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

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Introduction

Section 1.2 is based on:

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A large network of blood vessels, called the circulatory system, supplies the body with oxygen and nutrients, and removes the waste products of metabolism. The circulatory system starts to develop early on during embryonic development when groups of cells form primitive networks that later connect to form the circulatory system. Disturbance of blood vessel formation during the early stages of development is often lethal because without blood vessels organs do not develop properly. After birth, blood vessel formation continues to facilitate growth and repair.

The smallest blood vessels, called capillaries, develop in a process that is called angiogenesis. This process plays an important role in the abovementioned physiological processes, but it is also important in many diseases. Two common examples of diseases that involve blood vessel growth are cancer [1] and eye diseases [2]. When a tumor grows too big to directly extract oxygen from its environment, tumor cells start secreting growth factors to attract blood vessels [1]. These new blood vessels supply the tumor with oxygen and nutrients that enable the tumor to grow [1]. Furthermore, the tumor vasculature acts as a conduit for tumor cells to enter the blood stream, which can result in tumor metastasis [1, 3, 4]. Blood vessel growth can cause damage to the retina and this results in an impaired vision or blindness. In various forms of retinopathy, such as proliferative diabetic retinopathy and retinopathy of prematurity, hypoxia induces the formation of new blood vessels [2]. These new vessels can obscure vision or damage the retina. In age-related macular degeneration blood vessels grow behind the retina which also damages the retina [2] and results in a loss of central vision.

Recently, blood vessel formation has also be studied outside of the context of diseases. In the upcoming field of tissue-engineering blood vessel formation is studied because larger tissues and organs can only function when they become vascularized [5–7]. When vascularization of a tissue engineered implant depends on blood vessel ingrowth from the host, vascularization takes a up to several weeks [8]. During this time the core of the implant is deprived of oxygen and nutrients and this could damage the implant. Recent studies have focused on improving blood vessel formation after implantation by using a scaffold or adding angiogenic factors, or on inducing blood vessel growth before implantation [7, 8].

Blood vessel formation is commonly studied in the wet lab, either *in vitro* or *in vivo*. An alternative approach to research blood vessel formation are computational models. Classically, computational models have been used to test or investigate hypotheses generated in the wet lab. This is achieved by translating the biological hypothesis into a model consisting of rules or equations. Then, by solving the equations or simulating the model, the hypothesis can be tested. If the hypothesis is supported by the model, the model can be used further study the hypothesis. Specific parts of the hypothesis can be

studied by altering the model components that describe these mechanisms. Furthermore, in contrast to wet lab experiments, the evolution of any model component, such as cells or chemical concentrations, can be observed without affecting the system. Therefore, computational modeling is good tool for studying blood vessels formation. Ideally, computational modeling is used alongside wet lab experiments. Computational models can help to steer experiments, while wet lab experiments are necessary to validate the model. Together, these two methods can help us to provide new insights in the development of blood vessels in health and disease [9].

In this introductory chapter we first discuss the biological processes by which blood vessels develop. Following this, we provide an overview of the computational modeling approaches used to better understand various aspects of blood vessel formation. Finally, we give an overview of the research that will be discussed in the remainder of this thesis.

1.1 Blood vessel formation

Blood vessels form via two processes: vasculogenesis and angiogenesis [10]. Vasculogenesis is the *de novo* formation of blood vessels, which occurs during embryonic development. During vasculogenesis endothelial cell precursors, called angioblasts, organize into primitive vascular networks [10]. Angiogenesis, is the formation of new blood vessels from existing ones. Via this process the primitive vascular networks that formed during vasculogenesis are remodeled and extended. After birth, angiogenesis is responsible for the formation of blood vessels in growing and healing tissue [10]. Altogether, vasculogenesis is limited to the early stages of embryogenesis while angiogenesis is the main process of blood vessel formation after early embryogenesis. Therefore, we will mainly focus on angiogenesis and the cells involved in this process.

There are two mechanisms of angiogenesis: 1) intussusceptive angiogenesis and 2) sprouting angiogenesis [11]. In intussusceptive, or splitting, angiogenesis vessels split along the longitudinal direction by forming intraluminal tissue [11, 12]. In this manner, blood vessels are remodelled with minimal endothelial cell proliferation and migration, and without proteolysis of extracellular matrix components [11, 12]. These new blood vessels are formed during sprouting angiogenesis, which precedes intussesceptive angiogenesis [12]. Sprouting angiogenesis, is involved in the formation of new blood vessels during growth and regeneration, and in pathological processes such as tumor vascularization and neovascularization of the eye. In sprouting angiogenesis a new vessel sprouts from the side of an existing vessel as is illustrated in Figure 1.1. The wall of a blood vessel consists of quiescent endothelial cells, called *phalanx cells* [13], and pericytes partially cover the outside of

the vessel (Figure 1.1**B**). When a tissue becomes hypoxic, it secretes growth factors that activate the phalanx cells in the vessel wall [14]. One of the cells becomes the leader cell, which is called the *tip cell* (Figure 1.1**B**) [15]. When the tip cell migrates further it is followed by other activated cells, which are called *stalk cells* [15], and simultaneously the pericytes detach from the vessel wall (Figure 1.1**C**) [14]. The sprout grows longer because the stalk cells directly behind the tip cell proliferate (Figure 1.1**D**) [15]. As the tip cell moves further away, the cells at the base of the new sprout become quiescent phalanx cells that develop a lumen and recruit pericytes (Figure 1.1**E**) [14]. When the sprout grows longer new sprouts may split of from the initial sprout resulting in branches. Such branches later on connect with other branches, from the same or another sprout, and thereby form a vascular network.

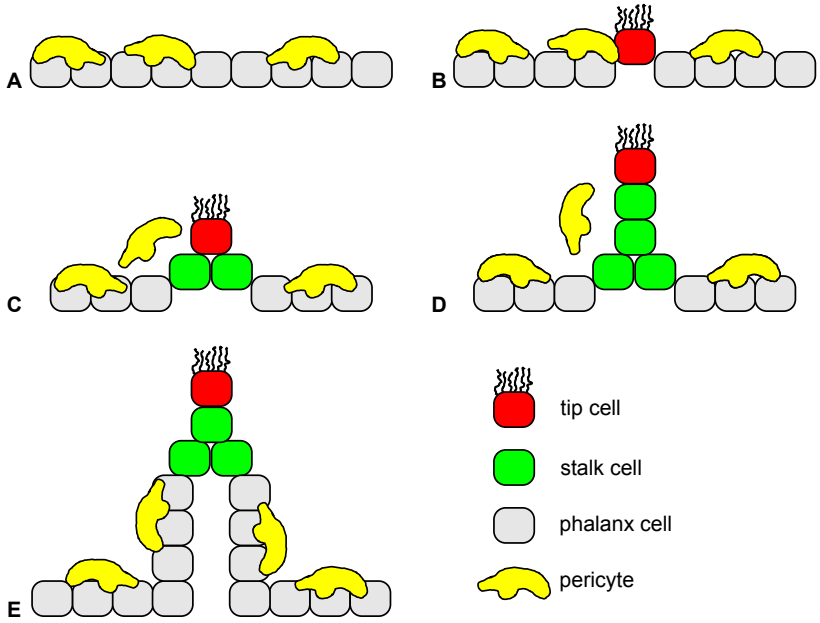

Figure 1.1: Sprouting angiogenesis. **A** row of phalanx cells (gray) that line a the blood vessel and pericytes (yellow) that partially cover the vessel on the outside. **B** one cell is activated and becomes a tip cell that migrates outwards. **C** the stalk cells (green) follow and the pericyte next to these stalk cells dissociates from the vessel. **D** the sprout elongates because the tip cells migrates further and the stalk cells proliferate. **E** at the base of the sprout a lumen forms and pericytes are recruited.

Angiogenesis nor vasculogenesis requires spatial prepatterning or genetic predetermination [9, 16]. Instead, endothelial cells migrate and thereby respond to and change their own environment. This, can affect the behavior of other endothelial cells, either close by or further away. For example, for

sprouting angiogenesis it does not matter which endothelial cell becomes the tip cell. As long as one cells becomes a tip cell the sprout can develop. As the sprout growth, the environment changes and an endothelial cells in the sprout may become a tip cell as well. This cell may either take over the tip [17, 18] or start a new sprout [15]. Thus, endothelial cell collectively self-organize into blood vessels. Whereas genes do not predetermine the vessels that are formed, genes do affect cell behavior. Genes are part of the pathways that regulate cell migration and behavior. These pathways integrate signals from outside, such a membrane-bound ligands of adjacent cells or diffuse ligand, with the genes expressed in the cell. In this manner, cell behavior is adapted to the environment. However, to understand the mechanisms involved in angiogenesis and vasculogenesis, it is not necessary to know all these pathways. Instead, the cell can be used as the main level of abstraction. In this manner we can first understand which cell behaviors are important, and then focus on the source of these behaviors. Therefore, in this thesis we will study angiogenesis with the cell as base unit. We study how certain cell behaviors and interactions to differentially behaving cells affect the patterns that form.

1.2 Computational models of angiogenesis

Computational models have been used to study angiogenesis since the 1980s. Angiogenesis research benefits from this approach in three ways. Firstly, computational models help to gain an overview in this complex system 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 [19–35]. Computational models are therefore not only useful to gain mechanistic understanding of angiogenesis, but also to find new therapeutic targets. In this manner, computational models can be used to direct experimental studies. Secondly, computational models can discriminate between and select from alternative hypotheses [36–39]. 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 hypotheses 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 [17, 40–44]. 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, computational models include processes at multiple scales, like extracellular matrix, cells, and cell-regulation simultaneously. Such a model can than be used to model angiogenesis as it happens in the body and predict how modifications at any scale

affect angiogenesis.

The earliest models of angiogenesis were continuum models that describe angiogenesis in terms of the spatial density of cells. The cell density is described by a set of equations that may include processes such a random cell movement, proliferation, chemotaxis, etc. The main advantage of these models is that they can often be solved analytically, but they are often too abstract to mimic angiogenesis realistically. More complex techniques allow for a more detailed description of angiogenesis, which yields more realistic models. Such techniques include discrete methods such as particle based modeling that describe cells as point-like particles and cell-based models that also explicitly model the cell shape and membrane. These discrete methods are often combined with continuum models, creating a hybrid model in order to utilize the strength of both methods.

In this section we review the contribution of computational modeling to angiogenesis research. First, we discuss how computational modeling resulted in several hypothetical driving mechanisms for vascular network formation. Second, we describe several computational models that are used study specific aspects of sprouting angiogenesis. Finally, we review how the knowledge gained from simple computational models and experiments is used to build large multi-scale models, which can for example be used to study the effects of anti-angiogenic drugs.

1.2.1 Network formation

During early vascular development endothelial cells join into a primitive vascular network. Vascular network formation can be mimicked *in vitro* by seeding endothelial cells on a suitable matrix containing nutrients and angiogenic factors [45]; for example Figure 1.2A shows human umbilical vein endothelial cells (HUVECs) seeded on Matrigel matrix forming a network-like pattern. The conditions in *in vitro* network formation experiments differ greatly from *in vivo* angiogenesis. Yet, specific cases of angiogenesis result in similar vascular networks such as angiogenesis in the yolk sac and retinal angiogenesis [46].

In vitro experiments showed that, after the network is formed, almost all matrix is located beneath the cells [47]. This led to the hypothesis that cells pull on the matrix, resulting in matrix accumulation below cell clusters. The pulling forces of the cells also cause the formation of tension lines, radiating from the clusters, in the surrounding matrix, along which cells migrate [21]. This model assumes that cells can exert traction on the matrix, which results in matrix deformation and heterogeneity of strain in the matrix. Cells preferentially move along the orientation of high stress. The model suggests that matrix remodeling suffices for network formation.

Namy and coworkers [22] combined the effects of cell traction with hap-

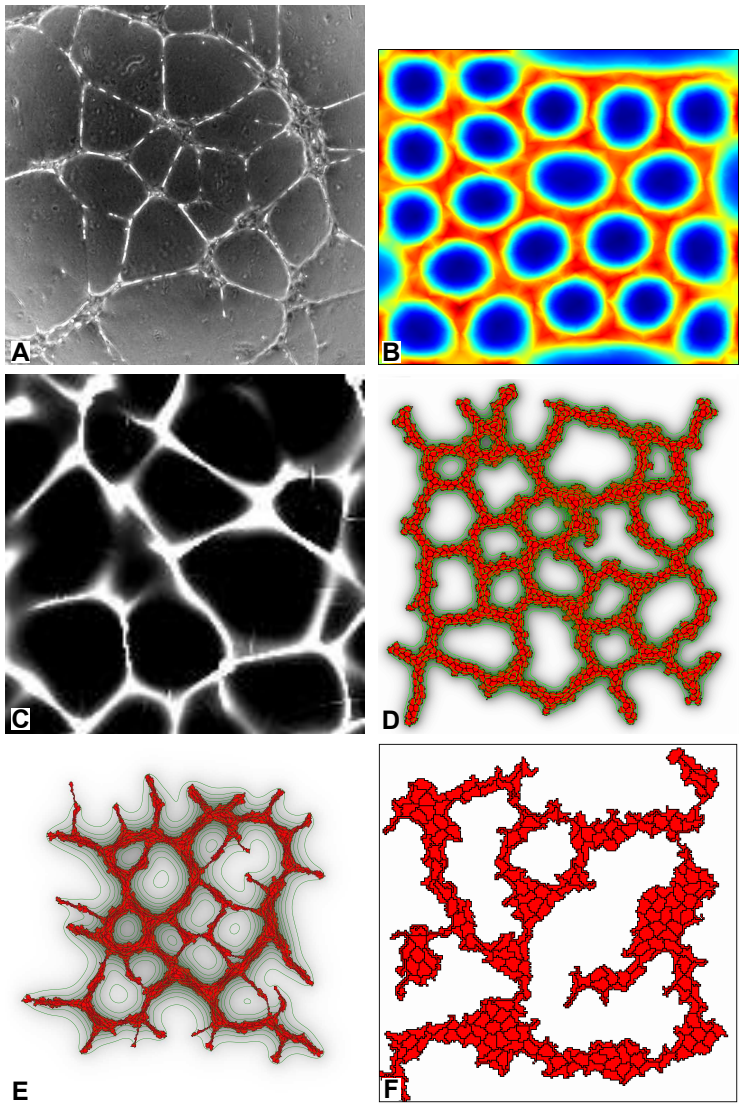

Figure 1.2: Overview of vascular network formation. **A** vasculature developed *in vitro* with HUVEC on Matrigel. **B** networks formed with the mechanical continuum model [22], **C** the chemical continuum model [20], **D** cell-based model with contact inhibition [28], **E** the cell-based model with cell elongation [25]. **F** the cell-based model with preferential attraction to elongated structures [31]. All images were reproduced with the publishers' permission.

totactic cell migration along matrix gradients (Figure 1.2**B**). They found an optimal cell density at which networks can be created, corresponding with experimental observations [48]. Similarly, a range of matrix stiffness, which is linked to the fibrin density of the experimental matrix, was tested. This model suggested that active cell migration may be required for network formation which contradicts the observations by Manoussaki *et al.* [21].

Both previous models consider mechanical interactions between cells and the matrix to be the driving forces for network formation. Serini *et al.* [19, 20] proposed that chemotaxis is the driving force of network formation [20]. In the *in vitro* models cells move predominantly towards regions of high cell density suggesting that the cells are attracted by a chemoattractant secreted by the cells. Therefore, Serini *et al.* built a the computational model in which cells secrete a chemoattractant to which cells move preferentially. This model produces network-like patterns as shown in Figure 1.2**C**. Two important predictions are made based on this model. First, the model predicts an optimal cell density for the formation of stable vascular networks and second, the size of the meshes in the network depend on the diffusivity and decay rate of the chemoattractant.

The mechanical and chemical hypotheses for vascular network formation have also been combined in one mechanochemical model [37]. This continuum model hypothesizes that network formation consists of two stages. First, cells move upwards chemical gradients. Second, at higher local cell density, the cells do not sense the gradient, but the high cell density signals them to start remodeling the matrix. This then attracts cells to the high density regions. The mechanochemical model showed that these assumptions indeed lead to network formation and that chemotaxis drives the formation of networks while mechanical interactions stabilize the formed network. However, the mechanochemical model cannot reproduce all observations from both the chemical and mechanical angiogenesis models. Therefore, a more detailed description of the matrix mechanics is required that also influences early cell migration.

Clearly, multiple hypotheses can be used to explain the experimentally observed network formation. Moreover, model observations and predictions for both the mechanical and the chemotaxis model could be reproduced *in vitro* [20, 22]. The mechanical models show that matrix thickness and stiffness may be determining factors in network formation, as has been show experimentally [47]. The chemical models reproduce the VEGF dependence that has be observed *in vitro* [20] as well as a characteristic length of the networks that depends on the diffusivity of the chemoattractant [49]. Both models only produce one similar prediction; there is an optimal cell density for network formation, below this density cells disconnect and above this density cells aggregate [48]. Therefore, it remains unclear whether the two mecha-

nisms are involved in angiogenesis in different environments, or that the two mechanism act consecutive or simultaneously during angiogenesis.

The models discussed so far use a continuum description for both cells and mechanical or chemical fields, meaning that cells and fields are described as densities. This kind of description is appropriate for mechanical and chemical fields; for example, the concentration of a specific chemical can be measured at a specific position and can have any value. However, generalization of cells into cell densities ignores cell behavior, cell size and shape, and cell-cell interactions, which are often key to morphogenic processes such as angiogenesis. Therefore, cells should be the basis of an angiogenesis model. Cell-based models incorporate detailed cell-cell interactions as well as cell shape and size, which can also be measured experimentally for quantification of the parameters and the predictions of the models [9]. Dynamic cell properties and behavior can be added by extending each cell with regulation networks, such as signaling or genetic pathways. Altogether, cell-based models are a solid basis for computational angiogenesis models that can be used to explain tissue effects at the cell level [16].

Various hypotheses for vascular network formation have been modeled using cell-based models. One of these models is a hybrid cell-based model, using the cellular Potts method (CPM), which is based on the assumption that cells chemotact toward a chemoattractant that they themselves secrete [25–29]. This assumption is similar to the assumption used for the continuum chemotaxis model [20]. In this cell-based model the cells' shape, size and membrane surface are described explicitly, and chemicals are described as continuous fields. One of the main advantages of this cell-based model is the more realistic chemotactic response of cells. This cell based model can be used to simulate network formation solely by defining cell behavior and properties. When only autocrine chemotaxis is included, network formation only occurs for narrow parameter ranges: strongly adhering cells or steep chemical gradients [26, 27]. However, three alternative hypotheses are proposed that allowed network formation for a much wider range of parameters: contact inhibition [28], cell elongation [25] and ECM-chemoattractant binding [29].

The contact inhibition hypothesis proposes that cells only respond to the autocrine chemoattractants where the cell membrane is not in contact with other cells. This exclusive sensing is thought to be mediated through the dual function of VE-cadherin; it acts as a homophilic trans-membrane cell-adhesion molecule and it plays an inhibitor role in the VEGF signaling pathway [50] which increases cell motility. Therefore, cells that are surrounded by other cells do not respond to VEGF. This process appears to contribute to both network formation (Figure 1.2**D**) and sprouting angiogenesis. The reasons for this are best understood in the context of sprouting angiogenesis and will

therefore be discussed in section 1.2.2.

The cell elongation hypothesis is based on the biological observation that cells elongate during network formation. In this model, the combination of elongated cells with autocrine chemotaxis results in network formation [25]. The final network, which can be observed in Figure 1.2E, is similar to *in vitro* networks. When cell elongation is omitted, cells aggregate instead of forming network, indicating that cell elongation drives network formation in this model. The evolution of network properties over time, such as the number of nodes and meshes, corresponds with data from *in vitro* experiments with HUVECs on Matrigel. This suggests that cell elongation may play an important rule during network formation. In this model network formation occurs at two time-scales. First, cell elongation induces a persistent movement along the long axis of the cell. This causes the formation of thin branches of connected cells. Second, the network coarsens by fusion of branches and mesh collapse. This is driven by the chemotaxis that enables slow migration of cells along their short axis.

The ECM-chemoattractant binding hypothesis is based on binding of the chemoattractant to the ECM. VEGF is a known chemoattractant for endothelial cells and it has heparin binding domains that cause VEGF to bind to the ECM [51]. Köhn-Luque *et al.* [29] developed a model based on this hypothesis using the CPM. In this model unbound VEGF is produced everywhere and ECM molecules that bind VEGF are produced by cells. Unbound VEGF can bind to the ECM molecules resulting in bound VEGF. When cells respond more to bound VEGF than unbound VEGF, a network forms that is similar to the network that develops during the first two ours of HUVECs on Matrigel [52]. Interestingly, cells in the branches of the network elongate by themselves. This may suggest that cell elongation is a second step in network formation that drives the formation of longer branches and network coarsening.

An alternative hypothesis that is not based on chemotaxis was proposed by Szabó *et al.* [30, 31, 38]. Their experiments suggested that neither mechanical interactions nor chemotaxis are required for network formation [30] and that cells move preferential towards elongated cells. From these observation they propose that network formation is driven by the *preferential attraction to elongated structures*. This hypothesis has been used as a basis for both a particle based model [30] and a cell-based model [31, 38]. In the particle based model cells are represented by point particles that diffuse and are attracted by their neighbors. While this model lacks some key cell properties, including cell shape, it suffices as a *proof-of-concept* model for preferential attraction to elongated structures. The more detailed cell-based models are used to investigate network formation from dispersed cells [31] and sprouting from a blob of cells [38]. This model suggests that cells can indeed form networks due to short range cell-cell interactions, as is shown in Figure 1.2F.

Sprouts formed in these networks only become stable when they connect to other sprouts, suggesting that anastomosis stabilizes the formed network.

Because they all produce similar morphological patterns, none of the modeled hypotheses can be ruled out as a driving force for network formation. Cell-based models [25–28] suggest that autocrine chemotaxis, combined with cell properties such as contact inhibition of cell elongation, may drive angiogenesis. Other cell-based angiogenesis models [30, 31, 38] have suggested that chemotaxis may not be necessary at all. Furthermore, mechanical interactions between the cells and the matrix may facility network formation. It is likely that each of these mechanisms plays a role in vascular network formation and that it depends on the circumstances which mechanism is dominant.

1.2.2 Sprouting

Sprouting angiogenesis is the formation of new vessels by creating a sprout from the wall of the existing vessel (Figure 1.1). This form of angiogenesis is often observed in the vicinity of hypoxic tissue that secretes angiogenic factors, *e.g.,* a growing tumor, which activates and attracts endothelial cells from the existing vessels [53]. By stimulating the formation of a new vasculature, a tumor is able to grow and proliferate. The mechanisms underlying the dynamics of sprouting angiogenesis are still poorly understood. What mechanisms guide the growing sprout? How do biochemical and biomechanical interactions of the ECM with cells effect sprouting? Is proliferation required and where is proliferation located in the sprout? How are tip cells selected in the vessel and what causes sprouts to branch? Computational models have contributed to a better understanding of these issues.

Experiments in corneal angiogenesis show that migration of activated endothelial cells and initial sprouting precedes stalk cell proliferation [55] and this proliferation is necessary for sprout elongation [56]. These observations indicate that sprouts will not reach the tissue that induced sprouting when endothelial cells cells are not able to divide. A continuum model [32] describes the change in cell density over time due to cell migration driven by cell diffusion, chemotaxis and haptotaxis. The initial configuration of the simulation consists of a blood vessel at one side and a tumor at the other side of the simulation domain. This tumor secretes a chemoattractant, resulting in a gradient of chemoattractant that attracts cells towards the tumor. Haptotaxis is induced by fibronectin that the cells secrete themselves. The highest levels of fibronectin are present where the cell density is maximum. Therefore, haptotaxis and chemotaxis work in opposite directions. The continuum model suggests that, in absence of proliferation, the sprouting is restricted. The authors propose that this is caused because haptotaxis outweighs chemotaxis and increasing the number of cells would increase the chemotactic response.

A drawback of this model is that it describes cells as a density field, hence it

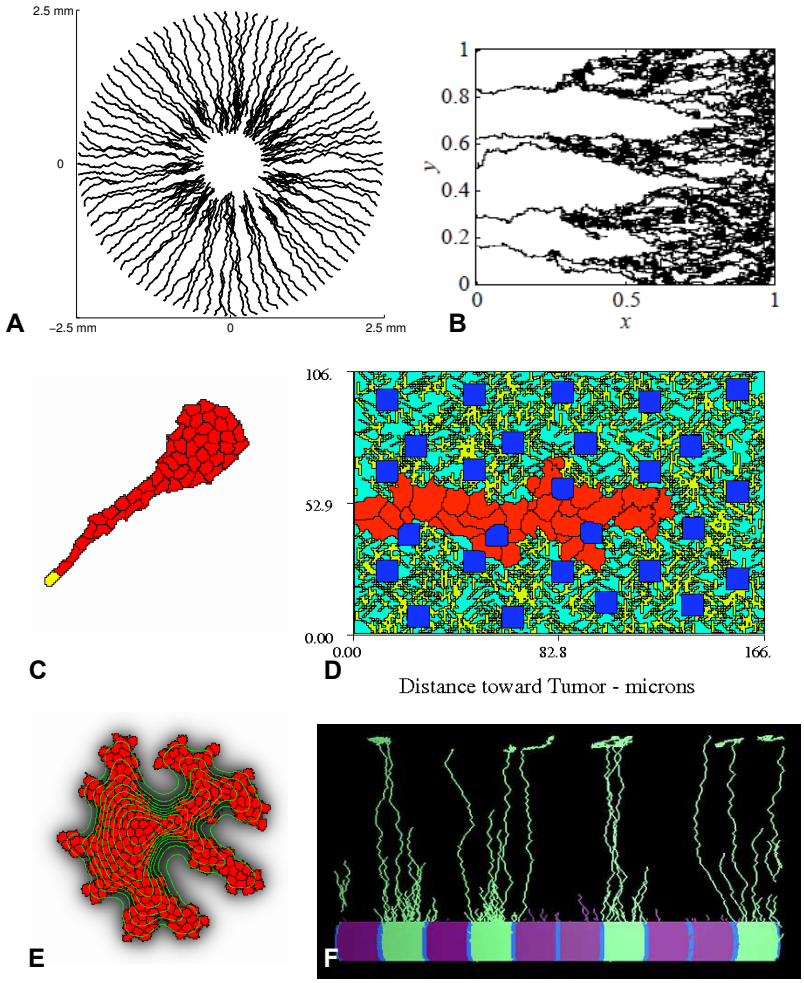

Figure 1.3: Overview of the computational models of angiogenic sprouting. **A** corneal angiogenesis as modeled in the discrete model based on tip cell elasticity and stalk cell adhesion [35]. **B** networks formed with the discrete model with chemotaxis and fibrinectin induced haptotaxis [54]. **C** sprouting induced by preferential attraction to elongated structures in a cell-based model [38]. **D** sprouting angiogenesis in a cell-based model with a heterogeneous ECM [34]. **E** sprouting induced by contact inhibition of chemotaxis [28]. **F** tip cell selection and sprouting in an agent-based model [41]. All images were reproduced with the publishers' permission.

cannot describe how the sprout breaks up due to lack of proliferation. Therefore, a discrete modeling approach has been introduced to study cell proliferation in the sprout [35]. As illustrated in Figure 1.3**A**, the model mimics a cornea with a lesion in the center from which VEGF is secreted. A sprout grows from the periphery and consist of multiple cell types; one leading tip cell and multiple following stalk cells. The tip cell is attracted by VEGF and therefore migrates towards the center of the cornea. Tip cell migration is limited by the elasticity of the tip cell and the strength of the adhesion between stalk cells. Adding proliferation enables unlimited sprout extension. This model suggests that basic cell properties can explain the need for proliferation in sprouting.

The previous two sprouting models only considered cell behavior and chemical fields, ignoring all ECM and stromal tissue. Anderson and coworkers [54] created a particle based, hybrid model describing sprouting angiogenesis. In this model cells are represented as point particles on a grid while the chemotactic and haptotactic fields are still described as continuum equations. This model was used to investigate how the balance of haptotaxis and chemotaxis influences branching and anastomosis. As shown in Figure 1.3**B** branching and anastomosis occur in the model, but these behaviors only occur when cells are able to move perpendicular to the chemotactic field, which is enabled by haptotaxis. When the haptotactic forces are strong enough branches can split and reconnect in order to form a functional vasculature.

Anderson *et al.*'s model [54] suggests that haptotaxis is key to branching, but it did not show how cells interact with their heterogeneous environment. A more recent, cell-based, model represents the ECM as a static, heterogeneous configuration of matrix fiber bundles, interstitial fluid and immobile tissue-specific cells [34]. The endothelial cells in the model are motile and adhere stronger to matrix fibers than to the surrounding matrix. Immobile cells act as obstacles that hinder the migration of endothelial cells. The tip cell is influenced by a chemoattractant field and it degrades ECM components. Degradation of the extracellular matrix during sprouting enables cells to migrate and branch off the main sprout as shown in Figure 1.3**D**. The model suggests that a heterogeneous composition of the matrix is necessary for the formation of branches; the inhomogeneities in the matrix enable cells to split from the main branch. Furthermore, the model suggests that the proliferation region determines sprouting dynamics but does not affect the final sprout morphology.

A follow-up model was used to investigate cell-ECM interaction in more detail [33]. In this model all cells respond to the chemoattractant and that the immobile tissue cells are removed, *i.e.*, only fibers cause matrix heterogeneity. The model suggests that sprouting only occurs in a specific range of matrix densities, which corresponds with experimental observations. Moreover, simulation results suggest that low fiber density results in cell elongation.

Similar changes were observed when the random fibers were replaced by a specific fiber pattern, for example long fibers cause cells to elongate in the same direction as the fibers. The authors propose that contact guidance, due to cell-matrix interactions, is key to role in vascular sprouting because it enables sprout branching in an inhomogeneous matrix.

All of the discussed models assumed an external source that induces sprouting. In contrast, both Szabó *et al.* [38] and Merks *et al.* [28] have proposed that cells can form sprouts in absence of external signals and in a homogeneous matrix. They supported their hypothesis with cell-based models that describe cell shape, cell membrane and cell migration in much more detail than the models discussed before.

The model by Szabó *et al.* [38] incorporates preferential attraction to elongated structures, cell polarity and self-propulsion (i.e. persistence of motion). The model also differentiates between tip and stalk cells. The tip cell is polarized, causing directed movement in the direction of the polarization vector. The results shown in Figure 1.3**C** suggest that both preferential attraction and self-propulsion are necessary to reproduce realistic sprouting behavior. Cell polarization may be regulated by cell-cell contacts and VE-cadherin may be a key player for this. Moreover, the model suggest that differential behavior at the sprout tip may drive sprout formation.

In contrast, Merks suggested that sprouting can occur in absence of matrix heterogeneity or differential cell behavior [28] (1.3**E**). The model assumes cells are attracted towards an autocrine chemoattractant, using similar rules as in chemotaxis-based network model (section 1.2.1). Contact inhibition mediated by VE-cadherin causes cells to be only sensitive to the chemoattractant at positions of the cell membrane adjacent to the ECM. Sprouting occurs in two ways. First, when cells are arranged in an aggregate, only the outer layer of cells sense the chemoattractant. These cells tend to migrate towards the center of the aggregate causing a buckling instability that induces sprouting. This effect enables cells, even those with a low motility, to move against the chemotactic gradient. Second, another mechanism may explain sprouting for highly motile cells. To move away from the parent vessel and form a sprout, cells must migrate *against* a steep gradient of self-secreted chemoattractant. Once a small sprout is created by a motile cell, the gradient around this outgrowth is less steep than the rest of the gradient, so cells within the sprout have higher motility than elsewhere, causing an instability.

Several of the previously discussed models defined the cell leading a sprout as the tip cell [34, 35, 38]. However, tip cell fate is regulated during angiogenesis via intercellular Dll4-Notch [57–60] signaling and VEGF signaling [60–63]. Bentley *et al.* [41] investigate the molecular and biophysical mechanisms driving tip and stalk cell differentiation using an agent-based, computational model [41] of a single row of endothelial cells. In this model, tip

cell fate is induced by VEGF and regulated by lateral inhibition of tip cell fate via Dll4-Notch signaling. When a cell becomes a tip cell, it starts extending long filopodia away from the blood vessel (Figure 1.3F). Based on this model Bentley *et al.* proposed that the balance between VEGF signaling and Dll4-Notch determines the transition from normal to abnormal sprouting [41, 42]. An extension of the same model was used to study tip cell competition in angiogenic sprouts. Time-lapse microscopy has shown how stalk cells migrate along the sprout and compete with the leading tip cell [17]. Cells that are treated such that they express higher levels of the VEGF receptor VEGFR2 are found more often at the sprout tip than wild-type cells. Blockage of Notch signaling neutralized this effect. Based on these observations Jakobsson *et al.* [17] proposed that tip cell compete for the tip position and that this is controlled by both VEGF signaling and Dll4-Notch signaling. In the computational model this hypothesis reproduced tip cell shuffling, suggesting that this mechanism may explain tip cell shuffling.

1.2.3 Predictive angiogenesis models

The models discussed so far, all isolated specific aspects of angiogenesis. To build predictive models of *in vivo* angiogenesis, we must incorporate the interactions with the rest of the body in a multi-scale model. Angiogenesis is induced by hypoxic tissue which, for example, can be a tumor or an active muscle. The change in oxygen and nutrient supply due to the new vasculature changes the signals coming from the tissue, resulting in a dynamic feedback loop between angiogenesis and the needs of the tissue. Also blood flow may be key to this feedback. Dysfunctional vessels are not able to support blood flow and do not contribute to the perfusion of the tissue. Endothelial cells change their behavior due to the shear stresses induced by blood flow [64]. The inclusion of these processes in a multi-scale angiogenesis model would be a great tool to study pathological processes either involve excessive or insufficient blood vessel growth. Such multi-scale models can be used to formalize and validate hypotheses, and to predict the effects of pro- or anti-angiogenic therapies on the vasculature and the other tissues involved.

In order to build these multi-scale models, researchers often extend existing models. For example, the particle-based sprouting model by Anderson *et al.* [54] has been extended with blood flow [65]. This model suggested that most vessels are not perfused due to the lack of anastomosis, and thus drugs can not reach the target. More complex approaches have been used to combine more detailed angiogenesis models with blood flow and the kinetics of oxygen and VEGF [44, 66, 67]. With these models vascularization in a heterogeneous ECM [44] and in skeletal muscle [43] has been simulated.

Also, cell-based models have been extended to include multiple tissues. Shirinifard *et al.* [68] combined cell-based models of blood vessel formation and

tumor growth to investigate how tumor growth and vascular remodeling interact. In this model the tumor induces angiogenesis in a peripheral vasculature. The new blood vessels supply the tumor with oxygen and growth factors and thereby affect the tumor development. Another example of a multi-scale, cell-based model was presented by Kleinstreuer *et al.* [40]. They modeled vasculogenesis including several types of cells and proteins in order to test the effects of various toxins on vascular development. For this they classified the effects of the toxins on the behavior of a single cell and included this in the model. The model was able reproduce the pattern formed in HUVEC cultures treated with various toxins, showing that this approach is useful to further study the effects of toxins on vascular development.

These first approaches on multi-scale, cell-based modeling of blood vessel formation indicate that this is a suitable approach for predictive modeling. However, extra steps, such as including mechanical interactions with the ECM and blood flow, subcellular signaling, and interactions between endothelial cells, perivascular and stromal cells, should be included to create reliable, predictive models of angiogenesis. Before, such effects can be included, they should be studied thoroughly in more simple, cell-based models.

1.3 Thesis outline

In this thesis we focus on the role of specialized cells in angiogenesis. This includes cells with distinctive behaviors, such as elongated cells, or cells with a specific role, such as tip cells or pericytes. These specialized cells are observed *in vivo* [15, 69–71], but *in vitro* blood vessel formation there specific properties are dispensable. Therefore, we aim to better understand the role of these specialized cell types in blood vessel formation. For this we use simple, cell-based models of vascular network formation, either via sprouting angiogenesis or vasculogenesis. In these models we focus on the cell scale and therefore we do not include the cause of the cell behaviors in the models or incorporate any high-level rules. Furthermore, we assume that the environment of the cells does not provide any structural, mechanical or chemical guidance to the cells. In this manner we build models in which we isolate cell behavior, similar to what happens in various *in vitro* models of blood vessel formation.

The cell behaviors we study in our models is not limited to the behavior that is described in the literature. Potential cell behaviors may still be undiscovered. And, often cell behavior is described at a level that is not applicable for our modeling approach. With our model we can assign any kind of behavior to the cells and study how this affects morphogenesis. In this manner we can predict the behavior of cells involved in blood vessel formation that have not been described in the literature. In order to study large ranges of cell behav-

iors with cell-based models, large parameter sweeps were necessary. Unlike classic methods, such as PDE models, no standard methods are available for this. Therefore, in chapter 5, we present a protocol for setting up, running and analyzing large scale parameter sweeps with cell-based models. In chapter 5 we demonstrate how this protocol was used to obtain the results presented in chapter 2. Furthermore, we show how the method can be applied to an alternative cell-based model.

In chapter 2 we further analyze the formation of blood vessels by elongated cells. Previously, it was shown that elongated cells form networks [25], but the precise mechanisms by which elongated form networks remained unclear. To better understand how elongated cells form networks, we quantify the alignment of cells during network formation and show that elongated cells align over time. Using these analyses we show that elongated cells align and that due to this alignment the rotation of the cells becomes limited. As a result the cells form a network-like structure that is in a state of dynamic arrest.

In chapters 3 and 4 we study how mixing of specialized cells, *i.e.* cells with different behaviors, affects vascular network formation. In chapter 3 we study how pericytes, a kind of perivascular cell, affect vasculogenesis. For this we combined *in vitro* experiments with a cell-based, computational model. With our model we studied which interactions between endothelial cells and pericytes could be responsible for the patterns observed *in vitro*. In this manner we showed that during blood vessel formation pericytes and endothelial cells may attract each other by diffusing chemoattractants. In chapter 4 we used our computational modeling approach to gain new insights in the molecular and behavioral differences between tip cells and stalk cells. With a large parameter sweep we searched for those cell behaviors that could make tip cells lead and affect network formation. In this manner we found that tip cells that respond less to an autocrine chemoattractant lead sprouts and affect network formation. This result seemed to contradict with the literature because tip cells are described as highly motile cells that respond more to chemoattractants. However, the chemoattractants to which tip cells are reported to respond more are chemoattractants secreted by hypoxic tissues, while in our model the chemoattractant is secreted by the endothelial cells. Furthermore, a literature study of tip cell gene expression in tip and stalk cells indicated that tip cells do not express the receptor for the endothelial cell chemoattractant Apelin. Blocking Apelin signaling in *in vitro* sprouting assays reduces sprouting, but only when tip cells were present in the spheroids. This support the hypothesis that the differential response of tip and stalk cells to Apelin could cause tip cells to lead sprouts.