters. A model is only valid when the parameters for which the model fits to experimental data are realistic for that experiment. Using our approach of high-throughput simulations, we can explore the parameters of each of the models and find parameter sets for which a certain model fits experimental data. Note that ideally, this fit not only concerns the final network, but also the network development over time. Then, for each model and parameter set we should determine whether the parameter values are realistic for the experimental data that fits with the model outcome. If a model reproduces experimental patterns with realistic parameters, the modeled mechanism may be the cause of those patterns. In this manner we could link the various proposed mechanisms for angiogenesis to specific experimental setups and use the correct model for further studies related to that experimental setup.