Chapter 12
Particle Simulations of Growth: Application to Angiogenesis

Florian Milde, Michael Bergdorf, and Petros Koumoutsakos

1 Tumor Induced Sprouting Angiogenesis

Sprouting angiogenesis describes the process of new capillaries growing from a preexisting vasculature and can be observed in the human body under various conditions. In a physiological context, angiogenesis mainly takes place during embryogenesis and fetal development. Under pathological conditions, angiogenesis can be observed to take place during wound healing, thrombosis and tumor growth (Folkman 2007). Whereas new capillaries grow in a controlled manner during wound healing and thrombosis and stop growing once the pathology has been alleviated, this is not the case for tumor-induced angiogenesis (Folkman 2007).

Angiogenesis as induced by tumors can persist for years, leading to the formation of a disorganized and leaky vasculature (Folkman 2007). Despite the inefficiency of this vasculature, it supplies the tumor with nutrients and growth factors, which allow for increased tumor cell proliferation and growth. In addition, the leaky vasculature enables single cancer cells or cell clusters that detach from the primary tumor to enter the vascular system and metastasize to remote organs.

Anti-angiogenic therapy, although comparatively young, has already been established as the fourth pillar of cancer therapy (Folkman 2006) (next to surgery, radiation and chemotherapy). Regulation of tumor-induced angiogenesis can help establishing more efficient pathways for drug delivery in the naturally leaky and inefficient blood vessels (Saharinen and Alitalo 2003). Inhibition, on the other hand, restrains nutrient supply, decreasing the growth rate of the tumor and reduces the risk of metastasis by preventing cell clusters from entering the vasculature (Folkman 2006). However, complete inhibition of angiogenesis promoting hypoxia (state of
oxygen shortage) inside the tumor could lead to the occurrence of aggressive migrating tumor cell phenotypes (Axelson et al. 2005; Pennacchietti et al. 2003).

Tumor growth in the avascular stage, e.g. in the absence of a tumor associated vasculature, is restricted to a size of about 1 mm$^3$ (Folkman 2006). In this case, tumor growth is limited by the shortage of oxygen and nutrient supply transported by means of diffusion from the surrounding tissue. If the tumor grows beyond this size of roughly 1 mm$^3$, cells in the core of the tumor suffer from oxygen shortage, become hypoxic and eventually die, forming a necrotic region at the core of the tumor. In this avascular state, tumors can remain for a long time (Folkman 2006). However, induced by hypoxia, tumor cells can acquire the ability to secrete angiogenic growth factors (a discrete step (or steps) referred to as “angiogenic switch” (Hanahan and Weinberg 2000)) initiating sprouting angiogenesis at the tumor surrounding vasculature. Different growth factors are involved in the process of angiogenesis.

Vascular Endothelial Growth Factors (VEGF) have been identified to be one of the key components (Ferrara et al. 2003). Upon release by hypoxic tumor cells, VEGF’s diffuse through the extracellular matrix (ECM), a fibrous construct occupying the space between the cells and establish a chemical gradient between the tumor and the nearby vessels. Once VEGF binds to specific receptors located on the endothelial cells (EC) lining the blood vessel walls, a cascade of events initiating vessel sprouting is set off.

Initially, ECs stimulated by VEGF start releasing proteases that degrade the basal lamina, a fibril structure supporting the vascular wall, enabling ECs to leave their position in the vessel wall and enter the ECM. Triggered by VEGF, further inter- and intracellular signaling pathways regulate increased EC proliferation and coordinate the selection of migrating tip cells located at the sprouting front. The migrating tip cells extend filopodia in order to probe their environment and migrate guided by the VEGF gradient towards regions of higher VEGF levels, a directed motion referred to as chemotaxis. Increased proliferation of ECs located behind the migrating tip cells leads to an extension of the sprouting blood vessel.

Fibronectin, distributed in the ECM and at the same time released by the migrating tip cells establishes an adhesive gradient guiding endothelial cells that follow behind, a movement referred to as haptotaxis. Next to the chemotactic and haptotactic cues, the fibrous structures present in the ECM influence cell migration by facilitating movement along fiber directions. Anastomosis, the formation of loops in the vascular network is the result of repeated branching and fusion of the tip cells as they migrate through the ECM. Finally, lumen formation within the strands of endothelial cells establishes a network that allows for the circulation of blood.

Maturation, the final stage of angiogenesis, is associated with the rebuilding of a basal lamina and the recruitment of other cell lines such as pericytes and smooth muscle cells that stabilize the vessel walls. The disorganized and leaky vasculature in combination with a growing tumor that exerts pressure on the fragile capillaries and thus suppressing temporal and local blood delivery, induces ever new regions of acute hypoxia. Under these conditions, tumor-induced angiogenesis never comes to a complete stop and full maturation is impaired.
1.1 Vascular Endothelial Growth Factors

Next to the predominant soluble isoform of VEGF, the presence of binding VEGF isoforms has been identified to drastically affect the morphology of capillary network formation (Lee et al. 2005; Ruhrberg et al. 2002). VEGF isoforms expressing a binding site for heparan sulphate proteoglycans found on cell surfaces, in the ECM and in body fluids can establish very localized chemotactic cues. These bound VEGF isoforms can be cleaved from the ECM by matrix metalloproteinases (MMPs) (Lee et al. 2005), attenuating the local gradients. MMPs can be expressed both by tumor cells and migrating ECs. Furthermore, it is noted that tumor cells are not the sole source of VEGF. Cells of the tumor microenvironment such as inflammatory cells stimulated by the tumor can also release VEGF and contribute to the chemotactic cues ECs react to.

1.2 Extracellular Matrix

The extracellular matrix plays an important role in cell migration and growth factor gradient formation. It describes any material that occupies the space between cells in metazoans (including the space between the tumor and the sprouting vasculature). Roughly 30% of the ECM are occupied by fibers such as collagen, laminin and fibrilllin coiled up into bundles that serve as a guiding scaffold for migrating cells (Davis and Senger 2005; Friedl and Bröcker 2000). The structure is subject to remodeling by endothelial tip cells (Kirkpatrick et al. 2007). The restructuring greatly facilitates cell migration through the ECM and plays a crucial role in lumen formation. Furthermore, the fibers constituting the ECM present binding sites for molecules such as fibronectin and certain VEGF isoforms that can be cleaved by MMPs.

2 Computational Modeling of Angiogenesis

In computational models of tumor-induced angiogenesis, only a limited number of the involved biological processes is accounted for. The availability of biological data and the understanding of the key processes that account for the phenomena under investigation dictate the choice of model processes. Existing models of angiogenesis can be classified into three broad categories:

1. cell-based models with the aim to capture the behavior of individual biological cells (Bauer et al. 2007),
2. continuum models that model the average, large scale behavior of cell populations (Anderson and Chaplain 1998; Levine et al. 2001)
3. discrete models that capture the explicit vascular networks morphology as determined by the tip cells migration (Chaplain 2000; Sun et al. 2005).
In Bauer et al. (2007), a two-dimensional, Cellular-Potts-based model was developed to study the formation of interconnected networks of endothelial cells. The model explicitly considers migration, division and adhesion of endothelial cells. Two types of EC’s are distinguished: migrating tip cells with the capability to degrade the matrix fibers, and proliferating stalk cells located behind the migrating tip cells. Branching and anastomosis of blood vessels can be observed without explicitly defined rules. Two dimensional, continuum models of angiogenesis with a probabilistic modeling of the capillary density have been presented in Anderson and Chaplain (1998). The models have been extended to generate discrete capillaries as masked points on a grid. Branching is modeled as a probabilistic process depending on the sprout age, growth factor concentration levels and the cell density. An extension of the model to three dimensions has been presented in Chaplain (2000). Another discrete two-dimensional model of sprouting angiogenesis has been introduced in Sun et al. (2005). A capillary indicator function is employed to capture the network structure. Branching is modeled to depend on the sprout age and the anisotropy of the ECM. In summary, cell-based models aim to describe angiogenesis at cell level resolution. However, due to their computational cost they are difficult to extend to macroscopic systems exploring large scale network formation processes. On the other hand, continuum-based models bypass these limitations by modeling the evolution of cell densities at the expense of detailed cell–cell interactions.

In this work, we propose two models, following a purely continuum, diffuse interface approach able to capture sprout morphologies and a hybrid particle-continuum-based approach. In both models, endothelial cell migration is considered to be influenced by chemical gradients inducing chemotaxis (VEGF), haptotaxis (Fibronectin) promoting cell–cell and cell–matrix adhesion and the structure of the ECM. VEGF is modeled to appear in a soluble and a matrix bound isoforms. The soluble isoform is released from an implicit tumor source and is subject to diffusion, cellular uptake and decay. The matrix bound VEGF isoform is initially distributed throughout the computational domain and can be cleaved by MMPs released at the sprouting tip (Sect. 1.1). Both soluble and cleaved VEGF isoforms contribute to the migration cues sensed by the ECs (see Fig. 12.1). Most existing models of sprouting angiogenesis account for chemotaxis induced by a soluble VEGF isoform (Anderson and Chaplain 1998; Chaplain 2000; Sun et al. 2005; Bauer et al. 2007). In Bauer et al. (2007) a matrix bound isoform of VEGF has been implicitly accounted for. To the best of our knowledge, the proposed model is the first to include an explicit cleaving mechanism and the presence of both soluble and matrix bound isoforms. Fibronectin is modeled to be released at the sprout tips, building up an adhesive gradient for the EC’s (Anderson and Chaplain 1998; Chaplain 2000; Sun et al. 2005; Bauer et al. 2007). In addition, we consider the binding of fibronectin to the ECM localizing the haptotactic cues to a confined area around the sprouts. The ECM is represented by a distribution of randomly oriented fiber bundles. The fiber bundle direction and density modulate the direction and speed of the tip cell’s migration. Other models explicitly considering an ECM have been introduced in Bauer et al. (2007) and Sun et al. (2005). In the Former, fiber bundles, structural cells and the interstitial fluid build the constituents of the ECM influencing cell migration.
Fig. 12.1 Conceptual sketch of the different VEGF isoforms present in the ECM. Soluble and cleaved VEGF isoforms freely diffuse through the ECM. Matrix-bound VEGF isoforms stick to the fibrous structures composing the ECM and can be cleaved by MMPs secreted by the sprout tips through adhesive forces. In the latter, a random anisotropic conductivity field accounts for the matrix structure and affects the migration velocity and branching behavior of the sprout tips.

3 A Continuum Modeling Approach

In the following, we propose a continuum, diffuse-interface model for mesenchimal-like cell migration and growth. We investigate on the models capability to reproduce angiogenesis-like growth that in contrast to prior models (except for the approach of Bauer et al. (2007)), does not rely on any probabilistic branching and fusion rules to generate blood vessel morphologies. The presented simulations have been conducted in 2D, as many in vitro experiments are essentially 2D. We note that all the methods described herein have also been extended to 3D. Next to the application of the model to the specific case of sprouting angiogenesis, the study might provide some hints on the relevance of the forces in play at the core of mesenchymal cell cluster migration as observed in organogenesis and infiltrating tumor growth.

3.1 Endothelial Cell Representation

When representing an agglomeration or cluster of cells, similar to the representation of multiphase flow, we can chose to represent the cells by a density function or by
a level set representing the interface between the cell cluster and the extracellular domain. We refer to the former case as “diffuse interface approach” and the later as “sharp interface approach.” A prerequisite of the level set approach is the definition of a narrow band of several grid spacings around the interface location. Under these conditions, to represent highly elongated agglomerates of cells as observed in angiogenesis, the level set approach is less favorable as the requirements for the resolution are much more demanding than for a corresponding diffuse interface representation.

3.2 The Extracellular Matrix

The extracellular matrix serves as an adhesive scaffolding in between cells. Migrating cells make use of this fibrous structure to exert forces and propel themselves. So far, the effect of the ECM has rarely ever been explicitly accounted for in continuum simulations of cell migration. We propose to model the extracellular matrix as a collection of randomly distributed bundles that facilitate but also bias migration. The procedure for constructing the matrix is as follows:

We distribute $N_F$ fibers as lines of thickness $b_p$. The start point $(x_{\text{start}}^p, y_{\text{start}}^p)$ is drawn from a uniform distribution inside the computational domain $\Omega$. The endpoint $(x_{\text{end}}^p, y_{\text{end}}^p)$ is given as

$$\begin{align*}
(x_{\text{end}}^p, y_{\text{end}}^p) &= \left( x_{\text{start}}^p + l_p \sin(2 \pi \alpha_p), y_{\text{start}}^p + l_p \cos(2 \pi \alpha_p) \right),
\end{align*}$$

with $\alpha_p \sim \text{uniform}(0, 1)$, and $l_p$ is the length of the fiber

$$l_p = 12^m \zeta, \text{ with } \zeta \sim \mathcal{N}(0, 1),$$

with simulation parameters $l$ and $m$, and the fiber thickness given as $b_p \sim \text{uniform}(b_{\text{min}}, b_{\text{max}})$. In order to get a smooth, differentiable field, we first discretize the fibers onto the ECM grid $e$ using Bresenham’s line rasterization algorithm before we filter $e$ $N_{\text{filter}}$-times with a second-order B-spline kernel.

3.3 Cell–Cell Adhesion

Tissue formation, stability and breakdown, cell sorting and tissue invasion, all these processes are largely influenced and determined by the fundamental biophysical mechanism of cell–cell adhesion. Adhesion of one cell to another, a substrate or the ECM is mediated by specific adhesion molecules on the cell membrane such as integrins and cadherins binding to collagens and fibronectin (in the ECM) or
cadherin molecules on other cells, respectively. It is a very local reaction as it is established upon contact. We postulate a set of requirements reflecting the key characteristics of cell adhesion. Cell adhesion is a short-range force that gives rise to a movement towards the entity the cell adheres to. The movement induced by adhesion will decrease as the cell density approaches the close-packing density. At close-packing density, no residual movement caused by adhesion is accounted for. Given this set of requirements, we model cell adhesion as an autocrine (in the case of cell–cell adhesion), or paracrine signal \( f \) (in the case of cell-ECM adhesion).

In the absence of other influences, we can formulate the evolution of a cell density \( \rho \) subject to cell–cell adhesion as:

\[
\frac{\partial \rho}{\partial t} = -\nabla \cdot (a^{c/c} \rho) + d\Delta \rho,
\]

\[
a^{c/c} = \kappa_f L(f, df) \nabla f,
\]

\[
\frac{\partial f}{\partial t} = -\mu f + \alpha \left(1 - \frac{f}{f_{\text{max}}}ight) \rho + D_f \Delta f. \tag{12.3}
\]

\( a^{c/c} \) denotes the adhesion force, \( \mu, \alpha \) and \( D_f \) the decay, release and diffusion parameters for the adhesion signal \( f \) and \( f_{\text{max}} \) defines the threshold value for the production of signal \( f \). The cutoff function \( L(f, df) \) is introduced to keep the magnitude of the gradient bounded by \( df \) in order to bound the migration velocity of the cells:

\[
L(f, df) = df \left(\text{max}(df, |\nabla f|)\right)^{-1}. \tag{12.4}
\]

The model is easily extended to account for different cell type populations \( \rho_i \)

\[
\frac{\partial \rho_i}{\partial t} = -\nabla \cdot \left(\sum_j a^{c/c}_{ij} \rho_j\right) + d_i \Delta \rho_i,
\]

\[
a^{c/c}_{ij} = \kappa_{ij} L(f_j, df) \nabla f_j,
\]

\[
\frac{\partial f_i}{\partial t} = -\mu_i f_i + \alpha_i \left(1 - \frac{f_i}{f_{i,\text{max}}}ight) \rho_i + D_i \Delta f_i. \tag{12.5}
\]

Here, \( \kappa_{ij} \) and \( a^{c/c}_{ij} \) denote the homotypic \((i=j)\) and heterotypic \((i \neq j)\) adhesion strength and adhesion force.

### 3.4 Close-Packing Density

So far, the model does not incorporate any repulsive effects that might delimit the local cell density. We introduce such effects by adding the following pressure-like term to the velocity:

\[
a^p = -\kappa_p H (\rho - \bar{\rho}) \nabla \rho \left|\nabla \rho\right|^{-1}, \tag{12.6}
\]
with the overall cell density $\rho \equiv \sum \rho_i$, a constant regulating the influence of cell pressure $\kappa_p$ and the Heaviside function $H$. We note here that the introduced forcing terms $a^x_i$ are additive and can be combined to build up a specific model.

### 3.5 Cell Sorting

In an attempt to explore our model of cell–cell adhesion, we perform a study of cell sorting, testing against the “Differential Adhesion Hypothesis” (see Steinberg 2007; Steinberg and Takeichi 1994 and references therein). The “Differential Adhesion Hypothesis” postulates that cellular sorting is an effect induced by differences in the intercellular adhesion strength amongst different cell populations. In our simulations, we consider two different cell types $\rho_1$ and $\rho_2$ which differ in their inter- and intracellular adhesion parameters $\kappa_i, \kappa_j$ and $\kappa_{ij}$. We observe different sorting behaviors (see Fig. 12.2) such as complete sorting, mixing or engulfment of one population by the other, depending on the choice of the adhesion parameters. Along with the effects of cell–cell adhesion, we explore the influence of the pressure/repulsion effects as introduced earlier (see case b in Fig. 12.2). Despite the fact that we model contact-adhesion via an indirect adhesion signal, the proposed model successfully recovers the different sorting behaviors.

### 3.6 The ECM, Chemo-, and Haptotaxis

We propose to model the haptotactic influence of the ECM structure on the cell migration velocity as the combinatory effect of the following assumptions. A cell will crawl along fiber bundles that are aligned with the chemotactic cue ($\nabla \phi$) in order to maximize its migration velocity. To propel itself along the scaffold provided by the ECM, cells rely on the presence of fibers. In the absence of fibers ($e = 0$) cell migration is impaired ($e_0 \ll 1$). On the other hand, if the fiber density is too high ($e \approx \rho_{cpd}$), cells have to degrade the matrix fibers before they are able to migrate and are slowed down. These assertions are formulated as:

$$a^{ ECM, \phi} = \left[ \left( 1 - \frac{\nabla e}{|\nabla e|} \cdot \frac{\nabla \phi}{|\nabla \phi|} \right) \nabla e + \nabla \phi \right] \left( e + e_0 \right) \left( \rho_{cpd} - e \right),$$

and illustrated in Fig. 12.3. We note, that the modeling of the chemotactic response as $a^{ ECM, \phi}$ is but the most simple one as it ignores many effects such as receptor saturation and activation thresholds.
Fig. 12.2  Top row depicts solution at \( t = 40 \), middle row at \( t = 160 \), bottom row depicts a cut through the domain at \( t = 160 \). Columns: \( a \) engulfment, \( \kappa_{11} = 0.25, \kappa_{22} = 0.025 \), and \( \kappa_{12} = \kappa_{21} = 0.05 \); \( b \) engulfment with pressure, \( \kappa_{11} = 0.25, \kappa_{22} = 0.025 \), and \( \kappa_{12} = \kappa_{21} = 0.05 \); \( c \) sorting, \( \kappa_{11} = 0.25, \kappa_{22} = 0.25 \), and \( \kappa_{12} = \kappa_{21} = 0.00 \); \( d \) mixing, \( \kappa_{11} = 0.25, \kappa_{22} = 0.09 \), and \( \kappa_{12} = \kappa_{21} = 0.2 \).

Fig. 12.3  A cell will move “onto” a fiber if the fiber direction is not transverse to the chemotactic gradient, i.e., the gradient of adhesion is not aligned with the chemotactic direction.

3.7 Angiogenesis-like Migration

We now combine the models of cell–cell adhesion, cell–matrix adhesion and chemotaxis in order to assemble a system that captures migration of cells through the ECM:
Fig. 12.4  Effect of increasing the length of the fibers (in (12.2)), \( m = 0.25, 1.0, \) and 4.0

Fig. 12.5  Effect of increasing the matrix density: 31\%, 51\%, and 75\%

\[
\frac{\partial f}{\partial t} = -\mu f + \alpha \rho \left( 1 - f_{\text{max}}^{-1} f \right) + D_f \Delta f, \\
\frac{\partial \rho}{\partial t} = -\nabla \cdot (\mathbf{a} \rho) + d \Delta \rho, \\
\mathbf{a} = \kappa_e \left( 1 - \left| \frac{\nabla e \cdot \nabla \phi}{|\nabla e|} \right| \right) \nabla e + \kappa_\phi \nabla \phi + \kappa_f \nabla \nabla \phi,  \tag{12.8}
\]

where \( \nabla_r \mathbf{x} \) denotes the regularized gradient \( L(\mathbf{x}, d\mathbf{x}) \) as defined in (12.4). For greater lucidity we omit here the effects of fiber density that we have introduced in (12.7). Figures 12.4–12.6 illustrate the effects of modifying the model parameters on the resulting vessel morphology. The growth factor concentration \( \phi \) in the reported simulations is kept constant throughout the simulation, linearly increasing towards the right side of the computational domain.
3.8 Matrix-bound Growth Factors

Until now, we have only considered the effect of purely soluble growth factors freely diffusing through the ECM, establishing a global growth factor gradient. It is however known that growth factors can exist in different isoforms, some having the ability to bind to heparine binding sites in the ECM. These isoforms can dynamically bind and unbind to the matrix and do not diffuse freely. Amongst other cell lines, endothelial cells secrete matrix metalloproteinases (MMPs) that have the capability to cleave the matrix binding domain from the rest of the VEGF molecule. The cleaved part of the VEFG molecule becomes diffusible again while retaining its angiogenic signaling potential. We extend our model to account for these processes by distributing small pockets of matrix-bound VEGF at the beginning of the simulation. ECs are modeled to secrete a compound that cleaves the matrix-bound VEGF. Upon cleavage, the bound VEGF become diffusible and adds to the global gradient established by the purely soluble VEGF isoform. Simulation results of such a situation are reported in Fig. 12.7.

Although a setting like this leads to the formation of localized chemotactic cues, we do not observe an increase in branching as observed in both in-vitro and in-vivo models of angiogenesis and vasculogenesis (Ruhrberg et al. 2002; Lee et al. 2005). If we look at the size of the distributed VEGF pockets in the order of a cell diameter, we must realize that the size of the pockets is much larger then what would be found in a real ECM. In the formulation of our continuum model, however it is not possible to explicitly account for localized structures that are of a size of one to two orders in magnitude smaller than cell size without decreasing the computational mesh size drastically. We therefore suggest to introduce a subgrid-scale modeling approach.

From a mesoscopic point of view the effect of localized chemotactic cues that are smaller than the description scale will clearly not propagate into any distinguishable residual localized movement. However, we can expect to see the cumulative effect on one cell, which from a mesoscopic point of view will result in increased random
Fig. 12.7  Simulation with matrix-bound growth factors using pockets of matrix-bound VEGF blue distributed in the matrix. The endothelial cells red release MMPs that cleave the bound growth-factors and make them soluble (diffuse blue cues)

motion. It has been shown that microscopic random motion can be modeled as diffusion from a macroscopic view point. We therefore extend the present model to include a spatially varying diffusion term in the equation of EC density, negligible in the absence of matrix-bound VEGF and increasing depending on the local concentration of matrix-bound VEGF. In this model, both release of MMPs and the cleaving of the bound VEGF is modeled implicitly via an increase in EC diffusivity. Results of the modified system showing an increased branching behavior in the presence of matrix-bound VEGF are depicted in Fig. 12.8.

3.9  Summary

The model of sprouting angiogenesis presented in this work relies on a pure continuum, diffuse interface model description. It integrates key aspects of cell
We capture cell–cell adhesion effects indirectly via the introduction of an artificial autocrine adhesion signal. Despite the simple formulation, the model manages to recover aspects of cell sorting behavior as formulated in the “Differential Adhesion Hypothesis.” In comparison to existing continuum models of cell–cell adhesion (Armstrong et al. 2006), the presented model is less intuitive but in turn benefits from an easier and more efficient implementation.

As a result of the explicit matrix representation that exerts an adhesive force on the migrating endothelial cells, the model recovers the branching behavior of migrating endothelial cells. The branching is an output of the model, and does not rely on the formulation of any heuristic branching rules.
In addition to soluble chemotactic cues, the model has been extended to also consider matrix-bound cues, modeled using a subgrid-scale approach. The consideration of these localized migration cues leads to an increase in the observed vessel branching, reproducing similar morphological features as observed under experimental conditions.

In its general formulation, the proposed method could easily be adapted to addressed other phenomena involving mesenchymal cell migration. One further tumor related phenomenon that might be addressed with the proposed model is the infiltrating growth of cancer cells as observed in glioblastoma (see Fig. 12.9).
4 A Hybrid Model of Sprouting Angiogenesis

This chapter is largely based on the work presented in Milde et al. (2008). For a more detailed description, we refer the reader to the original article. The deterministic model of sprouting angiogenesis presented in this section follows a hybrid approach, combining a continuum description of VEGF, MMPs, fibronectin and endothelial stalk cell density with a discrete, agent-based particle representation of the tip cells. We make use of particle-mesh interpolation methods (see Chapter Particle Simulations of Growth: Application to Tumorigenesis, Sect. 2.3) to derive an implicit three-dimensional level-set representation. The level-set description of the capillary sprouts is an extension of the discrete indicator functions considered in existing models. The consideration of particle based, grid-independent tip cell migration was initially proposed by Plank and Sleeman (2004).

The model explicitly considers the presence of both localized matrix-bound and freely diffusing VEGF isoforms. Endothelial tip cells are modeled to release proteases (MMPs) that can cleave the matrix-bound isoforms. We present studies that highlight the effects observable on the vessel geometries.

Further, the model considers the explicit modeling of the ECM to influence EC migration and branching. This approach renders the model completely deterministic in contrast to existing modeling approaches (McDougall et al. 2006; Plank and Sleeman 2004; Chaplain 2000). In Sun et al. (2005) the ECM was addressed by a heterogeneous and anisotropic description of conductivity. Similar to the work presented in Sun et al. (2005), the ECM model presented herein acts as a tensor on the migration cues established by the chemotactic and haptotactic gradients. Additionally, the present work considers the fibers to offer binding sites for matrix-bound VEGF and fibronectin.

We model tip cell branching events to occur in response to diverging directional cues as mediated by the VEGF and fibronectin gradients as well as the ECM fiber orientation. The local divergence of the migration cues is sensed via the explicit modulation of tip-cell filopodia. We note that no additional branching probabilities need to be introduced (Anderson and Chaplain 1998; Chaplain 2000; Plank and Sleeman 2004). Other models not depending on probabilistic branching rules are presented in Sun et al. (2005); Bauer et al. (2007). An overview of the considered model entities is given in Table 12.1.

4.1 Vascular Endothelial Growth Factors

Of the several growth factors involved in tumor-induced angiogenesis, VEGF has been identified as the key regulator (Ferrara et al. 2003). The release of soluble VEGF (sVEGF) from tumor cells in this model is modeled implicitly via a source term. In addition, a matrix-bound VEGF isoform (bVEGF) is considered. bVEGF is assumed to be locally distributed in the ECM and does not diffuse (Ruhrberg et al. 2002; Taraboletti et al. 2006).
Table 12.1 Molecular species, cells, cell densities and matrix property definitions

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>([bVEGF])</td>
<td>Concentration of matrix-bound VEGF</td>
<td>(12.9)</td>
</tr>
<tr>
<td>([cVEGF])</td>
<td>Concentration of cleaved matrix-bound VEGF</td>
<td>(12.10)</td>
</tr>
<tr>
<td>([VEGF])</td>
<td>Concentration of soluble VEGF</td>
<td>(12.11)</td>
</tr>
<tr>
<td>([FIB])</td>
<td>Concentration of unbound fibronectin</td>
<td>(12.12)</td>
</tr>
<tr>
<td>([bFIB])</td>
<td>Concentration of matrix-bound fibronectin</td>
<td>(12.14)</td>
</tr>
<tr>
<td>([MMP])</td>
<td>Concentration of matrix metalloproteinases</td>
<td>(12.15)</td>
</tr>
<tr>
<td>([EC])</td>
<td>Endothelial tip cell density</td>
<td>(12.12)</td>
</tr>
<tr>
<td>(\rho)</td>
<td>Endothelial cell density lining capillary walls Sect. 4.7</td>
<td></td>
</tr>
<tr>
<td>(K)</td>
<td>Vector field representing the fiber orientation in the ECM Sect. 4.5</td>
<td></td>
</tr>
<tr>
<td>(E_\rho)</td>
<td>Fiber density in the ECM Sect. 4.5</td>
<td></td>
</tr>
<tr>
<td>(E_\chi)</td>
<td>Fiber indicator function in the ECM Sect. 4.5</td>
<td></td>
</tr>
</tbody>
</table>

Matrix-Bound VEGF \([bVEGF]\) On initialization, Matrix-bound VEGF is distributed in spherical clusters throughout the computational domain. The pockets of \(bVEGF\) are mollified by gaussian filtering and multiplied with the matrix indicator field \(E_\chi\) to dispose of \(bVEGF\) not associated with any matrix fibers. MMPs released from migrating ECs can cleave the bound VEGF (Lee et al. 2005).

The evolution of Matrix-bound VEGF is given by

\[
\frac{\partial[bVEGF]}{\partial t} = -v_{bV}[MMP][bVEGF],
\]

with cleaving rate \(v_{bV}\).

Cleaved VEGF \([cVEGF]\) Once cleaved, the released VEGF (cVEGF) diffuses through the ECM. Both cleaved VEGF as well as soluble VEGF are subject to natural decay at a rate given by \(d_V\).

\[
\frac{\partial[cVEGF]}{\partial t} = k_V \nabla^2[cVEGF] + v_{bV}[MMP][bVEGF] - v_V[cVEGF][\rho] - d_V[cVEGF].
\]

Endothelial cells express surface receptors that bind VEGF molecules (Kearney et al. 2004), enabling the cells to sense VEGF gradients in their vicinity and to trigger chemotaxis (Gerhardt et al. 2003). Receptor ligand binding and the internalization of VEGF is captured in the model via VEGF uptake by the ECs at a rate \(v_V\). The endothelial cell density is given by \(\rho\).

Soluble VEGF \([sVEGF]\) Upon release, the soluble VEGF proteins diffuse through the ECM and are subject to endothelial uptake and decay. The diffusion constant is given by \(k_V\).

\[
\frac{\partial[sVEGF]}{\partial t} = k_V \nabla^2[sVEGF] - v_V[sVEGF][\rho] - d_V[sVEGF].
\]
4.2 Fibronectin

Unbound Fibronectin \([\text{FIB}]\) Fibronectin is released by the migration ECs depending on its local concentration (Alberts et al. 2002). We consider fibronectin to bind to integrins located at the EC membrane and to ECM fibers, establishing an adhesive interaction force between the fibers in the ECM and the cells (Serini et al. 2006). When not bound, fibronectin diffuses through the ECM with a diffusion rate \(k_F\) and decays at a rate given by \(d_F\).

\[
\frac{\partial [\text{FIB}]}{\partial t} = k_F \nabla^2 [\text{FIB}] + \gamma_F \mathcal{G}(F_{th}, [\text{FIB}])[\text{EC}] 
- \nu_{bF}[\text{FIB}] \left( E_x bF_{th} - [\text{bFIB}] \right) - d_F [\text{FIB}],
\]

with creation rate \(\gamma_F\) and creation function

\[
\mathcal{G}(C_{th}, [C]) = \frac{C_{th} - [C]}{C_{th}} ,
\]

depending on the creation threshold level \(F_{th}\). The rate of fibronectin binding to the ECM is given by \(\nu_{bF}\). \(bF_{th}\) is a constant introduced to account for binding site saturation in the ECM.

Matrix-Bound Fibronectin \([\text{bFIB}]\) Bound to the ECM, fibronectin establishes a haptotactic gradient for the endothelial cells (Paweletz and Knierim 1989). Matrix bound fibronectin does not diffuse, is subject to degradation by MMPs at degradation rate \(\delta_{bF}\) and decays at a rate given by \(d_{bF}\).

\[
\frac{\partial [\text{bFIB}]}{\partial t} = \nu_{bF}[\text{FIB}] \left( E_x bF_{th} - [\text{bFIB}] \right) - \delta_{bF} [\text{bFIB}] \mathbb{MMP} - d_{bF} [\text{bFIB}],
\]

4.3 Matrix Metalloproteinases (MMPs) \([\text{MMP}]\):

MMPs are proteases involved in the degradation of the ECM, cleaving matrix bound proteins such as bVEGF isoforms from the binding sites in the ECM (Lee et al. 2005). Migrating ECs are assumed to release the MMPs at a rate depending on the local MMP concentration (Iruelaarispe et al. 1991; Mignatti and Rifkin 1993). Upon release, MMPs are assumed to diffuse through the ECM and are subject to natural decay at a rate given by \(d_M\). MMP release is bounded by a predefined threshold level \(M_{th}\). The release and diffusion constants are given by \(\gamma_M\) and \(k_M\), and \(\text{EC}\) denotes the endothelial tip cell density. Evolution of the MMP concentration is defined as:

\[
\frac{\partial [\text{MMP}]}{\partial t} = k_M \nabla^2 [\text{MMP}] + \gamma_M \mathcal{G}(M_{th}, [\text{MMP}])[\text{EC}] - d_M [\text{MMP}],
\]
4.4 *Endothelial Cells*

Endothelial cells are the cells lining the interior wall of the blood vessels. Proliferating and migrating into the extracellular space, ECs are actively involved in the formation of new blood vessel sprouts and capillary networks. During angiogenesis, ECs can be observed to occur in three different states: Quiescent cells lining the vessel walls of the matured vasculature. Proliferating cells located in a small region behind the sprouting front and migrating tip cells extending filopodia, actively moving into the extracellular space.

**Tip Cell Migration** While proliferating cells in the sprout stalk provide the cells necessary for capillary growth, the migrating tip cells at the sprout front determine the morphology of the growing capillaries (Gerhardt et al. 2003). Chemotactic and haptotactic cues in the matrix are established via VEGF and fibronectin gradients and determine the migration direction (Gerhardt et al. 2003; Ferrara et al. 2003; Paweletz and Knierim 1989). As the VEGF level increases in the proximity of the tumor, EC surface receptors become occupied, attenuating the cells ability to sense the chemotactic cues (Kearney et al. 2004; Gerhardt et al. 2003). The function $\mathcal{W}$ is introduced to capture the effect of receptor saturation. Tip cell acceleration driving migration is defined as:

$$ a = \alpha \left( E_\rho \right) \mathcal{T} \left( \mathcal{W} \left( \left[ \text{VEGF} \right] \right) - \left[ \text{VEGF} \right] + w_F \nabla \left[ \text{bFIB} \right] \right), $$

(12.16)

where

$$ \mathcal{W} \left( \left[ \text{VEGF} \right] \right) = \frac{w_V}{1 + w_{V2} [\text{VEGF}]}, $$

(12.17)

with chemotactic parameters $w_V$ and $w_{V2}$ and

$$ [\text{VEGF}] = [s\text{VEGF}] + [b\text{VEGF}] + [c\text{VEGF}]. $$

(12.18)

The matrix density has a direct influence on the migration velocity. The presence of fibers ($E_\rho$) provides a scaffold for migrating tip cells, thus enhancing migration speed of ECs (Friedl and Bröcker 2000). A very dense matrix on the other hand builds up a barrier for migrating ECs that has to be degraded for cells to migrate through, effectively slowing down tip cell migration (Davis and Senger 2005). We capture this effect in the function

$$ \alpha \left( E_\rho \right) = (E_0 + E_\rho) (E_1 - E_\rho) C_1, $$

(12.19)

where a threshold $E_0$ is introduced to define the migration factor in the complete absence of fibers, $E_1$ the maximal fiber density completely blocking migration, and $C_1$ is the ECM migration constant. To model the directional cues the fiber bundles exert on cell migration, a tensor $\mathcal{T}$ is introduced acting on the migration velocity:

$$ \{ \mathcal{T} \}_{ij} = (1 - \beta \left( E_\chi \right)) \{ \mathcal{I} \}_{ij} + \beta \left( E_\chi \right) K_i K_j, $$

(12.20)
with

$$\beta(E_\chi) = \beta_K E_\chi,$$  \hspace{1cm} (12.21)

with the ECM strength $\beta_K$ and $K$ being the vector field the tensor is applied on.

Particle positions $x_p$ are updated according to:

$$\frac{\partial x_p}{\partial t} = u_p, \quad \frac{\partial u_p}{\partial t} = a_p - \lambda u_p,$$  \hspace{1cm} (12.22)

with $u_p$ and $a_p$, the velocity and acceleration, respectively, at particle location $x_p$ and $\lambda$ the drag coefficient.

**Branching** In this model, we consider branching of tip cells to depend on the presence of diverging migration cues in the ECM (Ruhrberg et al. 2002). Endothelial cells probe their surrounding environment for chemo- and haptotactic cues extending filopodia equipped with cell surface receptors (Gerhardt et al. 2003). Locations of high anisotropy suggesting a locally diverging migration acceleration field $V$ are detected by a curvature measure:

$$k(x) = \frac{||\hat{L}(x) \times \tilde{L}(x)||}{||\hat{L}||^3}.$$  \hspace{1cm} (12.23)

with $V = (u, v, w)$, $\hat{L}(x) = V(x)$ and $\tilde{L} = uV_x + vV_y + wV_z$ (Weinkauf and Theisel 2002).

If a sprout tips age exceeds the threshold level $s_{ath}$ necessary for branching, and the local curvature $k$ is greater than the defined threshold level $ai_{th}$, branching occurs. In order to determine the exact branching direction in 3D, we introduce a satellite particle-based filopodia model to sense the migration cues in the vicinity of the tip cell. 6 satellite particles are distributed radially around the tip cell in a plane perpendicular to the migration direction (Fig. 12.10). We then measure the velocities at the satellite positions and compute the angle between velocities at opposing satellite points. The branching direction is defined by the location of the two satellite particles with the largest angle between velocities pointing away from the migrating direction. A new tip cell is then created and the particle velocities $u_p$ on the right-hand side of (12.22) are updated

$$u_p' = \frac{u_p}{1 + \beta} \left( \frac{a_s}{|a_s|} + \beta \frac{x_s - x_p}{|x_s - x_p|} \right),$$  \hspace{1cm} (12.24)

where $u_s$ denotes the satellite velocity at satellite position $x_s$ and $\beta = 0.8$. This results in a short acceleration of the two tip cells towards the satellite positions.

**Anastomosis** The formation of loops in the growing capillary network can be observed after the sprouts have extended some distance into the ECM and branching has taken place (Paweletz and Knierim 1989). We consider two distinct fusion
Fig. 12.10 The figure shows satellite particles placed in the plane perpendicular to the sprout migration direction. $u_1$ through $u_6$ describe the local migration cues at sprout particle location.

events, the fusion of two tip cells and the fusion of a tip cell with an existing sprout. When a fusion event takes place, a loop is closed and migration stops for the fusing sprout tips.

4.5 ECM

We model the ECM as a collection of fiber bundles randomly distributed throughout the computational domain (see also Sect. 3.2). We introduce a three-fold representation of the ECM given by the grid-functions $F$ describing the fiber orientations, $E_x$, a smooth indicator function and the fiber density field $E_\rho$ used to regulate the migration speed as defined in (12.17).

The procedure generating the fiber field follows the one described in Sect. 3.2. For the field $K$ the fiber directions are rasterized onto the grid, and for $E_x$ we tag the grid points at the fiber locations with a value of 1.

$K$ and $E_\rho$ are filtered with a Gaussian filter to obtain a smooth matrix representation. The same is not possible for the vector field $E_x$, so the field is constructed using smoothed fibers. For overlapping fibers, the maximum value of the two fibers is retained.

4.6 Methods

We suggest the application of a fractional step algorithm in order to solve the coupled reaction-diffusion system efficiently. In the suggested algorithm the nonlinear
and linear reaction parts of the equations are solved simultaneously using explicit Euler steps. To solve the linear diffusion part of the system, we employ implicit time stepping.

The reaction-diffusion system can be safely decoupled from the tip-cell advection, as EC cell migration occurs on a much larger time scale than molecular diffusion. Steady state can be assumed for the source and sink of the molecular species. Spatial gradients and the curvature measure are calculated on the grid using second order finite differences. We apply Mesh-Particle interpolations using the \( M^4 \) kernel (see Chapter Particle Simulations of Growth: Application to Tumorigenesis, Sect. 2.3) in order to approximate the acceleration and curvature at the tip particle location \( \mathbf{x}_p = (x_p, y_p, z_p) \).

The Particle-Mesh interpolations of the sprout tip density onto the grid are performed using a 4th order B-spline kernel, guaranteeing positive values for SE at all grid locations.

### 4.7 Endothelial Cell Density

Capillary sprouts are defined by the endothelial cell density \( \rho \). We obtain the endothelial cell density by interpolation of the sprout tip cell density \( Q_p \) at \( \mathbf{x}_p \) onto the grid using a 4th order B-spline kernel \( B_4 \). The interpolation is done every time step, \( \rho \) is updated to the maximum of the interpolated sprout tips and the \( \rho \) field at the previous time step.

\[
\rho_{ijk}^{n+1} = \max \left( \rho_{ijk}^n, \sum_p B_4(ih - x_p) B_4(jh - y_p) B_4(kh - z_p) Q_p \right), \tag{12.25}
\]

with particle weight \( Q_p \), and mesh size \( h \). The grid value \( \rho_{ijk} \) denotes the cell density at grid point \([i, j, k]\) and \( n \) denotes the \( n \)th time step.

### 4.8 Parameters

All model equations are nondimensionalized by scaling the molecular concentrations \( c \) with a maximum threshold concentration \( c_0 \) of the respective species. The endothelial cell density [EC] is scaled with the close packing cell density \([EC]_0\). Space and Time variables are scaled with the domain size \( D_1 \) and \( D_1^2 / k_V \), respectively, where \( k_V \) is the VEGF diffusion coefficient.

We report the set of initial parameters in the table below. For a detailed description of the parameter values, we refer to Milde et al. (2008) and the works referenced therein.
Fig. 12.11 The figure shows a conceptional $x - z$ plane through the computational domain. Five sprout tips are initially placed on the $y - z$ plane lower end of the domain in $x$ direction, a tumor source of soluble VEGF is modeled at the upper end in $x$ direction outside the computational domain.

\begin{align}
    k_V &= 1.0 & v_{bV} &= 100.0 & v_V &= 0.0 & d_V &= 0.675 \\
    k_F &= 0.05 & \gamma_F &= 113.0 & d_F &= 67.5 \\
    v_{bF} &= 1.0 & \delta_{bF} &= 10.0 & d_{bF} &= 0.675 \\
    k_M &= 0.1 & \gamma_M &= 113.0 & d_M &= 0.1 \\
    bF_{ih} &= 0.5 & F_{ih} &= 0.001 & M_{ih} &= 1.0 \\
    w_V &= 0.1 & w_{V2} &= 0.5 & w_F &= 0.001 \\
    D_l &= 1.0 & Fb_l &= 10.0 & sa_{ih} &= 5.0 & ai_{ih} &= 15.0 \\
    bV_l &= 0.25 & bV_r &= 0.008 & Q_p &= 1.0
\end{align}

Unless indicated along with the specific simulations, the results reported in this section are based on simulations using the above parameter value set.

### 4.9 Initial and Boundary Conditions

We define the computational domain as a cube of size $D_l^3$ discretized with a $128^3$ uniform grid. At the beginning of the simulation, five vessel sprouts are placed along a line at $z = 0.5$ on the $y - z$ plane at $x = 0.0$, equally distributed in $y$. The precise locations of the sprouts are at $y = 0.15625, 0.3125, 0.46875, 0.625, 0.78125$ (see Fig. 12.11).

The tumor source of VEGF is modeled via a Dirichlet boundary condition in the $x$ direction, with $[s\text{VEGF}] = 1.0$ at $x = 1.0$ and $[s\text{VEGF}] = 0.0$ at $x = 0.0$. All other concentrations are subject to homogenous Neuman boundary conditions.
in the $x$ direction. In $y$ and $z$ directions we apply periodic boundary conditions for all concentrations and the capillary sprout tips. Matrix bound VEGF pockets are distributed throughout the domain according to the distribution given by

$$
\begin{pmatrix}
  x \\
  y \\
  z
\end{pmatrix} = \left( \begin{array}{c}
  0.1 + 0.9 \sqrt{r_1} \\
  r_2 \\
  r_3
\end{array} \right) \text{ with } r_1, r_2, r_3 \text{ u.a.r } [0, 1].
$$

(12.27)

The increase in concentration along the $x$ axis accounts for the tumor source of VEGF. All sprouts leaving the domain in $x$ direction are eliminated from the simulation.

### 4.10 Results

In the context of large scale parametric studies, we investigate the effects of the structure of the ECM and the distribution of matrix-bound VEGF and present results on the morphology of the generated vessel networks. The presented three-dimensional simulations are novel as they include explicitly soluble and bound growth factors. They account for the structural properties of the ECM and its binding sites and investigate their effects on endothelial cell migration. We quantify the simulation and report statistics on the branching and fusion events in combination with observable measures on the vascular trees such as the branch length distribution. We note that the statistics provide a quantitative, comparative analysis that may guide future experiments and simulations.

**Matrix Structure** In a first set of simulations, we assess the effect of the ECM density on the generated capillary networks. Different number of fiber bundles are distributed at random to create ECMs of increasing densities: 15,000 fibers resulting in a volume density of 6%, 30,000 fibers (11%), 70,000 fibers (26%), 100,000 fibers (38%), and 200,000 fibers (75%) (see Fig. 12.12). We report the normalized volume density of the matrix as the average density of the fiber density field. For each case, we performed 32 simulations with a different random seed for the fiber placement.

Stopping the simulations at time $T = 22$ and comparing the number of branch points found in the computational domain, we find a linear increase of the number of branches for a logarithmic increase in the fiber density up to a threshold density around 40% (Fig. 12.13). After this saturation point, the number of branches can be observed to decrease again. We report examples of vessel network morphologies as observed for different matrix densities in Fig. 12.14. We note that in a low density ECM, branching is largely reduced while in very dense ECMs, branching events can be observed to happen very frequent. In the case of a very high fiber density (75%), we observe that the fiber density is high enough to impair cell migration, leading to shorter capillary networks (Fig. 12.14, E).
Fig. 12.12 Slice of the [ECM] field for five different densities: A 6%, B 11%, C 26%, D 38%, and E 75%.

Fig. 12.13 Influence of the matrix density on the number of branches of the vessel network (error bars represent standard deviation of data).
Matrix-Bound VEGF Focussing on the effect of matrix-bound VEGF pockets, we vary the amount of matrix-bound VEGF, by increasing the number of pockets, distributing 500, 1,400, 3,700 and 10,000 pockets holding a VEGF level of $bV_l = 0.25$. Investigating the effect of matrix-bound VEGF we decrease the threshold age for branching $s_{a_{th}} = 4$ and increased the branching threshold level of anisotropy $a_{i_{th}} = 45$ in all simulation. The parameters governing MMP evolution are set to: $k_M = 0.01; \gamma_M = 250$ and $d_M = 100$. In order to focus on the effects induced by matrix-bound VEGF, haptotaxis is completely switched off: $w_F = 0.0$. We observe that an increase in the number of pockets leads to an increase in the number of branching events (see Fig. 12.15).

The pockets induce strong local gradients (Fig. 12.16), increasing the measured curvature $k$ at the pocket location. For high pocket densities, the probability for cells to hit a pocket location is increased, consequently leading to an increase in branching. The presence of a higher pocket density, the probability of a sprout tip hitting a pocket is amplified, consequently leading to an increase in branching.

In a second set of simulations, we varied the VEGF level, comparing runs with constant number of 3,700 pockets containing a VEGF level of $bV_l = 0.01, 0.03, 0.09$ and 0.25 (not shown here) (Milde et al. 2008). Again, we
Fig. 12.16  Effect of MMPs cleaving matrix-bound VEGF on chemotactic cues and anisotropy at pocket location for $bV_n = 3,700$ and $bV_l = 0.25$: $t = 13.21$ (A), $t = 16.89$ (B) and $t = 20.63$ (C). The white clouds in the left figure denote the MMP concentration, the orange dots indicate pockets of matrix-bound VEGF. The figure in the middle shows the evolution of the acceleration field at pocket location. The results indicate that not only the density of bound VEGF but also the pocket level has an effect on branching behavior. Here, the effect can not be attributed to the increase in the probability of a sprout tip hitting a pocket but to the MMPs cleaving the matrix-bound VEGF, effectively reducing the chemotactic cues together with the level of anisotropy at pocket location (see Fig. 12.16).

As pockets are being cleaved, their influence on the branching and migration behavior is reduced. In the case of low pocket VEGF concentrations, the cleaving removes most VEGF from the pocket before the sprout tips get there. Thus, the
influence of bound VEGF levels on the observed branching behavior. As for the branch length distributions in the absence of bound VEGF as compared to the case of 100,000 distributed pockets (see Fig. 12.17), no significant variation is observed in the mean.

However, the distributions for the two cases vary notably, showing a narrower distribution of the branch lengths in the presence of bound VEGF pockets. Concerning the probability of anastomosis, we found that an increase in the number of distributed VEGF pockets leads to an increase in the number of fusion events (not shown here).

A similar effect has been observed for an increase in the weight of haptotactic cues (results reported in Milde et al. (2008)). If however we compare the probability of anastomosis against the number of branches, we find that the two simulation settings having quite a different effects on the fusion probability (see Fig. 12.18).
Fig. 12.19 Effect of pocket number of matrix-bound VEGF ($bV_n$) on the vessel network morphology: $bV_n = 500$ (A), $bV_n = 1,400$ (B), $bV_n = 3,400$ (C), and $bV_n = 10,000$ (D)

Fig. 12.20 Evolution of angiogenesis (red) in the presence of matrix bound VEGF (blue). Bound VEGF is cleaved by MMPs (not shown)

The morphology of the vessels (Figs. 12.19, 12.20) suggests that the distributed VEGF pockets attract the migrating sprout tips, leading to a deviation in the migration projectory, whereas the haptotactic cues introduce directed deviation towards existing sprouts (not shown here) (Milde et al. 2008).

4.11 Conclusions

We present a three-dimensional model of sprouting angiogenesis that incorporates the effects of the extracellular matrix structure on the vessel morphology and considers both soluble and matrix-bound growth factor isoforms. Conducted over large scale parametric studies, we report observations on the vessel network morphology as influenced by the structure of the ECM and the distribution of matrix-bound VEGF. The reported results stress the importance of the implicit consideration of an ECM, as its structure and density directly affect the morphology, expansion speed and the branching dynamics of the computationally grown capillary networks. As has been observed experimentally in Friedl and Bröcker (2000); Davis and Senger (2005); Serini et al. (2006), the simulations reflect the strong influence of the ECM composition on the process of angiogenesis. Simulation results on sprout morphology and branching patterns presented for sprouting angiogenesis
in the presence of matrix-bound VEGF isoforms are consistent with the findings made in Lee et al. (2005); Ruhrberg et al. (2002). We would like to stress that with the number of branches depending on the matrix structure and the presence of matrix-bound VEGF isoforms, the model may be easier to tune against experiments compared to branching probabilities that most individual-based methods employ. The grid independent particle representation of migrating tip cells leads to the generation of smooth capillary networks. The coupling of the particle and mesh-based representation is straightforward in the context of Particle to Mesh and Mesh to Particle interpolation schemes. Limitations of the current model are related to the explicit initialization of tip cells at predefined locations. New sprout tips can only emerge at the sprouting tip via branching.

References


Paweletz N, & Knierim M (1989) Tumor-related angiogenesis; Critical Reviews in Oncology/ Hematology, 9(3):197–242


