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Computational modeling of angiogenesis : from matrix invasion to lumen formation

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Introduction

This chapter is based on:

- Sonja E. M. Boas, Margriet M. Palm, Pieter Koolwijk and Roeland M.H. Merks, *Computational Modeling of Angiogenesis: Towards a Multi-Scale Understanding of Cell-Cell and Cell-Matrix Interactions*(2013) Mechanical and Chemical Signaling in Angiogenesis, Studies in Mechanobiology, Tissue Engineering and Biomaterials Volume 12, 161-183
- M. G. Dallinga and Sonja E. M. Boas *et al.*, *Tip cells in angiogenesis* (2015) Tip Cells in Angiogenesis. In: eLS. John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0025977
- Sonja E.M. Boas, Yi Jiang, Roeland M.H. Merks, Sotiris A. Prokopiou and Elisabeth G. Rens *Cellular Potts model: applications to vasculogenesis and angiogenesis* In: Louis, P.-Y. and Nardi, F.R. (Eds.) Probabilistic Cellular Automata: Theory, Applications and Future Perspectives. Springer. (in press)

1.1 Angiogenesis and vasculogenesis

Blood vessels supply tissues with oxygen and nutrients and carry away waste products. A network of blood vessels is formed during embryogenesis [1, 2]. Vascular networks form *de novo* from endothelial progenitor cells, a process called vasculogenesis [1, 2]. A well studied model system is vasculogenesis in the retina [3, 4], in which circulating progenitor cells organize into early retinal vessels. Angiogenesis, the formation of new blood vessels from existing vessels, then proceeds to extend the retinal vasculature to the periphery [1, 3, 4]. Throughout our lives, angiogenesis is important in many physiological processes, such as the menstrual cycle and wound healing [1, 5].

Angiogenesis is also involved in many pathological conditions [1, 6]. Tumor growth is highly dependent of sufficient blood supply [2]. The core of a growing tumor becomes necrotic and sends out signals to attract new blood vessels. Similarly, blood vessels are attracted towards the inflamed region in rheumatoid arthritis [7]. As a result, the inflammation is sustained by the increased delivery of inflammatory cells, nutrients, and oxygen for the proliferating inflamed tissue [7]. In age-related macular degeneration (AMD), pathological ocular neovascularization can cause severe vision loss [8]. Whereas medical therapies focus on inhibition of angiogenesis in cancer, rheumatoid arthritis and AMD, diabetic patients can suffer from wound healing defects caused by reduced levels of angiogenesis [6, 9]. A better understanding of the mechanisms of angiogenesis aids the development of such medical therapies.

Besides for medical therapies, a true understanding of vasculogenesis and angiogenesis is important for tissue engineering [10–12]. Over the last decades, major steps have been taken in the field of tissue engineering, aiming for the *in vitro* growth of entire organs [13]. Oxygen supply through simple diffusion is insufficient for such large tissues and requires vascularization of the tissue. Creation of a functional vasculature is challenging and would benefit from a better understanding of angiogenesis [10–13].

In this thesis, we use computational modeling to gain insights in the mechanisms of angiogenesis.

1.2 The mechanisms of angiogenesis

Driven by the goal to improve medical therapies and tissue engineering, angiogenesis has been extensively studied. As holds for most biological processes, angiogenesis showed to be a very complex process. Figure 1.1 shows an overview of the major components involved in angiogenesis. A blood vessel wall consists of quiescent endothelial cells, also known as phalanx cells [14]. Phalanx cells can be activated by signals from nearby regions that demand more blood supply, such as wounds, tumors or inflamed tissues. Vascul-

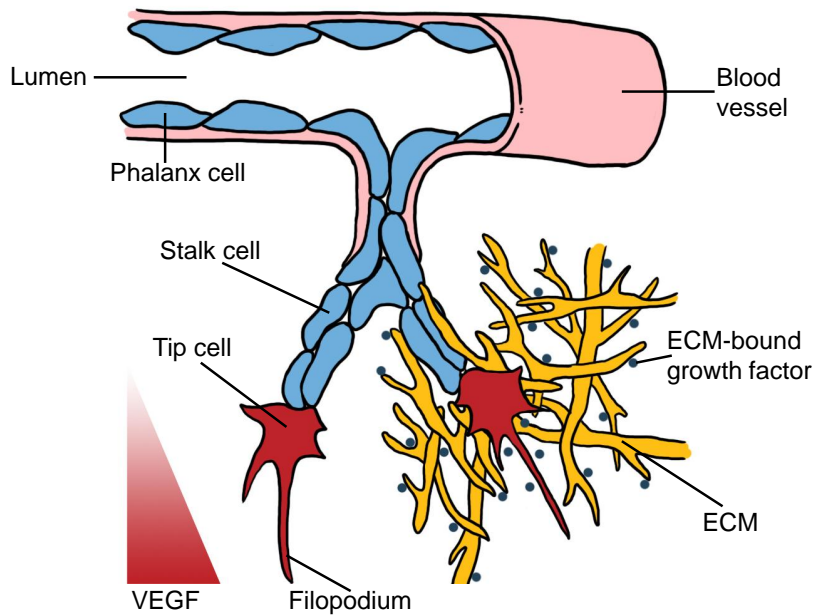


Figure 1.1: Overview of the mechanisms of blood vessel formation. The quiescent endothelial cells that form the capillary blood vessel are called phalanx cells. An external signal, such as the chemoattractant VEGF that is secreted by hypoxic tissue, can activate phalanx cells in a nearby vessel to form a sprout towards higher concentrations of the signal (gradient indicated by red triangle). Upon activation, endothelial cells differentiate into tip cells and stalk cells. Tip cells form long filopodia to sense the environment and lead the sprout. Blood vessels are surrounded by an extracellular matrix, composed out of a web of fibers and matrix-bound growth factors. Endothelial cells invade the matrix by degrading it, which can result in the release of matrix-bound growth factors to stimulate further sprouting. This figure was adapted from [16]

lar endothelial growth factor (VEGF) is a well known growth factor that serves this purpose [3, 15]. VEGF activates phalanx cells in nearby vessels and promotes sprouting of new blood vessels towards higher concentrations of the growth factor, the growth factor gradient is indicated by the red triangle in Figure 1.1. Here, we discuss the major steps of angiogenesis: matrix degradation, tip cell selection and vessel maturation. Each of these steps are the focus of a chapter in this thesis.

1.2.1 Matrix degradation

During sprouting, there is an intensive interaction with the extracellular environment. Blood vessels are surrounded with extracellular matrix (ECM, see yellow fibers in Figure 1.1), the non-cellular component present within all tissues and organs that provides structural support [17]. The extracellular matrix is a web of interlinked fibers, such as collagen, laminin or fibrin fibers [17]. In addition, many proteins such as growth factors bind to the extracellular matrix [18]. Sprouts invade the surrounding matrix by creating a tunnel through it. This tunnel is made by active proteolytic degradation of the matrix fibers [19]. For this purpose, cells secrete proteolytic enzymes that cut the matrix fibers. This can result in the release of growth factors that were bound to the matrix (see blue dots in Figure 1.1), which can modulate sprouting behavior [20]. A new hypothesis for the interactions between proteolytic degradation and invasion of the matrix is introduced in Chapter 2.

1.2.2 Tip cell selection

Upon stimulation of phalanx cells with a growth factor from the extracellular environment, activated endothelial cells differentiate into one of two fates: 'tip cells' or 'stalk cells' [3, 21–23]. In Figure 1.1, tip cells are colored red, whereas phalanx cells and stalk cells are colored blue. Tip cells are the leaders of sprouts and have long filopodia to sense signals from their environment, such as the VEGF gradient [3]. Stalk cells form the base of the sprout and elongate the sprout by proliferation [14]. Selection of tip and stalk cells occurs by lateral inhibition through Dll4-Notch signaling [3, 21–23]. Dll4 is a ligand on a tip cell membrane that interacts with the Notch receptor on a neighboring stalk cell. Activation of Notch result in the cleavage of the Notch-intracellular domain (NICD), which then travels to the nucleus for transcription of Notch target genes [21]. Eventually, cells with low Notch activity (low Notch/high Dll4) become tip cells and cells with high Notch activity (high Notch/low Dll4) become stalk cells [3].

It was long thought that once endothelial cells have differentiated into tip and stalk cells, that the tip cell present at the sprout tip would stay the leader of the sprout [3]. In contrast, more recent experimental studies show that there is a continuous competition between cells for the sprout tip position [24, 25], a process called tip cell overtaking. In Chapter 3, we study the biological function of tip cell overtaking.

1.2.3 Vessel maturation

Newly formed sprouts are not yet fully functional. To close circulation, the sprout needs to connect to a nearby sprout or blood vessel, a process called anastomosis [26]. Perivascular cells, referred to as pericytes, vascular

smooth muscle cells or mural cells, are recruited to sprouts to envelop the surface of the vascular tube and promote stabilization and maturation of the sprout [27].

The cord of cells that forms the sprout needs to hollow such that blood can flow through. The mechanisms of hollowing or lumen formation have been debated for centuries. Experimental research has led to two main hypotheses: vacuolation [28–31] and cell-cell repulsion [32, 33]. During vacuolation, vacuoles are suggested to form by the fusion of pinocytotic vesicles. Initially, lumens were thought to form intracellularly by spanning the cell with a large vacuole that then fuses to the cell membrane on both sides of the cell [28, 29]. Later, lumens were also suggested to form extracellularly by the secretion of vacuoles between cells [30, 31]. During cell-cell repulsion, cell membranes of adjacent cells are suggested to repulse each other to form an extracellular lumen between the cells [32, 33]. Both hypotheses are supported by 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, separately and in combination.

Each of these steps in angiogenesis are intensively studied in the laboratory as well as with mathematical or computational models. The next section summarizes the experimental assays that have been used for these studies. In the following section, we discuss the usefulness of modeling and introduce computational models of vasculogenesis and angiogenesis. Finally, we give the outline of this thesis.

1.3 Experimental models of angiogenesis and vasculogenesis

Experimental models of angiogenesis and vasculogenesis can roughly be categorized in cell cultures, organ cultures, and *in vivo* models. A good overview of these experimental models is given in [34–37], here we discuss the assays that are most relevant for the validation experiments in this thesis.

1.3.1 Cell cultures

Cell cultures are well-suited to study specific steps in angiogenesis, such as proliferation, matrix invasion, cell migration, and tube formation [34–37]. In two-dimensional cell cultures, cells are placed on a plastic dish that is coated with ECM proteins for adhesion [37]. This assay is often used to study cell proliferation or migration. It is also suitable to study vasculogenesis, since vasculogenesis in the retina is considered a two-dimensional process.

Tube formation assays are developed to investigate angiogenesis in a three-dimensional environment (e.g. the assay by Koolwijk *et al.* [38] in Chapter 2).

1. Introduction

The endothelial cells are seeded on top of a three-dimensional matrix, invade the matrix and form tubular structures [38, 39]. Different substances can form the extracellular matrix. Matrigel is a popular matrix for angiogenesis assays, because it naturally contains pro-angiogenic growth factors. It is secreted by mouse tumor cells and is composed of a mixture of ECM proteins, such as laminin and collagen. Alternatively, cells are cultured on purified matrices, such as collagen matrices [40] or fibrin matrices [38, 39], of which the composition is more controlled.

Instead of seeding a monolayer of endothelial cells on top of a matrix, embryoid bodies of endothelial cells can be placed within an extracellular matrix [37]. Subsequently, sprouts grow out from these embryoid bodies. Jakobsson *et al.* [24] monitored the cell migration trajectories during sprouting in this assay to study tip cell overtaking.

1.3.2 Organ cultures

In comparison to cell cultures, organ cultures contain multiple cell types and there is a larger heterogeneity between the cells [34–36]. Commonly used organ cultures are retinal explants and the rat aortic ring assay [36]. In the former assay, the retina is dissected, covered with a collagen matrix and stimulated with VEGF. In the latter, a segment of the aorta is cultured *in vitro*. This assay was for instance used by Arima *et al.* [25] to study sprouting dynamics and tip cell overtaking.

1.3.3 In vivo models

The environmental conditions of angiogenesis *in vivo* are far more complex than in the isolated cultures. This makes *in vivo* experiments more complicated to interpret, but also essential for an understanding of the complete system. The mouse retina model is extensively used to study angiogenesis [41]. The retinal vasculature is immature in mouse pups and pruning of these easily accessible, developing vessels has been of great value to gain a mechanistic understanding of angiogenesis [41]. The *in vivo* ischemia retina model is commonly used to study retinopathy [36]. Mouse pups are exposed to high-oxygen condition to induce vessel regression, and angiogenesis into avascular regions is studied after elevation hereof. In the hindlimb ischemia model, angiogenesis is triggered in the leg muscle after ligation of the main artery in the thigh. Zebrafish embryos form a particular well model to study embryonic processes due to their transparency in this stage. This allows for live time-lapse imaging of blood vessel formation over long periods, as was e.g. done by Kamei *et al.* [29] and Blum *et al.* [30] to study lumen formation.

1.4 Computational models of angiogenesis and vasculogenesis

Angiogenesis and vasculogenesis research can benefit from computational modeling in three ways. Firstly, computational models help to gain overview in such complex systems by testing which components and interactions are minimally required. These components and interactions can then be examined to understand their function and predict their effects. By modeling mechanisms on a lower level of organization, e.g the cell, predictions can be generated on a higher level of organization, such as the blood vessels in vasculogenesis. This way, computational models are not only useful to gain mechanistic understanding of angiogenesis, but also to propose new therapeutic targets.

Secondly, computational models can discriminate between and select from alternative hypotheses. Often, more than one hypothesis explains a biological observation, such as network formation from dispersed endothelial cells. Computational models can test the sufficiency of each hypothesis to reproduce the biological observations. Predictions that result from these models can be validated experimentally to support or reject the tested hypotheses.

Thirdly, computational models can connect and combine knowledge on single proteins and mechanisms to examine angiogenesis as a system. Experimental research is often limited to a specific step or protein in angiogenesis and does not grasp how this part is integrated in the whole. Ultimately, multi-scale computational models would include processes at multiple scales, such as tissues, cells, and molecules simultaneously.

1.4.1 Modeling techniques

Several modeling techniques can be used to model angiogenic sprouting and vasculogenesis. We categorize them in continuous models, single-particle models and multi-particle models [42].

1.4.2 Continuous models

Continuous models describe variables as continuous in time and space and can be described using ordinary differential equations (ODEs) or partial differential equations (PDEs). ODEs predict the change in concentration of e.g. proteins, without considering the spacial component. Ventrakaman *et al.* [43] used a system of ODEs to represent the concentration of proteins that are completely mixed in solution. They showed a bistability in the concentrations of plasmin, which degrades fibrin. ODEs can also describe the protein reactions within cells. Collier *et al.* [44] used this technique to develop the first mathematical model of pattern formation due to Dll4-Notch dependent lat-

eral inhibition in a static monolayer of cells [44]. A system of ODEs described the level of Notch activation and the level of Delta activity in each cell. They obtained alternating patterns of cells with high and low Delta expression using a simple rule to describe lateral inhibition: "the more intense the inhibition a cell receives, the weaker its ability to deliver inhibition must become" [44]. Sprinzak *et al.* [45, 46] developed a more refined ODE model of Delta-Notch signaling to study the effect of cis-interactions, the mutual inactivation of interacting Delta and Notch of the same cell, on lateral inhibition [45, 46]. They showed that cis-interaction between Delta and Notch speeds up the patterning dynamics and amplifies the feedback in lateral inhibition.

If the spatial aspect is relevant for a question, one can model the system as a continuum or density function using the conservation of mass equation to formulate a PDE. PDEs are often used to model the migration of cells, with cells represented as densities [47–50]. Anderson & Chaplain [47] used a PDE model to explain why endothelial cells in capillary sprouts stop migrating towards a tumor in absence of mitosis. Cell migration depends on diffusion, chemotaxis towards a tumor-derived angiogenic factor, and preferential migration towards higher concentrations of extracellular matrix proteins (haptotaxis). The model suggest that mitosis is required to desensitize endothelial cells for the angiogenic factor when they become saturated in regions near the tumor where there are high concentrations of angiogenic factor. Desensitized cells can reestablish chemotaxis-driven angiogenesis towards the tumor.

Chaplain & Lolas [48] examined the dynamics of the plasminogen-plasmin system during matrix invasion by modeling cancer cells with a PDE. The model includes the density of tumor cells, ECM proteins, the ECM protease plasmin, the plasmin-activator uPA, and the inhibitor PAI-1. Cells secrete uPA and PAI-1. Production of plasmin requires the proximity of uPA and cells, to resemble activation of uPA by binding to cell-bound receptors. The matrix is degraded by plasmin and PAI-1 inhibits matrix degradation by scavenging uPA and plasmin. Cells invade the matrix by random motility, biased with chemotaxis towards uPA, chemotactic repulsion by PAI-1, haptotaxis and proliferation.

Manoussaki [50] studied the role of chemical and mechanical forces in blood vessel formation. In the model, cells exert forces on the extracellular matrix. Cells migrate by passive advection along with the matrix deformation, by a preferential bias along high matrix strain representing aligned fibers, and by chemotaxis towards an exogenously supplied chemical stimuli. Their modeling results suggest that purely mechanical interactions between cells and the matrix can suffice for pattern formation. Besides strain-biased migration due to cell-traction forces, Namy *et al.* [49] also included haptotaxis in their model. This model reproduces experimental observations of the effect of changes in the concentrations of seeded endothelial cells and of matrix

protein concentrations on vasculogenesis.

1.4.3 Single-particle models

In contrast to continuous models, cell-based models represent cells as individual entities and can be categorized in single-particle models and multiple-particle models [42]. Single-particle models represent cells as points or ellipsoids in space. Angiogenesis can be represented at the 'vessel-level', with individual cells modeled as discrete particles. Milde *et al.* [51] combined a discrete particle representation for tip cells with a continuum approximation of VEGF, proteolytic enzymes, matrix and endothelial stalk cells. Tip cells migrate and deposit a track of ECs during migration. Tip cells sense the environment by 'filopodia' and are biased in their direction of migration by the matrix density, haptotaxis and by VEGF. This work shows sprout branching due to the release of VEGF from localized pockets of a matrix-bound VEGF isoform by proteolytic enzymes that are secreted by the tip cell.

In the model by Qutub *et al.* [52], sprouts are composed of connected nodes. The leading node represents the tip cell and the following stalk cells consist of two nodes and can elongate the segment in-between to represent proliferation. Their model setup allows for the integration of modules that describe blood-flow, oxygen transport, VEGF gradients, and tissue geometry to study angiogenesis on a multi-scale level.

In single-particle models, based on Lagrangian dynamics, sprouting occurs when cells follow a random walk biased by attractive and repulsive forces resulting from interactions with nearby cells [53, 54].

1.4.4 Multi-particle models

Single-particle models do not include cell shape or interaction surfaces between cells for signaling. In multiple-particle models, a collection of particles represent a cell and its shape. A cell can for instance be represented by a collection of spheroids that stay in proximity of one other due to higher attractive forces between intracellular particles than between particles of different cells [55].

Alternatively, some multiple-particle models only represent the cell membranes; nodes in the membrane can be connected by vertexes [56, 57] or springs [58]. To study tip cell selection during sprouting, Bentley *et al.* [58, 59] developed a computational model in which the membrane of each endothelial cell is composed of nodes that are interlinked by springs. Filopodia can grow out of nodes and are assumed to extend towards higher concentrations of vascular endothelial growth factor (VEGF) [59]. The model predicted that tip cell patterning will stabilize faster in VEGF gradients than in uniform VEGF environments and that high VEGF levels induce oscillation of the alter-

nating tip-stalk cell pattern. Anastomosis led by filopodia can create new cell-cell junctions with new Dll4-Notch signaling opportunities, which can make tip and stalk cells within the sprout switch fate [58]. Although the cell membrane is composed out of multiple nodes, cells function as single, immobile agents that can switch fate, thus this model might better be categorized as a single-particle model. To study the mechanisms of tip cell overtaking, Bentley *et al.* [60] extended their model with a true multiple-particle representation of cells by using the cellular Potts Model [61, 62] to represent the shape and movement of the cells, thereby explicitly modeling cell-cell adhesion and junctional reshuffling.

The cellular Potts model (CPM) [61, 62] is a commonly used model to study *de novo* sprouting [16, 63–66]. It represents cells as motile patches of lattice sites and includes cell shapes, cell-cell interactions, cell-matrix interactions, and cell motility. The cellular Potts model can easily be coupled to PDEs to describe concentration fields of e.g. proteases, growth factors or matrix proteins [16, 63–66]. In addition, a system of ODEs can be coupled to each cell to model protein interactions within cells and intercellular signaling pathways, such as Delta-Notch signaling [67]. For these reasons, the cellular Potts model is an excellent modeling framework for all questions addressed in this thesis, which involve mechanisms that depend on growth factor gradients, cell-cell signaling pathways and cell-matrix interactions.

1.5 CPM-based models of vasculogenesis and angiogenesis

Since all models of angiogenesis in this thesis are based on the cellular Potts model, we will pay some extra attention to CPM-based models that have already been developed to study which cell behaviors drive sprouting and network formation. This self-organization into sprouts and networks requires communication between the cells. We will discuss three types of communication used for this purpose: chemical signals, cell-cell interactions and cell-matrix interactions.

1.5.1 Chemical signals

Cells can respond to chemical signals, such as VEGF, by migrating towards higher concentrations of it [3], a process called chemotaxis. VEGF is a chemo-attractant known to be secreted by ECs and to attract ECs [70]. Merks *et al.* [64] hypothesized that dispersed ECs form vascular networks when they only chemotact towards EC-secreted VEGF at regions of their membrane that are not adhering to other cells with VE-cadherins, a mechanism called VE-cadherin mediated contact-inhibited chemotaxis. This hypothesis was based

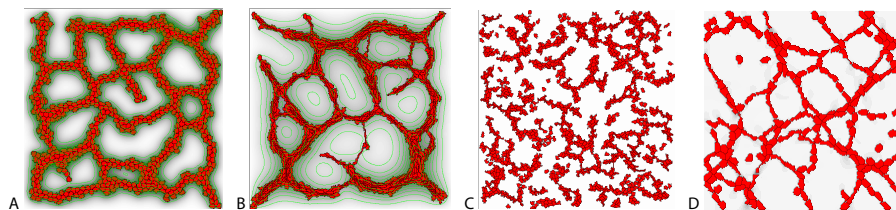


Figure 1.2: Vasculogenesis models. Simulation results of vasculogenesis driven by (A) contact-inhibited chemotaxis [64], (B) cell elongation with chemotaxis [63], (C) preferential adhesion to elongated cells [68], and (D) by mechanical cell-matrix interactions [69].

on the biological findings that cells adhere to each other with VE-cadherins and that VE-cadherins can inhibit VEGF signaling by interacting with the VEGF receptor 2. Indeed, the modeling results showed self-organization of endothelial cells into vascular networks (Figure 1.2A). By the same mechanism of contact-inhibited chemotaxis, sprouts can grow out from spheroids of cells. The latter setup models angiogenesis, rather than vasculogenesis, and for instance represents the *in vitro* assay in which embryoid bodies of endothelial cells are seeded within a matrix.

Another mechanism that can drive network formation is a combination of cell elongation and regular chemotaxis to an auto-secreted chemoattractant [63] (Figure 1.2B). This mechanism also suffices to model angiogenesis from a spheroid of cells.

Although both chemotaxis-based models can reproduce vasculogenesis and angiogenesis, Köhn-Luque *et al.* [71] noted that the diffusion speed assumed for VEGF by Merks *et al.* [64] is much lower than reported for most VEGF isoforms. Köhn-Luque *et al.* [71] proposed an alternative CPM-based model for vascularization in which VEGF, containing ECM-binding domains, is secreted by the underlying endoderm. Endothelial cells scavenge VEGF by the secretion of ECM and subsequently chemotact more strongly to ECM-bound VEGF than to soluble VEGF, resulting in network formation.

1.5.2 Cell-cell interactions

Computational modeling suggests that chemical signals are not always required for sprouting, cell-cell adhesion might suffice [68, 72]. Palm & Merks [72] showed that elongated, adhesive cells can self-organize into vascular structures. Cells aggregate into elongated structures that can only rotate very slowly, while connected in the branch points. If the model would run for infinity, the cells would form a spheroid, but this process is so slow that the cells dynamically arrest in a network-like pattern.

Based on experimental observations of elevated cell motility within the pres-

ence of elongated structures [68], Szabó *et al.* proposed that endothelial cells organize into vascular networks by preferential adhesion to elongated structures [68]. Indeed, this mechanism could drive network formation from initially dispersed cells *in silico* (Figure 1.2C).

1.5.3 Cell-matrix interactions

An interesting form of cell-matrix interactions are cell-derived mechanical signals that are transferred through the matrix [69, 73, 74]. Cells can pull on the matrix to generate strains and respond to strain by preferentially moving towards higher strains [69, 73, 74]. This results in a feedback loop of strain generation and migration that can drive sprouting and vascular network formation [69](Figure 1.2D).

Bauer *et al.* [16] explicitly model matrix fibers and tissue-specific cells and showed that inhomogeneities in the ECM lead to branching and anastomosis of sprouts. Daub & Merks [65] showed that branching can be stimulated by ECM-guided cell migration, or haptotaxis, towards high ECM concentrations. Sprout migration speed, sprout integrity and branching is also affected in this model by haptokinesis, which assumes that cells have an optimal motility at intermediate ECM concentrations. Cells can manipulate these optimal ECM conditions themselves by secreting proteolytic enzymes for matrix degradation.

The effect of proteolytic degradation of the matrix on sprout morphology was extensively studied in one of our computational models [75] that represents an *in vitro* assay of angiogenesis in fibrin matrices [38]. In this assay, sprout morphology ranged from narrow sprouts, to cyst, to the lowering of the complete monolayer. We used computational modeling to study how uPA and MMP secretion by tip and stalk cells affects sprout morphology. MMP degrades the basement membrane and uPA degrades fibrin. A simulation is initiated with a monolayer of endothelial cells, with a single tip cell in the middle, on top of a fibrin matrix with a basement membrane in-between. We assume that the tip cell secretes uPA and MMP at a maximal rate, but that the secretion hereof by stalk cells can be stimulated by the addition of angiogenic factors or inflammatory factors. Figure 1.3 gives an overview of the simulation results as a function of the secretion rates of uPA and MMP by stalk cells relative to the maximal secretion rate by the tip cell. Because the simulations are stochastic, variation is seen between simulations with the same parameter settings; representative simulations were selected for the morphospace in Figure 1.3. Sprout morphology can be grouped in four categories: sprouts, solid round cysts, hollow cysts and monolayers. Sprouts have a cord-like orientation of cells, whereas cysts are more round and multi-cellular. A high secretion of both uPA and MMP (Figure 1.3D) by stalk cells results in lowering of the monolayer. Sprouts are formed for low secretion of uPA (Figure

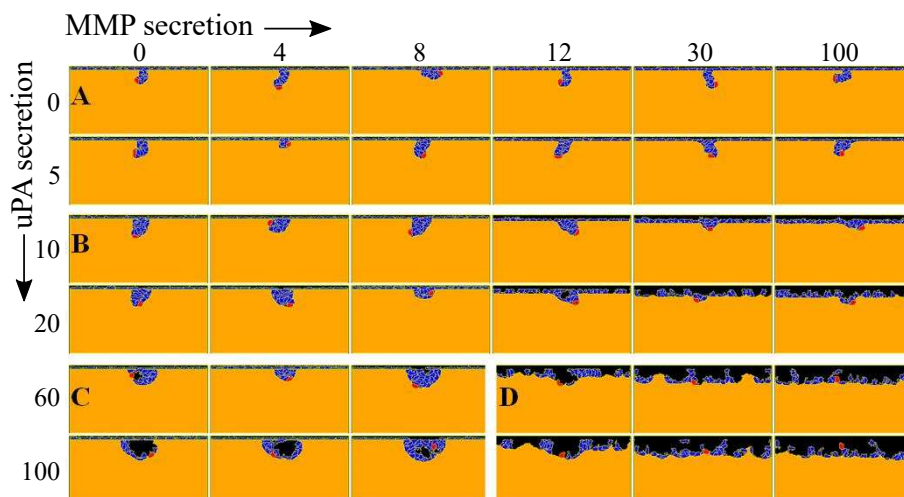


Figure 1.3: Morphospace of proteolytic enzyme secretion by stalk cells. Tip and stalk cells secrete uPA and MMP to degrade fibrin and the basement membrane respectively. The secretion of uPA and MMP by stalk cells is expressed as a percentage of the maximal secretion rate as secreted by the tip cell. This results in different sprout morphologies: (A) sprouts, (B) solid cyst-like structures, (C) hollow cyst-like structures and (D) monolayers.

1.3A), whereas solid cyst-like structures are formed for medium levels of uPA secretion (Figure 1.3B) for all MMP secretion levels by stalk cells. High secretion of uPA and low secretion of MMP (Figure 1.3C) results in hollow-cyst like structures. Occasionally (6 out of 128 simulations), no sprouting occurs for low levels of MMP secretion by stalk cells because stalk cells position themselves between the tip cell and the BM and thereby prevent degradation of the basement membrane. An interesting transition is seen between a hollow cyst-like structure and monolayer lowering for a secretion of MMP between 8% and 12%. The hollow cyst-like structures can be formed since the BM remains intact for attachment of endothelial cells before this transition. These structures are likely to collapse if gravity was included in the model. Experimentally, tubular structures can also disappear due to excessive fibrinolysis [76]. Thus, the intensity and the distribution of proteolytic enzyme secretion over different cell types (tip and stalk cells) seems to be a sufficient explanation for the tissue behavior observed in the laboratory. However, the proteolytic degradation system was strongly simplified in this model and does not include inhibitors of degradation. In Chapter 2, this model is extended with a detailed description of fibrin degradation by the plasminogen-plasmin system.

1.6 Thesis outline

In each chapter of this thesis, there is an overarching question: "How do the behaviors and properties of individual cells affect the collective cell behavior during angiogenesis". With this question in mind, we used CPM-based models to study several aspects of angiogenesis throughout this thesis.

The sprouting process during angiogenesis can be categorized in three themes: extracellular matrix invasion, sprouting dynamics and lumen formation. This thesis highlights each of them:

- In Chapter 2, we study what mechanisms select endothelial cells in a monolayer seeded on an extracellular matrix to form local ingrowth spots and then further invade the matrix to form sprouts. For this purpose, we developed a computational model that represents an *in vitro* model of tube formation [38]. Initial ingrowth and subsequent sprouting is driven in this model by a local, positive feedback loop: cells locally degrade the fibrin matrix and are stimulated in degradation by activators that are released from the matrix by this degradation.
- In Chapter 3, we asked whether the experimentally observed competition of cells for the sprout tip position, called tip cell overtaking, has a biological function or is a side effect of sprouting dynamics. We compared cell trajectories and tip cell overtake rates during sprouting in simulations, with and without regulation by Dll4-Notch signaling, with experimental data.
- In Chapter 4, we used computational modeling to validate two hypotheses of lumen formation. Although there is an ongoing debate on which hypothesis is accurate, our model indicates that both hypotheses might function synergistically and provides a possible explanation for the origin of the debate.
- In Chapter 5, we introduce a workflow for analyzing multi-factorial, non-linear models, such as the CPM-based models in this thesis, with a global sensitivity analysis. So far, such models are mostly studied by changing the parameter values of one or two parameters at the time. This can lead to misinterpretations of the modeling results, because the effect of the other parameters that were kept constant is not taken into account. A global sensitivity analysis studies all parameters simultaneously and determines which parameters or parameter combinations have the largest impact on the model output. A simple model of angiogenesis, based on contact-inhibited chemotaxis, is used as a case study.

Each model in this thesis addresses a biological question that originated from experimental work. Why do only a few cells in a monolayer start to form sprouts? Does tip cell overtaking require regulation by a VEGF-Dll4-Notch pathway? Do lumens form by vacuolation or by cell-cell repulsion? The modeling results give new insights in these questions and generate predictions that can lead further experimental research in this field.