Continuum Models of Mesenchymal Cell Migration and Sprouting Angiogenesis

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INTRODUCTION

Cell migration is fundamental to a number of physiological processes such as embryogenesis, organ growth, inflammation, wound healing, and tumor-induced angiogenesis. In embryonic development, the blastocyst cells migrate to form layers (gastrulation) and later migrate to target destinations in the developing embryo to specialize and become components of organs. This process of developmental migration continues in the adult, as some cells in our bodies are born, migrate, and die on a daily basis. Wound healing is another example of collective cell migration, involving the proliferation of existing cells and the migration of cells close to the wound towards each other to close the cleft. In the pathological context, enhanced cell migration is key to invasive growth of tumor cells, which is the basis of metastasis. This invasion involves the detachment of individual cells from their originating tissue and is followed by their propulsion through existing tissue.

A particular aspect of cell migration that has attracted increased attention recently is related to the process of angiogenesis as induced by tumor cells emitting vascular endothelial growth factors (VEGFs). Tumor-induced formation of new blood vessels in the context of sprouting angiogenesis is based on the orchestrated migration of clusters or cords of endothelial cells. The endothelial cells migrate through the extracellular matrix (ECM) following chemotactic and haptotactic cues. For these endothelial cells, the most prominent chemoattractant is VEGF [1]. VEGF gradients can be established in a variety of ways; soluble VEGF isoforms can be secreted by tumor cells, macrophages, or astrocytes [2]. This secretion can lead to long-range chemotactic signals. At the same time, some chemokines can also be sequestered in the ECM, where they represent highly localized cues. Another subset can be cleaved by matrix proteinases [3] and made soluble, or released as the matrix itself is degraded by heparinases or plasmin [4,5].

All these processes are characterized by a collective, directed motion of the cells in play. In many cases, the migration direction is aligned with a chemical gradient, for example, of nutrient or a growth factor: in this case, the migration is referred to as chemotaxis. There has been much
debate on how cells are able to persistently sense a chemical gradient of a chemottractant. Mathematical models have been used largely as qualitative indicators of cell motion. Their predictive value can be traced to their capability of differentiating between various mechanisms by which a cell might be able to sense a gradient and migrate in response to it, in a sustained fashion. A number of mathematical models have been proposed for gradient-driven cell motions. These models are predominantly based on mechanisms of “Local Excitation-Global Inhibition” (LEGI) [6] and its extension to “balanced inactivation” [7] (see Reference [8] for a review of chemotaxis models). The effect of noise on the directional sensing ability of these models has been studied by Ueda and Shibata [9], and Rappel and Levine [10], and may lead to testable predictions.

Morphogenesis In Silico

The simulations of sprouting angiogenesis can be broadly categorized as simulations of morphogenetic patterns that in turn correspond to a variety of computational models. These models often focus on a particular aspect of the patterning process and, in turn, their computational parameters are tuned to the process under consideration. For example, a vast body of literature exists on the mathematical description of bacterial chemotaxis (see the comprehensive review by Tindall et al. [11]). The patterning observed in developmental vasculogenesis has been successfully reproduced in silico by simulations that independently considered either purely chemical signaling [12] or purely mechanical effects [13].

While much of the theoretical work published on chemotactic systems focuses on modeling the gradient sensing response, the representation and interaction of the cells, or in reproducing final, static patterns, little attention has been paid to the environment in which the migration takes place. Models of vasculogenesis [13–15], either consider the ECM as a homogeneous viscoelastic tissue, or do not consider it at all (see review [16] for a survey of different vasculogenesis models). Although reported mathematical models of sprouting angiogenesis continue to achieve a great degree of sophistication [17], in these models the ECM plays a mostly secondary role and is assumed to be homogeneous. Heterogeneity is introduced through random components in the behavioral rules of the cells; for example, cells branch with a predefined probability [18,19]. Exceptions include the deterministic model of Sun et al. [20], where the ECM affects the migration by deflecting the migration velocity through a random anisotropy. On a microscopic scale, the ECM was structurally considered in [21] a Cellular
Potts approach. Due to the detailed cell representation, this model is only suitable for studying the dynamics of a handful of cells.

A grand majority of the models that describe to some extent the dynamics of collective cellular motion are based on cell-based representations. These formulations are appealing because they allow researchers to impose behavioral rules in an intuitive, cell-centered fashion. Also, the interpretation of the results is straightforward, as there is no abstraction layer; for example, instead of integrating a mean cell density, one can simply count the cells in the plot. A smaller subclass does employ continuum formulations; however, at a coarse macroscopic scale, where the output of the simulation has to be interpreted as a probability density function for the cell density, and therefore, morphological information is all but completely buried in the description. One reason why continuum models that can recover detailed morphological features are scarce is because some cellular interactions are difficult to model at the macroscopic scale. One basic problem is the representation of cell–cell adhesion. In cell-based models, cell–cell adhesion can conveniently be expressed in the shape of an intercell attraction. In mesoscale continuum models, the notion of a single cell does not exist, and we have to model the macroscopic effect of cell–cell adhesion. A handful of approaches have been proposed; in the context of tumor growth, cell–cell adhesion is often represented by a surface tension term that acts on the interface between tissues of different cell types [22,23]. Other continuum models have been proposed [24] based on the concept of deducing collective continuum behavior from an underlying discrete model [25], or on medium-range attractive forces [26]. One common drawback of these models is that they are computationally expensive. Surface tension calculations in continuum models require solving a global problem. The approach of Armstrong et al. [26] is local; it, however, requires performing a convolution with a kernel involving $O(20^d)$ neighbors.

In a recent work, we introduced the first hybrid model of sprouting angiogenesis, which explicitly considers the structure of the ECM [27]. In this model, matrix fibers are explicitly represented as a collection of fiber bundles that direct cell migration, and can sequester chemical cues leading to branching of blood vessels without requiring a priori defined branching probabilities. Painter [28] employed a similar approach to study cell organization and the incipience of fingering patterns. In the present article, we focus on the continuum formulation of a computationally efficient cell–cell adhesion model, and the interaction of in silico cells with the underlying artificial ECM.
Sprouting Angiogenesis

Sprouting angiogenesis, the process of new capillaries forming from existing vessels, can be observed in the human body under various conditions. Apart from angiogenesis in a physiological context, which mainly takes place during embryogenesis and fetal development, angiogenesis can be observed under pathological conditions, such as wound healing, thrombosis, and tumor growth [29]. In the case of wound healing and thrombosis, newly formed capillaries grow in a controlled manner and stop growing once the pathological condition has been alleviated; this is, however, not the case for tumor-induced angiogenesis [29].

Tumor-induced angiogenesis can persist for years, involving a disorganized, inefficient, and leaky vasculature [29]. Nonetheless, this vasculature supplies the tumor with nutrients and growth factors, which enable increased tumor cell proliferation and thus enhanced tumor growth. Furthermore, angiogenesis enables hematogenous spread of cancer: single cancer cells or cell clusters that detach from the primary tumor may enter the leaky vessels and use the vasculature to metastasize to remote organs. Regulating, or even inhibiting tumor-induced angiogenesis, can affect tumor growth. Inhibition of angiogenesis restrains nutrient supply, reducing the growth rate of the tumor and hindering migrating cell clusters from entering the vasculature, thus reducing the risk of metastasis [2]. However, a complete inhibition-promoting hypoxia (state of oxygen shortage) within the tumor could increase the occurrence of aggressive migrating tumor cell phenotypes [30,31]. Regulating capillary growth may help in establishing a more efficient pathway for drug delivery, as the leaky vessels and the high interstitial pressure in the proximity of the tumor prevent effective supply of drugs through the blood vessels into the core of the tumor [32]. Although anti-angiogenic therapy is comparatively young, it has already been established as a novel form of chemotherapy in cancer treatment [2] (next to surgery, radiation, and conventional chemotherapy).

Tumor-Induced Sprouting Angiogenesis

The maximal size a tumor can assume without relying on a vasculature for nutrient supply is restricted to 1 mm³ [2]. In this case—which is referred to as avascular growth—nutrients reach the tumor by the sole means of diffusion through the surrounding tissue. If the tumor grows beyond this size of about 1 mm³, cells in the core of the tumor cannot obtain enough oxygen to survive. These cells become hypoxic and eventually
starve, forming a necrotic region at the core of the tumor. Tumors can reside in this avascular state for a long time [2]. However, one of the many responses of tumor cells to hypoxia is that they start to secrete angiogenic growth factors that are responsible for initiating sprouting angiogenesis. Several growth factors are involved in the process of angiogenesis. VEGFs have been identified to be one of the main driving forces [1]. VEGFs, upon release by hypoxic tumor cells, diffuse through the ECM occupying the space between tumor and existing vasculature in the proximity of the tumor and establish a chemical gradient between the tumor and nearby vessels. Once VEGF has reached a vessel, it binds to receptors located on endothelial cells (EC), which line the blood vessel walls. This binding sets off a cascade of events.

In the early phase of angiogenesis, ECs stimulated by VEGF start releasing proteases that degrade the basal lamina, a fibril structure building the outermost layer of the vessel wall. This enables ECs to leave the vessel wall and enter the ECM. Further signaling pathways downstream of VEGF lead to an increase in EC proliferation and coordinate the selection of migrating ECs located at the tip of outgrowing sprouts. Migrating sprout tip cells probe their environment by extending filopodia and migrate along the VEGF gradient toward regions of higher concentration, a directed motion referred to as chemotaxis. ECs located initially behind the migrating tip cells proliferate, thus extending the sprouting blood vessel. Fibronectin, which is distributed in the ECM and at the same time released by the migrating tip cells, establishes an adhesive gradient that serves as another cue for the ECs following behind. Fibronectin released by the ECs binds to integrins located on collagens and other fibers, which occupy roughly 30% of the ECM. Matrix-bound fibronectin in turn can bind to transmembrane receptors located on the EC membrane. This autocrine signaling pathway, promoting cell–cell and cell–matrix adhesion, accounts for a movement referred to as haptotaxis. In addition to chemotactic and haptotactic cues, the fibrous structures itself present in the ECM also influence cell migration by facilitating movement in fiber directions.

After the initial sprouts have extended into the ECM for some distance, repeated branching of the tips can be observed. Sprout tips approaching others may fuse and form loops, a process called anastomosis. Anastomosis can be observed to occur in two ways, either sprout tips fusing with other sprout tips, stopping their migration completely, or sprout tips fusing with already established sprouts at some distance behind the tip. Along with anastomosis, the formation of lumen within the strands of endothelial cells
establishes a network that allows the circulation of blood. Maturation, the final stage of angiogenesis, incorporates the rebuilding of a basal lamina and the recruitment of pericytes and smooth muscle cells to stabilize the vessel walls.

In tumor-induced angiogenesis, the newly built vasculature is often disorganized and leaky, leading to high interstitial pressure and inefficient blood supply. Together with a growing tumor, which exerts pressure on the fragile capillaries and thus suppresses temporal and local blood delivery, ever-new regions of acute hypoxia arise, releasing VEGF, which sets off the process of angiogenesis anew. The process therefore never comes to a stop, and full maturation is impaired.

**Vascular Endothelial Growth Factors (VEGFs)**
In addition to the soluble isoform of VEGF, the presence of other VEGF isoforms has been identified to significantly influence morphology of capillary network formation [3,33]. Some VEGF isoforms express a binding site for heparan sulfate proteoglycans that is found on cell surfaces, in the ECM, and in body fluids; thus, such isoforms can be bound by the ECM. These “matrix-bound” VEGF isoforms can be cleaved from the ECM by matrix metalloproteinases (MMPs) [3], establishing very localized chemotactic cues. MMPs are expressed both by tumors and migrating ECs. Inflammatory cells stimulated by the tumor can also release VEGF and contribute to the chemotactic cues ECs react to.

**Extracellular Matrix (ECM)**
The ECM describes any material that occupies the space between cells in metazoans, including the space between the initial vasculature and the tumor. It plays an important role in cell migration. Fibers such as collagen, laminin, and fibrillin are distributed throughout the ECM, occupying roughly 30% of it. These fibers form bundles that serve as guiding structures for migrating cells [34,35]. The structures have been shown to be subject to remodeling by endothelial tip cells [36], facilitating migration through the matrix and playing a crucial role in lumen formation. The ECM further presents binding sites for fibronectin and matrix-bound VEGF isoforms that can be cleaved by MMPs.

**COMPUTATIONAL MODELING OF ANGIOGENESIS**
Computational models of tumor-induced angiogenesis address a limited number of the involved biological processes. The choice of the modeled
processes is dictated by the availability of biological data and by the understanding of the key processes for the phenomena under investigation. In the present model, we consider the motion of the ECs as affected by chemical gradients induced by VEGF, cell–cell adhesion, and the structure of the ECM.

In the present model, VEGF appears in soluble and matrix-bound isoforms. The soluble VEGF is released from an implicit tumor source, and diffuses freely through the ECM. The matrix-bound VEGF isoform can be cleaved by MMPs released at the sprout tips (section titled, “Vascular Endothelial Growth Factors”), and contributes to the migration cues of the ECs (see Figure 11.1). As an extension to the explicit modeling of matrix-bound growth factors, we present a subgrid-scale approach to account for the influence of matrix-bound VEGF on cell migration. Existing models of sprouting angiogenesis account for chemotaxis induced by the soluble isoform of VEGF [18,20,21,37], and a matrix-bound isoform of VEGF has been implicitly accounted for in a recent work by Bauer et al. [21], in which the ECM consists of fiber bundles, structural cells, and interstitial fluid, influencing EC migration through adhesive forces. In [20], the matrix is represented by a random anisotropic conductivity field that affects the

**FIGURE 11.1** 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 comprising the ECM and can be cleaved by MMPs secreted by the sprouting tips.
migration velocity of the sprout tips. Models of angiogenesis can be classified in three broad categories:

1. Discrete, cell-based models that aim to capture the behavior of individual biological cells [21]

2. Continuum models that describe the large-scale, averaged behavior of cell populations [37,38]

3. Discrete models that model explicitly vascular networks determined by the migration of tip cells [18,20].

Bauer et al. [21] developed a two-dimensional, Cellular-Potts-based model to simulate migration, division, and adhesion of endothelial cells establishing an interconnected network. They distinguished two types of ECs: migrating tip cells that degrade the matrix fibers, and proliferating stalk cells located behind the tip cells. This allows for branching and anastomosis of blood vessels without explicitly defined rules. Anderson and Chaplain [37] presented two-dimensional, continuum models of angiogenesis, with a probabilistic modeling of capillaries and a discrete extension to this model, with capillaries as masked points on a grid. Capillary branching is modeled through a branching probability, depending on the sprout age, the tumor angiogenic growth factor (TAF) level, and the endothelial cell density. The model was later extended to three dimensions [18]. Sun et al. [20] presented a deterministic, discrete two-dimensional model of sprouting angiogenesis. They used a capillary indicator function to describe the network structure and formulated branching as a function of the sprout age and the anisotropy of the ECM. In summary, cell-based models aim to describe angiogenesis at cell-level resolution, but they are difficult to extend to macroscopic systems due to their computational cost. Continuum-based models bypass these limitations by modeling the evolution of cell densities at the expense of detailed cell–cell interactions.

In the present paper, we report our investigations of angiogenesis-like growth using a continuum model, which does not rely on any heuristic rules (e.g., branching rules) to obtain blood vessel morphologies. This experiment might provide some hints on the relative significance of the forces at the core of angiogenesis and mesenchymal motion in general. The following presentation considers 2D systems. These systems are relevant as many in vitro experiments of angiogenesis are essentially 2D. We note that all the techniques described herein have been extended to 3D.
A Continuum Representation of Cell Migration

There are two basic choices to represent the endothelial cells: similar to the representation of multiphase flow, we can chose to represent the cells by a density function (diffuse interface approach), or by a level set that represents the boundary to the extracellular domain (sharp interface approach). In the case of cell migration, we need to be able to represent agglomerates of cells that are highly elongated. Therefore, the level set approach is less favorable as we always require a narrow band of several grid spacings around the level set, and the requirements for the resolution are much more demanding than for a corresponding diffuse interface approach.

A single cell population is represented by a density $\rho$. This density evolves in time according to

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\mathbf{a} \rho) = d \Delta \rho,$$

(11.1)

where $\mathbf{a}$ denotes the cumulative effect of cell–cell adhesion ($\mathbf{a}^{c/c}$), pressure ($\mathbf{a}^p$), and migration cues ($\mathbf{a}^{p^{\text{act}, \phi}}$) as defined in the following sections. The term on the right-hand side accounts for random fluctuations on the cell population modeled at a macroscopic scale by a diffusion term with diffusion coefficient $d$.

If more than one cell type is present, one density is used per type, that is, $\{\rho_i\}_{i=1}^{\#\text{CellTypes}}$. 

Cell–Cell Adhesion

Cell–cell adhesion is a fundamental biophysical mechanism. It is responsible for tissue formation, stability, and breakdown. It is involved in tissue invasion and metastasis of tumor cells. It is a crucial mechanism in embryogenesis, as it is the driving force of cell-sorting processes.

Cell adhesion to another cell or the ECM is established by specific adhesion receptors on the cell membrane, such as integrins, which may bind to collagens and fibronectin (in the ECM), and intercellular adhesion molecules such as cadherins. This reaction is very local, as it happens upon contact. In order to model cell adhesion, we state a set of requirements that reflect the main characteristics of the process: (1) cell adhesion is a short-range force; (2) cell adhesion will give rise to a movement of the cells toward the entity they adhere to; and (3) this cell movement will decrease as the cell density approaches the close-packing density, and at close-packing density we expect to find no residual movement caused by adhesion.
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Given these characteristics, we can model cell adhesion as an autocrine- (in the case of cell–cell adhesion), or paracrine-like signal $f$ (in the case of cell adhesion to the ECM) acting as an adhesive force $a^{\text{c/c}}$ on the cell population $\rho$. Consider the case of cell–cell adhesion: in the absence of other influences, we can model cell–cell adhesion forces as

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

$$\frac{\partial f}{\partial t} = -\mu f + \alpha \left(1 - \frac{f}{f_{\text{max}}}\right) \rho + D_f \Delta f,$$

Parameters $\mu$, $\alpha$, and $D_f$ are the decay, release, and diffusion parameters of the adhesion signal that define the range of the adhesive forces. The parameter $f_{\text{max}}$ denotes the threshold value for the release of $f$. $L(f, df)$ is a cutoff function that keeps the magnitude of the gradient bounded by $df$ in order to limit the migration velocity of the cells; here, we chose

$$L(f, df) = df \left(\max(df, |\nabla f|)\right)^{-1}.$$

The factor $\kappa_f$ determines the influence of the adhesive force on the cell population. In the case where we have populations of different cells $\rho_i$, the model (11.2) is easily extended to

$$a^{\text{c/c}}_i = \sum_{j} \kappa_{ij} L(f_j, df_j) \nabla f_j,$$

$$\frac{\partial f_i}{\partial t} = -\mu_i f_i + \alpha_i \left(1 - \frac{f_i}{f_{i,\text{max}}}\right) \rho_i + D_i \Delta f_i,$$

where $\kappa_{ij}$ describes the heterotypic ($i \neq j$) and homotypic ($i = j$) adhesion strength.

Close-Packing Density

The models (11.2) and (11.4) do not incorporate any repulsive effects that might limit the local cell density. Such effects are easily introduced by adding the following pressure-like term to the velocity:

$$a^p = -\kappa_p H(\rho - \bar{\rho}) \nabla \rho |\nabla \rho|^{-1},$$

where $H$ is the Heaviside step function.
where $\rho = \sum_i \rho_i, \kappa_p$ is a constant that determines the cell population response to pressure, $\bar{\rho}$ is the cell close-packing density, and $H$ is the Heaviside function.

The Extracellular Matrix

Continuum simulations of cellular motion rarely explicitly consider effects of the ECM on the migration. These effects are, however, crucial, as the ECM serves as a scaffolding with adhesive sites that the cells can use to exert forces and propel themselves. We propose to model the ECM as a collection of bundles of adhesive fibers that facilitate but also bias migration. The matrix is constructed as follows: We create $N_f$ fibers as lines of thickness $b_p$ from $(x_{\text{start}}, y_{\text{start}})$ to $(x_{\text{end}}, y_{\text{end}})$. The start point is drawn from a uniform distribution in the computational domain $\Omega$. The end point is given as

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

where, $\alpha_p \sim u.a.r \in [0,1)$ and $l$ is the length of the fiber, which is obtained as

$$
l_p = l z_p, \quad \text{with} \quad z \in N(0,1),
$$

The base fiber length $l$ and the fiber length variation $m$ are parameters of the simulation. The fiber thickness is given as $b_p \sim u.a.r \in [b_{\text{min}}, b_{\text{max}}]$. These fibers are then discretized onto the ECM grid $e$ using Bresenham’s line rasterization algorithm (See Figure 11.2). In order to get a differentiable field we filter $e$ $N_{\text{filter}}$ times with a second-order B-spline kernel.

Chemotaxis and Adhesion inside the ECM

In our model of chemotaxis, the migrating cells follow the concentration gradient of a chemoattractant $\phi$. The model extension presented here to account for cell–ECM adhesion is a formulation of the following assumptions: in order to maximize its migration velocity, a cell will crawl along fibers, if these fibers are not transverse to the chemotactic cue ($\nabla \phi$). If there are no fibers in its environment, that is, $e = 0$, then a cell will not be able to migrate efficiently ($e_0 \ll 1$); if the fiber density is too high ($e \approx e_\infty$),
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then cells have to degrade the matrix before they are able to migrate. These assertions are represented by

\[
\mathbf{a}_{\text{ecm},\phi} = \begin{pmatrix} 1 - \frac{\nabla e \cdot \nabla \phi}{|\nabla e| |\nabla \phi|} \end{pmatrix} \nabla e + \nabla \phi \left( e + e_o \right) \left( e_o - e \right),
\]

and illustrated in Figure 11.3. We would like to point out here that the modeling of the chemotactic response \( \mathbf{a}_{\text{ecm},\phi} \) is but the most simple one and ignores many effects, such as the saturation of receptor sites on the cell membranes. The parameter \( e_o \) defines the minimal migrative response in the total absence of an ECM, and \( e_\infty \) defines the matrix density threshold that completely blocks migration.

FIGURE 11.2 An example matrix \( e \) in the domain \( \Omega = [0,8] \times [0,1] \): base fiber length \( l = 0.201 \), \( N_{\text{filter}} = 3 \), \( b_{\min} = 4 \times 10^{-3} \), and \( b_{\max} = 2.7 \times 10^{-2} \).

FIGURE 11.3 A cell will move “onto” a fiber if the fiber direction is not transverse to the chemotactic gradient; that is, the gradient of adhesion is not aligned with the chemotactic direction.
FIGURE 11.4  (See color insert following page 40) (A) Cell sorting simulations of two distinct cell populations $\rho_1$ and $\rho_2$. Top row depicts solution at $t = 40$, middle row at $t = 160$ bottom row depicts a cut through the domain at $t = 160$. Columns: I engulfment, $\kappa_{11} = 0.25$, $\kappa_{22} = 0.225$, and $\kappa_{12} = \kappa_{21} = 0.05$; II engulfment with pressure, $\kappa_{11} = 0.25$, $\kappa_{22} = 0.025$, and $\kappa_{12} = \kappa_{21} = 0.05$; III sorting, $\kappa_{11} = 0.25$, $\kappa_{22} = 0.025$, and $\kappa_{12} = \kappa_{21} = 0.00$; IV mixing, $\kappa_{11} = 0.25$, $\kappa_{22} = 0.09$, and $\kappa_{12} = \kappa_{21} = 0.2$. (B) Simulation with matrix-bound growth factors using pockets of matrix-bound VEGF distributed in the matrix. The endothelial cells release MMPs that cleave the bound growth factors and make them soluble (diffuse blue cues). (C) Simulation with matrix-bound growth factors by the "subscale" model. Within the white circle, there are only soluble growth factors present, and outside of the circle a constant concentration of growth factors is bound to the matrix. As is apparent from the network structure, the matrix-bound growth factors lead to distinctive increased branching.
MODEL APPLICATIONS

We present simulation results for different scenarios of cell sorting and cell migration processes. We consider both dynamics of a single cell type as well as interactions among different cell types with varying adhesive properties.

Cell Sorting

In order to test our model of cell–cell adhesion, we performed simulations of cell-sorting processes. According to the “Differential Adhesion Hypothesis” (see References [39,40] and references therein), cellular sorting is induced by differences in intercellular adhesiveness. We consider here two different types of cells that differ in their adhesion parameters $k_{ij}$. Depending on the choice of adhesion within and across cell types, we obtain different sorting behaviors (see Figure 11.4A) such as engulfment of one population by the other, complete sorting, and mixing. We also considered the pressure/repulsion effects as introduced earlier in one of the cases (see case AII in Figure 11.4). As we model contact-adhesion through auto/paracrine signaling, the proposed model successfully recovers the different sorting behaviors, although indirectly.

Angiogenesis-Like Migration

We now assemble a system modeling the chemotactically driven migration of cells through an ECM. This model consists of (1) cell–cell adhesion, (2) cell–matrix adhesion, and (3) chemotaxis. Accordingly, we substitute $a$ in Equation 11.1 by $a = a^{cc} + a^{\phi 2}$ and define

$$a^{\phi 2} = \kappa_e \left(1 - \frac{\nabla e \cdot \nabla \phi}{|\nabla e|} \right) \nabla e + \kappa_\phi \nabla \phi.$$  \hspace{1cm} (11.9)

Note that for greater lucidity the system (11.9) ignores the effects of fiber density that we have introduced in Equation 11.8. Parameters $\kappa_e$ and $\kappa_\phi$ define the influence of cell–matrix adhesion and the response to chemotaxis.

Figures 11.5–11.7 illustrate the effects of modifying the model parameters on the resulting vessel morphology. The simulation outcome can be quantified by branch point and vessel section length dynamics as shown in Figures 11.8 and 11.9 in the case of varying matrix density and cell–cell adhesion.
A Multiscale Modeling Approach for Matrix-Bound Growth Factors

So far we have assumed that the growth factors are soluble and freely diffuse through the ECM. However, not all growth factors exist in an a priori soluble form; for example, it is well established that there are several different isoforms of VEGF, some that are soluble and some that can bind to heparin sites, which can be found in the matrix. These isoforms can bind to the matrix and do not diffuse freely. Endothelial cells secrete matrix metalloproteinases (MMPs) during angiogenesis. These MMPs have been shown [3] to cleave such matrix-bound VEGF isoforms, thus freeing them. We modeled this process by distributing small pockets of matrix-bound VEGF and by having the ECs secrete a compound that cleaves that matrix-bound VEGF. Once cleaved, this VEGF adds to the gradient established
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by the soluble VEGF present in the domain. Figure 11.4B shows such a situation. A setting such as this is able to provide localized chemotactic cues. However, we do not observe an increase in branching, which is what is observed in both in vitro and in vivo models of angiogenesis and vasculogenesis [3,33].

If we look at the distribution of matrix-bound VEGF, we must realize that it is very unlikely to find pockets of VEGF of that size in a real ECM.
Focus points of matrix-bound VEGF must be quite a bit smaller than the cell scale. In our continuum description, it is, however, not possible to explicitly incorporate localized structures that are truly microscopic. We must, therefore, resort to subgrid-scale modeling. From a mesoscopic point of view, what will be the effect of localized chemotactic queues that are smaller than the description scale? We will clearly not be able to distinguish any residual localized movement due to these cues. What we can expect to see is the cumulative effect on one cell, which from a mesoscopic viewpoint will be increased (apparently) random motion. It is well known that microscopic random motion manifests itself as diffusion from a macroscopic viewpoint. We therefore model the presence of matrix-bound VEGF by an increase in the spatially varying diffusion coefficient $d$ in Equation 11.1 for the EC density $\rho$. That is, if only soluble factors are present, the diffusion is zero; in the local presence of matrix-bound factors, the diffusion term is increased depending on the concentration of matrix-bound isoforms. So both the release of MMPs and the cleaving of matrix-bound VEGF are modeled implicitly by increasing the diffusion of the ECs. The result of this modification of the model is depicted in Figure 11.4C: this type of modeling of matrix-bound VEGF does lead to increased branching (see Figure 11.10). We employ the automated imaging
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software AngioQuant [41] to extract the network and branching statistics from the simulation results (see Figure 11.11).

FIGURE 11.10 Sprouting angiogenesis: Influence of bound VEGF distributed in the second half of the domain on the total vessel network length (filled circles) and the number of junction points (empty circles).

FIGURE 11.11 Extracted networks (using AngioQuant) from simulations with a bVEGF influence factor of 0 (top) and $1.6 \times 10^{-4}$ in the right half of the computational domain.

DISCUSSION

We have presented a pure continuum model of sprouting angiogenesis. This model considers several core aspects of mesenchymal motion: (1) cell–cell
adhesion, (2) the structure of the ECM, (3) cell–matrix adhesion, (4) chemotaxis, and (5) the effect of matrix-bound growth factors.

In this model, we simulated cell–cell adhesion by modeling it as an autocrine signal. Although indirect, this formulation is very simple and manages to recover aspects of the sorting behavior of cells. Compared to existing continuum models of cell–cell adhesion [26], the present model is less intuitive; however, it is more efficient and easier to implement. In the present work, the ECM is represented explicitly as a collection of fibers that exert adhesive forces. This method of representing the mechanical aspects of the cell microenvironment enables us to recover branching behavior as an output of the model, and hence we do not need to call on heuristic branching events. In addition to soluble chemotactic cues, we also consider matrix-bound cues, which are modeled using a subgrid-scale approach. The incorporation of these localized cues yields an increase in vessel branching, and thus results in the same morphological chances as observed in experiments. In its general form, the methods presented earlier can be used to simulate mesenchymal motion in general. The invasion of cancer cells into healthy tissue is another tumor-related phenomenon that is dominated by mesenchymal motion, and to which the present model may be applied (see Figure 11.12).

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