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Modeling Morphogenesis *in silico* and *in vitro:* Towards Quantitative, Predictive, Cell-based Modeling

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Abstract. Cell-based, mathematical models help make sense of morphogenesis—*i.e.* cells organizing into shape and pattern—by capturing cell behavior in simple, purely descriptive models. Cell-based models then predict the tissue-level patterns the cells produce collectively. The first step in a cell-based modeling approach is to isolate sub-processes, *e.g.* the patterning capabilities of one or a few cell types in cell cultures. Cell-based models can then identify the mechanisms responsible for patterning *in vitro*. This review discusses two cell culture models of morphogenesis that have been studied using this combined experimental-mathematical approach: chondrogenesis (cartilage patterning) and vasculogenesis (de novo blood vessel growth). In both these systems, radically different models can equally plausibly explain the *in vitro* patterns. Quantitative descriptions of cell behavior would help choose between alternative models. We will briefly review the experimental methodology (microfluidics technology and traction force microscopy) used to measure responses of individual cells to their micro-environment, including chemical gradients, physical forces and neighboring cells. We conclude by discussing how to include quantitative cell descriptions into a cell-based model: the Cellular Potts model.

Key words: morphogenesis, cell cultures, quantitative biology, cell-based modeling, cellular potts model, vasculogenesis, angiogenesis, chondrogenesis
AMS subject classification: 92C15, 92C17, 92C42, 82D99

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1 Introduction

How genetics encodes the growth and form of multicellular organisms is one of the most challenging questions in biology. To answer this question, we can look at multicellular organisms as huge colonies of individual cells. Cells behave according to a relatively small set of "rules" encoded by their gene networks, which they execute depending on their cell type and on the signals they receive from their neighbors and from the environment (*e.g.*, contact-dependent signals and chemoattractants). Morphogenesis then follows from the collective, non-centralized responses of the individual cells [29].

Therefore, analyzing and reconstructing the dynamics of the genetic regulatory networks alone does not suffice for unraveling biological development [14]. A complete study of development would require identifying the sets of cell behaviors the genetic networks regulate (including adhesion to neighoring cells, the division rate, cell shape, the response to signals from neighboring cells and tensions in the extracellular matrix, *etc.*), identifying when and where cells change their behavior in response to signals from other cells (*i.e.*, cell differentiation), and how the resulting collective behavior of the individual cells produces a growing tissue, organ, or animal [29]. This "cell-based" approach allows one to separate questions about genetic regulation from questions about developmental mechanics: it first experimentally characterizes the effects the genetic and metabolic networks have on cell behavior, and analyzes the mechanisms behind it. Then it studies the mechanisms by which single-cell phenomenology directs multicellular morphogenesis and physiology, and how phenomena occurring at the multicellular level (*e.g.* pattern formation, tissue mechanics) feed back on single cell behavior and gene expression. In this approach cell-based computational modeling is instrumental, as it captures single-cell behavior in simple, purely descriptive models, and predicts the multicellular phenomena, including pattern formation and development, that many individual cells produce collectively. Although whole biological organisms or organs (see *e.g.* [27, 18]) have been modeled successfully with cell-based approaches, many biological systems would be too complicated for such an approach. It would quickly become intractable to characterize the behavior of each cell type in interaction with each of the signals and cell types it may ever encounter *in vivo*.

Therefore, a successful approach of the multi-factorial mechanisms of morphogenesis would first simplify the system to its bare essentials both experimentally and theoretically. After analyzing the more simple system in full detail, one would gradually add more components to the experimental system and fit those into the computational and mathematical models. Such a combined experimental-theoretical project provides clear predictions, which can be tested *in vivo*. Experimentally, cell cultures provide useful and simple models of morphogenesis. Cell cultures allow quantitative characterizations of cell behavior and response to the microenvironment, including morphogen gradients and neighboring cells. Cell cultures also help study the autonomous patterning capabilities of cells, *i.e.*, the patterns that cells form in the absence of external guidance cues.

A challenge in cell-based modeling is the level of detail necessary for the description of single-cell behavior. Although for some systems the gene networks regulating embryonic patterning have been identified in detail [12], descriptions of gene networks rarely directly predict dynamic cell

behavior (*e.g.*, cell motility, cell adhesion, chemotaxis). Therefore cell-based models are typically based on experimentally plausible, qualitative descriptions of cell behavior.

It is not always possible to decide whether differences between phenomena observed in cell cultures and *in silico* models are due to incorrect model assumptions or to imprecise representations of conceptually correct cell behavioral descriptions. Recent experimental developments can quantitatively characterize mammalian cell behavior. These can become inputs for cell-based models, thus creating *quantitative* models of morphogenesis in cell cultures. Here we review current qualitative models for analyzing cell culture models, and discuss the steps needed to make these models more quantitative. We will propose how a combination of quantitative experimentation and quantitative cell-based modeling can help identify the patterning principles in cell culture systems, and how these insights can extrapolate to *in vivo* morphogenesis.

The remainder of this paper is organized as follows. In Section 2 we review recent cell-based computational models of *in vitro* cell culture models of biological development, focusing in particular on models of cartilage patterning and blood vessel development. We will show how different, qualitative models can equally plausibly explain developmental phenomena. Section 3 will discuss how recent experimental developments allow quantitative assessments of cell behavior, which will help us distinguish between mechanisms producing similar tissue-level patterns. Section 4 proposes how to use these quantitative descriptions of cell behaviors as input to quantitative cell-based models. Section 5 concludes our discussion on quantitative cell-based modeling.

2 From cell behavior to tissue patterning: insights from qualitative models

Figure 1 shows three typical cell culture models for morphogenesis, and the computational models developed to help explain their behavior. As a model of the development of mammary acini (gland modules), with polarized epithelial cells enclosing a lumen, Rejniak and Anderson studied the *in vitro* formation of epithelial acini using a cell-based model based on the immersed boundary method [42]. This method considers cells as visco-elastic bodies, modeling the cell surface as a set of connected springs and the cell body as an incompressible fluid. Rejniak and Anderson derived a plausible set of rules and conditions, including oriented cell division, differentiation, polarization and apoptosis, by which single cells can develop into well-organized acinar structures. Aiming to get a better control over the mechanical properties of tissue-engineered cartilage, Sengers and coworkers [46, 47] computationally assessed extracellular matrix (ECM) deposition by chondrocytes (Figure 1B,E). In a continuous interaction between cell culture and agent-based computational modeling work, the *Epitheliome* project (see *e.g.* [56, 50]) analyzes the cell behavioral rules governing self-organization of human keratocytes (skin cells) under different culture conditions, in particular for obtaining a better understanding of wound healing (Figure 1C,F). The remainder of this section reviews two specific cell-based models of cell cultures in more detail, and shows how different cellular behaviors can equally plausibly reproduce the *in vitro* system.

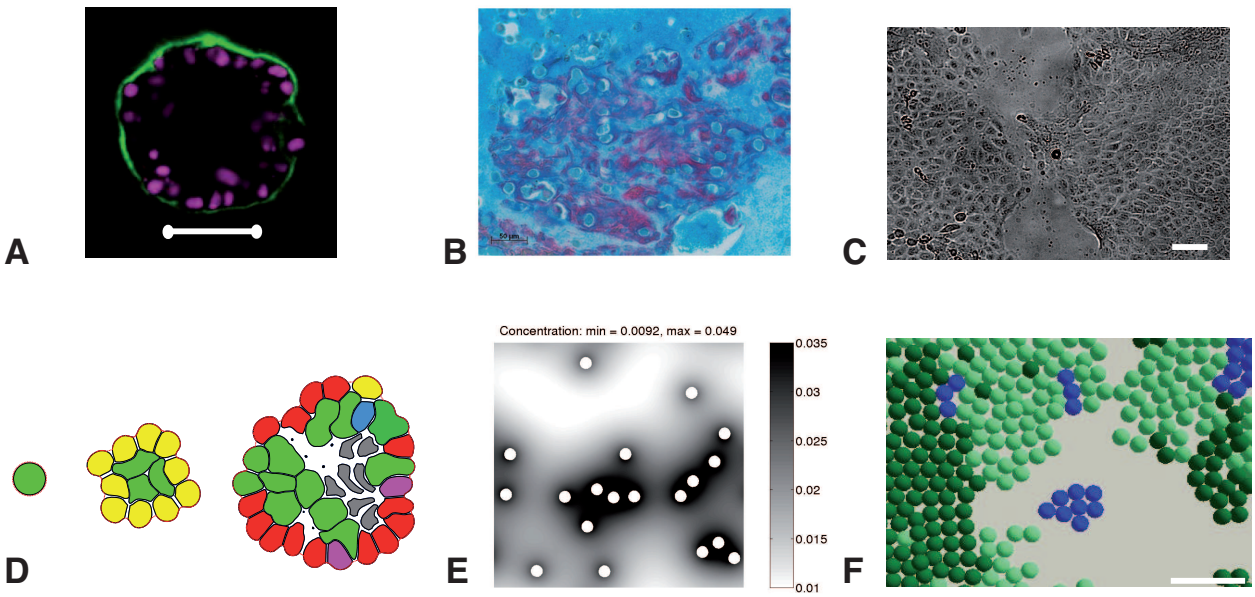

Figure 1: Collage of cell culture models (A-C) and their *in silico* derivatives (D-F). A. *In vitro* model of acinus formation: the hollow structures in glands where the product is secreted [42]. Scale bar, 50 μm. Reprinted with permission from [42]. Copyright (2008) by Springer; B. *In vitro* model of bone formation. Co-culture of human bone marrow cells and cartilage-secreting cells (articular chondrocytes). Scale bar, 50 μm. Reprinted with permission from [47]. Copyright (2007) by Elsevier; C. *In vitro* model of wound healing. Induced scratch in human keratocyte (skin cell) monolayer. Scale bar, 100 μm. Reprinted with permission from Ref. [50]. Copyright (2007) by The Royal Society. D. Computational, single cell-based model of acinus development [42]. Reprinted with permission from Ref. [42]. Copyright (2008) Springer. E. Computational simulation of ECM (osteoid) deposition by chondrocyte. Gray scale indicates concentration of extracellular matrix components in $\mathrm{g\ cm^{-3}}$. Reprinted with permission from [46]. Copyright (2004) by Springer. F. *Epitheliome* [56, 50], an agent-based model of scratch closure in keratocyte monolayers. Scale bar, 100 μm. Reprinted with permission from Ref [50]. Copyright (2007) by The Royal Society.

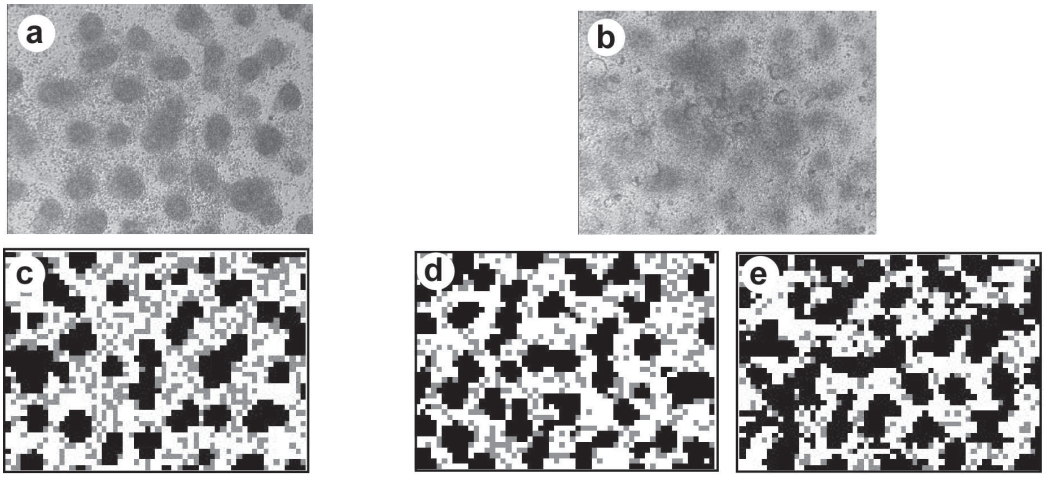

Figure 2: A computational model of chondrocyte cell cultures. The model aims to explain difference between (a) leg-cell cultures and (b) wing-cell cultures. (c) Simulation of leg-cell culture; (d) reduced fibronectin production or (e) reduced fibronectin production and reduced production of a lateral inhibitor may explain patterning differences. Reprinted with permission from Ref. [23]. Copyright (2004) by Elsevier.

2.1 Cell-based models of *in vitro* limb bone patterning

As a model for bone patterning in the embryonic limb, Kiskowski and coworkers [23] studied chondrogenic patterning in so-called *micromass* cell culture of dissociated mesenchymal cells (embryonic connective tissue). Micromass cell cultures are carried out in the absence of an external protein scaffold; the extracellular matrix proteins are produced by the cells themselves. In this cell culture system, precartilage cells aggregate into regularly-spaced focal condensations, which differentiate into cartilage nodules at a later stage (*in vivo* these nodules would be the precursor of the leg and wing bones). To analyze the mechanisms of chondrogenic patterning, Kiskowski *et al.* [23] developed a lattice-gas model with the following assumptions: i) the mesenchymal cells move around randomly, while adhesion to neighboring cells or the extracellular matrix slows them down, ii) the cells secrete a self-enhancing *activator*, TGF-β, and a hypothetical inhibitor (possibly FGF) that diffuses faster than the activator and inactivates the activator inside and outside the cells, iii) TGF-β induces cells to secrete fibronectin, an extracellular matrix protein which slows down movement of the cells.

The model reproduces the periodic patterns of cartilage nodules observed in micromass cultures of wing and leg chondrocytes well (Figure 2). There is no direct correspondence between the model parameters and physical parameters and the parameters are estimated by trial and error. Nevertheless, the model has predictive value: the authors could predict the system's response to a range of experimental perturbations. First, they ran their model for a range of increasingly diluted initial cell densities. The authors observed that reducing the initial cell densities also reduced the number of condensations, while the average distance between nodules increased.

Interestingly, similar, equally plausible models can explain the same phenomena. Kiskowski [23] and coworkers tested the role of the "activator" TGF-β in their model. Experimentally, TGF-β was administered transiently (5-6 h) to the micromass cultures, yielding nearly uniform cartilage condensations. Indeed, a pulse of activator production in the computational model produced also produced confluent nodules. In the same way, eliminating the inhibitor, which the authors hypothesize is secreted by the nodules, also yielded cartilage monolayers both *in silico* and *in vitro*. Christley and coworkers refined the initial model of chondrogenesis [10], accommodating a number of new experimental observations. In contrast to the observations of Kiskowski and coworkers, 1) cell tracking measurements indicated that chondrocytes do not slow down in cartilage condensations, and 2) cell density in chondrocyte micromass cultures is constant and nodules do not deplete cells in their surroundings; cells only round up in nodules. The refined model described chondrocytes using random walkers that accelerate within fibronectin patches; they produced activator and inhibitor according to a mechanism of local, self-enhancement and lateral inhibition, while the cells secreted fibronectin depending on exposure to the activator. The Christley and Kiskowski models reproduce the experimental observations equally well, although in the Christley model patterning might be independent of cell motility because cells distribute evenly throughout the simulation.

The mechanisms proposed by Kiskowski *et al.* [23] and Christley *et al.* [10] require a locally self-enhancing "activator" and a long-range "inhibitor", and indeed patterning in these models is due to a classic Turing-Gierer-Meinhardt mechanism. Although both papers propose plausible candidates for an activator and an inhibitor, their role has not been definitely pinned down. Interestingly, Zeng *et al.* [60] proposed an alternative mechanism that does not require diffusing activators or inhibitors. In their model, cells move randomly and periodically secrete extracellular matrix (fibronectin), which slows down the cells. After exposure to (external) fibronectin, the cells gradually produce cellular adhesion molecules (N-CAM), which "traps"" cells in the condensations. Thus patterning arises from positive feedback in fibronectin production, while cell adhesion gradually "freezes" the pattern. In the reaction-diffusion models proposed by Kiskowski, Christley and co-workers, the diffusion rates of the activator and the inhibitor set the average nodule size and the average spacing between nodules (i.e. the pattern wavelength). Zeng and Glazier's model does not have a fixed wavelength; the nodule patterns coarsen until cell adhesion "freezes" further cell movement. Thus this model makes a clear, experimentally testable prediction of how adhesion between mesenchymal cells would affect morphogenesis *in vivo* and *in vitro*: reduced cell adhesion should produce bigger and more widely spaced cartilage condensations, because it takes longer before the pattern freezes up, while increased cell adhesion should lead to more closely spaced, smaller cartilage condensations. Experimental work analyzing both the behavior of mesenchymal cells and the resulting patterns will be required to decide which of the presented mechanisms, if any, is responsible for *in vitro* chondrogenesis.

2.2 Cell-based modeling of *in vitro* vasculogenesis

Another cell culture system that has been widely studied using a combination of *in vitro* models and computational and mathematical modeling is *de novo* blood vessel growth. During the initial stages of embryonic blood vessel development, endothelial cells—the cells lining the inner walls of blood

vessels—aggregate into a network-like, protovascular system, a process called *vasculogenesis*. In the closely related process of *angiogenesis* new blood vessels form by sprouting and splitting of existing blood vessels. A popular *in vitro* model of both vasculogenesis and angiogenesis is a cell culture system of human umbilical vein endothelial cells (HUVEC; endothelial cells isolated from human umbilical cords) cultured in Matrigel, a mixture of isolated proteins produced by a mouse tumor cell line, mimicking the ECM. Stimulated by growth factors present in the Matrigel and in the growth medium, the endothelial cells aggregate and form polygonal networks of cells.

The mechanisms by which endothelial cells aggregate into vascular networks within such HUVEC-Matrigel cultures are largely unknown. Experimental and computational biologists have proposed a range of mechanisms, but currently each of these seems to reproduce *in vitro* patterning equally well. This section reviews several such mathematical and computational models of *in vitro* vasculogenesis. In addition, we will discuss how more detailed descriptions of single cell-based behavior could help decide which of these alternative mechanisms best reflects reality.

2.2.1 Chemotaxis-based models

Preziosi and coworkers proposed a chemotaxis-based mechanism for endothelial cell aggregation during angiogenesis. They developed a series of continuum models [2, 48, 15] whose results they compared to HUVEC-Matrigel cultures. The Preziosi model assumes that a) endothelial cells secrete a chemoattractant which attracts other endothelial cells, b) the chemoattractant diffuses in the extracellular matrix which gradually inactivates it, and c) endothelial cell motion is direction persistent. It takes the form:

$$\begin{aligned} \frac{\partial n}{\partial t} + \nabla \cdot (n\vec{v}) &= 0, \\ \frac{\partial \vec{v}}{\partial t} + \vec{v} \cdot \nabla \vec{v} &= \mu \nabla c - \nabla \phi(n), \\ \frac{\partial c}{\partial t} &= D\nabla^2 c + \alpha n - \tau^{-1} c, \end{aligned} \tag{2.1}$$

with n, the density of endothelial cells, $\vec{v}$, the velocity field, $\phi(n)$, a density-dependent friction term, and c, the chemoattractant concentration. Numerical simulations of these equations produce network patterns reminiscent of *in vitro* vasculogenesis (Fig. 3 A).

The Preziosi models describe the endothelial cells as a fluid whose motion is accelerated by chemoattractant gradients. This assumption describes the cells' direction persistence, *i.e.* the biologically-plausible assumption that a change of direction takes some time, because the cells need to remodel the cytoskeleton. However, this term also introduces a cellular inertia: in the absence of a chemoattractant gradient and at low densities of surrounding cells, cells continue moving at constant speed. Studies in other organisms and cell types show that cells, while they do exhibit persistence, do not accelerate in response to chemoattractant gradients, primarily because their maximum velocity is limited [43, 57].

Merks, Glazier and coworkers therefore derived a cell-based model from the Preziosi model to set the cell velocity rather than the cell acceleration proportional to the chemoattractant gradient.

These models feature spatially extended representations of endothelial cells, and assume that (as, *e.g.*, [33] observed) the response of the cell to the chemoattractant is local along the membrane rather than occurring at the cell center. When placed together in an *in silico* Petri dish these simulated ECs organize into disconnected, round "blobs" of endothelial cells instead of vascular-like networks. They therefore set out to see which additional, biologically plausible assumptions would suffice to reproduce endothelial cell self-organization into vascular networks.

Interestingly, the observation that endothelial cells elongate in response to the Matrigel culture conditions appears crucial for formation of blood-vessel-like structures: elongated cells organize into vascular networks with temporal dynamics closely matching that of HUVEC cultures (Figure 3C) [31]. The same model also reproduces formation of capillary-like structures in cultures of endothelial-cell-like bone marrow macrophages of patients with multiple myeloma, a cancer of plasma cells in the bone marrow [19]. Alternative additional mechanisms, including cell adhesion [30] and contact-inhibited chemotaxis [33] (Figure 3D) also suffice for network formation. Interestingly, the set of cell behaviors described in these cell-based models also suffice for reproducing endothelial sprouting from clusters of ECs, suggesting that vasculogenesis and aspects of angiogenesis are—at least partly—two sides of the same coin [32, 33].

2.2.2 Mechanical models

In a model based on the Murray-Oster mechanochemical theory, Manoussaki and coworkers [25] proposed that endothelial cells exert traction forces on the Matrigel (Figure 3B). The cells thus drag the Matrigel towards them, together with the attached endothelial cells which passively move along with the Matrigel. For patterning to proceed, the matrix must be sufficiently compliant or the EC traction forces sufficiently strong so the cells can indeed move the matrix. Namy and coworkers experimentally validated this critical value [34]. Using cultures of bovine endothelial cells in fibrin matrices (the primary protein forming blood clots) they showed that patterning only occurs if the matrix is sufficiently soft and if the layer of matrix is sufficiently thick, so cells can displace it relative to the culture dish. Namy *et al.* have extended the mechanical-traction model with haptotaxis, *i.e.* movement up ECM-density gradients. Haptotaxis allows for patterning at lower matrix compliances, because it amplifies cell aggregation: cellular traction creates initial cell aggregates surrounded by an ECM density gradient which guides cells towards the aggregates.

2.2.3 Preferential attachment to elongated structures

Szabo and coworkers [52, 51] observed that non-vascular (glia- and muscle related) cells can form irregular networks on rigid tissue culture substrates with continuously shaken culture medium, hence excluding traction-based or chemotaxis-based patterning mechanisms. Instead they proposed that cells are preferentially attracted to locally elongated configurations of cells [52] , or that cells adhere most strongly to elongated cells [51]. This mechanism produces vascular-like network patterns (Fig. 3E) similar to the ones produced by chemotaxis-based or cell-traction-based mechanisms. However, note that networks forming in matrix-free tissue cultures tend to have a more irregular aspect (see, *e.g.*, Figures 1C-D in ref. [52]) than those formed by endothelial cells

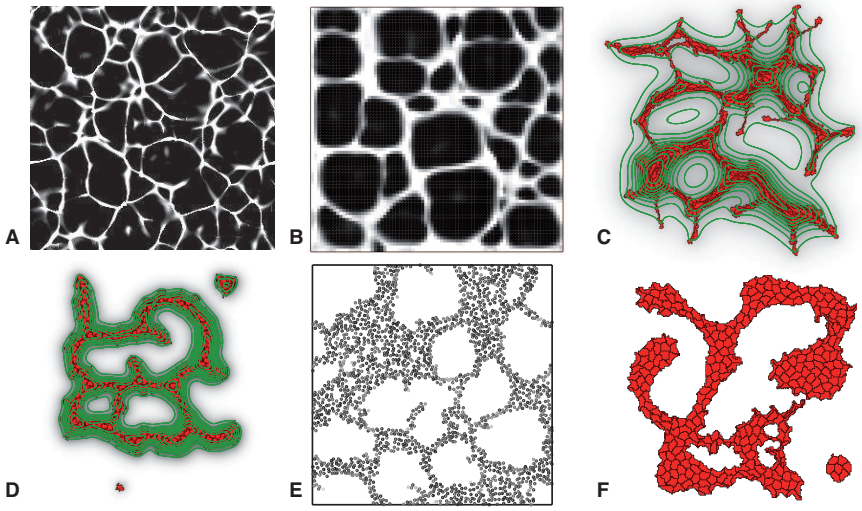

Figure 3: Different hypothetical mechanisms for *in vitro* vasculogenesis produce similar patterns. A. Persistent motion along gradients of an autocrinically secreted chemoattractant [15] (Figure courtesy of Prof. Luigi Preziosi, Politecnico di Torino, Italy). B. ECs exert traction forces on the substrate, pulling surrounding cells towards them [25, 24]. Reprinted with permission from Ref. [24]. C. Overdamped chemotactic motion of elongated endothelial cells [31]. D. Overdamped chemotactic motion of round endothelial cells with contact-inhibition of motility. E and F. Preferential attraction to elongated cellular configurations [52, 51]. Panel E reprinted with permission from Ref. [52]. Copyright (2007) by the American Physical Society.

cultured in Matrigel or fibrin, or those produced by the hypothetical mechanisms reviewed here (see Figure 3).

The *zoo* of computational models for chondrogenesis and vasculogenesis reviewed in this section shows that different mechanism can explain the same phenomenon. Thus simple visual comparison with experiments is insufficient for distinguishing different models. The mere observation that a model faithfully reproduces a cell culture does not necessarily mean that the underlying model correctly represents the underlying microscopic mechanism. Both chemotaxis models and mechanical models assume an isotropic attractive force between cells, which drives aggregation, but set of authors call it "chemotaxis", the other "mechanical traction". To make matters worse, both the chemotaxis and cell traction models produce clear predictions, which, when tested experimentally, have the expected outcome [48, 34]. Thus, to distinguish alternative mechanisms, much more detailed descriptions of individual cell behavior are needed, yielding quantitative predictions of morphogenesis in cell cultures.

3 Quantitatively characterizing cell behavior

As the many possible explanations for vascular-like patterning in *in vitro* cultures of endothelial cells illustrate, superficial pattern resemblance between model and experiment is insufficient for choosing between alternative explanations. The studies discussed in Section 2 were primarily qualitative: the rules describing cell behavior were based on observations described in the literature and many of the parameter values were unknown or based on best guesses. These models have provided important new insights into the generic principles of biological patterning and are a means to compare the outcomes of different, equally plausible scenarios with each other. However, to decide which of these alternative mechanisms best describes the experimental observations, we will need to describe cell behavior in much more detail. This section reviews experimental approaches that can deliver such quantitative cell behavioral descriptions.

Recent experimental developments have made it possible to identify many of the parameters required for quantitative cell-based computational models and to indicate where the models should be refined. New microscopy technology has made it possible to characterize the dynamics of the cell surface in detail and to measure the forces the pseudopods apply on the substrate and the neighboring cells. Microfluidics technology can help measure cellular responses to specifically controlled microenvironments, including chemoattractant gradients, extracellular matrix proteins and surrounding cells.

3.1 The mechanics of cell behavior

Many recent studies have characterized the mechanics of endothelial cell behavior in great detail. By measuring the deformation in response to a compression force, the mechanical properties of endothelial cells in response to compressive forces could be characterized [8]. Such measurements help parameterize the passive response of endothelial cells to compressive and traction forces exerted on the EC by neighboring cells. We also require data on the active behavior of endothelial cells, and we need to know how this active behavior depends on mechanical and chemical cues from the microenvironment. Several techniques can be used to characterize cell behavior on a substrate. Reinhart-King and coworkers [39] showed that the total force an EC exerts on a substrate increases with the cell's area, and they found that the rate of spreading increases with the density of integrin ligands in the substrate. At low ligand density ECs spread rhythmically with lamellipodia following extending filopodia, while at higher densities of ligand the ECs spread in "pancake-like" manner, possibly because they are more tightly bound to the substrate and thus detach less easily from the substrate.

Pillared substrates are also widely used for measuring the forces cells exert on a substrate. Most commonly such patterned substrates are covered with flexible pillars; the forces the cells apply on the substrate can then be derived from the pillars' deflection and their spring constants. Although pillared substrates may yield more detailed measurements of cell mechanics, their major disadvantage is the fact that mammalian cells can respond to substrate structure and will thus affect cell behavior [37]. However, good use for pillared substrates can be found in characterizing mechanical cell-substrate interaction, including contact guidance [54].

3.2 Microfluidics: cellular responses to the microenvironment

Detailed observation of cellular responses to the local environment, including growth factors, other cells and matrix proteins, is crucial for developing a quantitative understanding of the cell behavior responsible for multicellular patterning. A series of recent studies have characterized the responses of endothelial cells to their microenvironment in astonishing detail. They help us refine our cell-based models of angiogenesis. Chen and coworkers [9] designed a diffusion chamber to set up exponentially-decaying gradients of vascular-endothelial growth factor (VEGF), *i.e.*, the type of gradients formed due to secretion at a source, diffusion, and decay. After validating the shape of these gradients, they studied the sprouting of human dermal microvascular endothelial cells (MVECs) cultured on dextran beads, and found that 1) to sprout MVECs must bind a minimum number of VEGF molecules which varies per cell (at least $\sim 8 \times 10^4$ molecules per cell), 2) sprouts align to the gradient if the VEGF gradient is sufficiently steep (at least $\sim 100\,\mathrm{ng\,ml^{-1}\,mm^{-1}}$), 3) sprouting and alignment diminishes at concentrations of VEGF higher than $\sim 250\,\mathrm{ng\,ml^{-1}}$ due to receptor saturation. Interestingly, differences in VEGF binding between tip and non-tip cells diminished at higher VEGF concentrations. The observations by Chen and coworkers quantified the response of endothelial cells to VEGF gradients; thus receptor saturation may flatten out differential VEGF responses between cells.

Shamloo and coworkers [49] characterized the response of endothelial cells in more precisely controlled, linear gradients of endothelial cells. They have built a clever microfluidic device to set up precisely controlled VEGF gradients. The microfluidic device consists of two parallel channels in which a source solution (containing high concentration of protein) and a sink solution (with zero concentration of the protein) are continuously injected. Between these two channels is a cell culture chamber, connected to the source and sink channels with tiny capillaries. Using finite element simulations the authors predicted the solution injection rates needed to set up the required concentration gradient while keeping fluid flow in the culture chamber minimal, well below shear stresses known to induce behavioral responses in endothelial cells. The concentration gradient was experimentally validated using a fluorescent probe of approximately equal size to VEGF. Using this set-up they found that 1) endothelial cells require a gradient steeper than $\sim 14\,\mathrm{ng\,mL^{-1}\,mm^{-1}}$ to migrate to higher concentrations of VEGF, and 2) that the response of endothelial cells to chemotactic gradients does not depend strongly on absolute concentrations. Microfluidics technology is continuously evolving, making it possible to map out cellular responses in ever more tightly controlled microenvironments (see, *e.g.*, [1]).

3.3 Characterizing cellular interactions

Apart from the cells' individual behavior and its responses to the micro-environment, quantitative cell-based models of morphogenesis require quantitative descriptions of direct cell-cell interactions. Such direct interactions include cell adhesion and signal exchange. In a recent paper, Yin *et al.* [59] introduced a new, dielectrophoretic (DEP)-microfluidic device that allowed them to precisely position pairs of cells in a microfluidic chamber using electric fields. After they released the electric field, the cells' movement was tracked over time in a continuously replenished culture medium. The microfluidics device was used to quantify the interactions between HUVEC and

tumor cells. Interestingly, single HUVEC cells underwent more-or-less random walks, frequently retracing paths previously taken, whereas movement of cells in the vicinity of an immotile tumor cell was biased towards the tumor cells. Because the particular tumor cell type used in the experiment is known to secrete VEGF, the most obvious explanation would be that the ECs follow a VEGF gradient towards the tumor cell. However, when the researchers inhibited the cells' sensitivity for VEGF, the interaction between tumor cells and ECs was unaffected, but, unexpectedly, the movement of single HUVECs and HUVEC pairs became much more directed and persistent. Using fluorescent antibodies for collagen, Yin and coworkers demonstrated that ECs deposit collagen on the substrate and that they haptotactically move along these collagen traces. VEGF reduces the ability of ECs to sense these trails, possibly by inducing secretion of collagen-degrading enzymes, which would "wipe out" the trace; thus they reasoned that in this case cells cannot retrace their paths, resulting in more straight, persistent paths.

Recent experimental work suggests that endothelial cells may also interact or communicate mechanically. The mechanical compliance of the substrate affects endothelial cell response to each other in culture. On relatively soft matrices (500 Pa) the cells stay together after they touch, but on somewhat stiffer substrates (5500 Pa), cells repeatedly attach and detach, while they touch once and then move away from each other on stiff matrices (33000 Pa). Also the concentration of ligands for cell-ECM adhesion proteins (integrins) matters for the interactions between ECs: in matrices of relatively low stiffness (2500 Pa) and low concentrations of integrin ligands, interacting endothelial cells are most likely "pulled" together via neighboring attractive membrane tethers. However, at higher concentrations of integrin ligands, cells tend to touch and then move away when they encounter each other [40].

4 Towards quantitative cell-based models

The detailed, quantitative characterizations of cell behavior discussed in Section 3 provide precisely the kind of data required to bring cell-based models to the next level. Cell-based models predict the tissue-level patterning following from individual cell behavior and cellular interactions. If the resulting patterning mechanisms do match those seen in reality, the models will help us pinpoint the gaps in our understanding of cell behavior. Also, cell-based models will help us unravel how abnormal cell behavior—*e.g.*, due to a disturbed physiological environment or a genetic knock-out—can produce abnormal patterning; thus they may help us follow the sequence of events from a knock-out mutation *via* abnormal cell behavior, to abnormal patterning.

Based on their microfluidics experiments measuring the pairwise interactions between endothelial cells (see Section 3.3), Yin and coworkers [59] constructed an agent-based computational model to test if their observations provided sufficient explanation for angiogenesis. Their agents secreted ECM components, which serve as a guidance cue for cells to retrace their own trajectories. The model ECs altered their speeds based on collagen concentrations, and secrete VEGF, which reduces the cell propensity to follow ECM tracks. Additionally, they assumed that ECs chemotactically move to higher concentrations of VEGF. They found that in the vicinity of a hypothetical, VEGF-secreting tumor, the observed set of cell behaviors produced branched vascular beds with

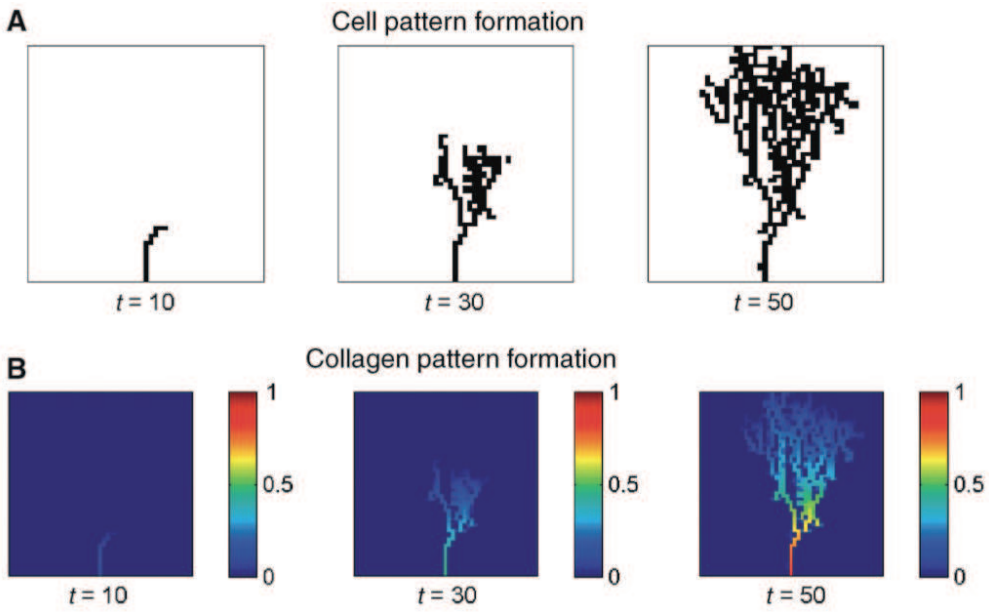

Figure 4: Agent-based model of tumor-induced angiogenesis. Endothelial cells secrete collagen tracks which they haptotactically follow depending on local VEGF concentrations. Agent behaviors derived from microfluidics experiments. A. Cell patterns. B. Scaled concentrations of collagen. Reprinted with permission from Ref. [59]. Copyright (2007) by Nature Publishing Group.

the branching frequency increasing for higher VEGF concentrations (Fig. 4). Thus these *in vitro* experiments suggest a set of cell behaviors that qualitatively reproduce *in vivo* characteristics of blood vessels during tumor angiogenesis.

A risk with many cell-based models is that alternative sets of cell behavior can produce comparable tissue-level behavior, as we have discussed in Section 2.2 Can comparing model outcomes with experimental data distinguish between alternative models? The coarse-grained descriptions of cell behavior typically used in cell-based models necessarily produce rather rough approximations of biological patterns. The resulting uncertainties with respect to the outcomes of alternative models may thus become so large that a comparison between model and experiment does not suffice for ruling out hypothetical mechanisms. Thus the observed differences between a model and the corresponding experiment may be due to incorrect assumptions about the mechanism, or an imprecise, but roughly correct description of cell behavior. Making descriptions of cell behavior more quantitative will reduce the uncertainty of the cell-based model's outcome, and hence more likely produce significant differences between model behavior and cell cultures. The remainder of this section outlines the steps to take for building such quantitative cell-based models.

4.1 Building quantitative cell-based models

During the last twenty years, a wide range of computational cell-based modeling methodologies has been developed, including methods representing biological cells as continuum fields, point

particles, grid points, spheres, ellipses, Voronoi domains, or collections of these (reviewed in [3]). However, because many of the cell behaviors relevant for biological morphogenesis involve subcellular mechanical events, including pseudopod extensions and retractions and cell shape changes, the cell-based model preferably describes cellular mechanics to some subcellular detail. We will therefore focus on the Cellular Potts model [17], a popular cell-based model that meets these criteria, but our arguments extend to similar cell-based modeling frameworks, including the subcellular element model [35, 44] and the related cellular particle dynamics model [13], the cellular immersed boundary model [41] and Brodland's cellular finite-element models [7, 20].

The Cellular Potts model (CPM), alternatively called Glazier-Graner-Hogeweg (GGH) model [16], is a popular cell-based method for simulating biological growth development (reviewed in [29]; more recent studies include [21, 18, 38, 33, 58]). The CPM was used to develop several of the *in vitro* vasculogenesis described in Section 2.2.1 (Figure 3C, D and F), in combination with partial-differential equations (PDE) to model the diffusion of secreted chemoattractants in the Matrigel. The CPM is a lattice-based Monte-Carlo approach that describes biological cells as spatially-extended patches of identical lattice indices $\sigma(\vec{x})$ on a regular (*e.g.* square or hexagonal) lattice, where each index uniquely identifies, or labels a single biological cell [17]. Intercellular junctions and cell junctions to the ECM determine adhesive (or binding) energies. Connections (links) between neighboring lattice sites of unlike index $\sigma(\vec{x}) \neq \sigma(\vec{x'})$ represent bonds between apposing cell membranes, where the bond energy is $J(\sigma(\vec{x}), \sigma(\vec{x'}))$, assuming that the types and numbers of adhesive cell-surface proteins determine J. A penalty increasing with the cells deviation from a designated target volume A_{target} imposes a volume constraint on the simulated cells.

We define an *effective energy* H which sums the effects of cell behaviors, including cell adhesion, cell shape changes, cell growth, and chemotaxis:

$$H = \sum_{\text{neighbors}} J((\sigma(\vec{x}), \sigma(\vec{x'}))(1 - \delta(\sigma(\vec{x}), \sigma(\vec{x'}))$$
$$+\lambda_{\mathrm{A}} \sum_{\sigma} (a(\sigma) - A_{\mathrm{target}}(\sigma))^2 + \text{cell shape, chemotaxis, cell traction, } \textit{etc.} \qquad (4.1)$$

where $\vec{x}$ and $\vec{x'}$ are neighboring lattice sites, $a(\sigma)$ is the current area of cell σ, and $A_{\mathrm{target}}(\sigma)$ is its target area, and λ_{A} represents cell resistance to compression. The Kronecker delta is $\delta(x, y) = \{1, x = y;\ 0, x \neq y\}$, which simply selects cell boundaries. The CPM algorithm models pseudopod protrusions by iteratively displacing cell interfaces using a Metropolis algorithm, with a preference for displacements that reduce the local effective energy H of the configuration; thus on average the algorithm will exchange stronger connections for weaker adhesive connections, while cells will stay close to their target area. To mimic these cytoskeletally-driven pseudopod extensions and retractions, the algorithm randomly chooses a source lattice site $\vec{x}$, and attempts to copy its index $\sigma(\vec{x})$ into a randomly-chosen neighboring lattice site $\vec{x}'$. For each copy attempt the algorithm calculates how much the effective energy would change if the copy were performed, and accepts the attempt with probability

$$P(\Delta H) = \begin{cases} \exp[(-\Delta H - H_{\text{dissipation}})/T] & \text{if } \Delta H \geq -H_{\text{dissipation}}, \\ 1 & \text{if } \Delta H < -H_{\text{dissipation}}, \end{cases} \tag{4.2}$$

where the system temperature, or *motility parameter*, T determines the acceptance rate for energetically unfavorable moves, *i.e.* it sets the extent to which the cells' active cell motility overcomes the constraints set by its local environment. $H_{\text{dissipation}}$ is the energy dissipated if the copy were accepted, for example due to resistance of the matrix to displacement.

The ECM is modeled as a generalized cell with $\sigma = 0$, without a volume constraint. Additional constraints set the shapes of cells (*e.g.* cell length by using $H = \lambda_L(L_i - l_i)^2$ and a connectivity constraint, see [31]), and implement chemotaxis (by favoring cell interface movements up chemical gradients [45]), or cell traction by adding an extra term $\Delta H(\vec{F})$ for movements along internally or externally generated forces. Although the CPM produces biologically-plausible simulations of biological development, its parameters do not straightforwardly represent experimentally quantifiable values; indeed the CPM is sometimes criticized for this reason (see *e.g.* [36]). However, the CPM can be parameterized and turned into a more quantitative model, as we will discuss in the next section that focuses on modeling vascular development.

4.2 Quantitative, cell-based modeling of vasculogenesis

A first step towards quantitative cell-based modeling is to ensure the cell behavior modeled by the CPM matches experiments exactly. Virtual cells in the CPM typically undergo a random walk with relatively short persistence lengths, while microfluidics experiments show that ECs have longer persistence lengths or even retrace their own paths [59]. Indeed, *in vitro* vasculogenesis may require endothelial cell persistence [2, 48, 15]. A number of authors have proposed CPM extensions for making cells directionally persistent [6, 5, 26]. These methods assign a target direction or target velocity to the cell and favor motility in the target direction. The target direction of motion can be pre-assigned [6, 26] or derived from the cells' instantaneous velocity [5]. In both methods the target direction becomes a running mean over the cells' past movements. In this way the cells' movements depend both on its past movement (persistence) and on local variables, including chemoattractants or the interaction with neighboring cells. Cell motility and cell persistence parameters are easily derived from cell tracking experiments (see, *e.g.*, [6]) but in more advanced methods we could make the persistence parameters a function of local signals. For example, Reinhart-King [40] and coworkers observed attractive and repulsive pairwise interactions between endothelial cells, depending on Matrigel compliance. Even without explicitly modeling the substrate as an elastic medium, by increasing or reducing persistence lengths in the vicinity of neighboring cells we could model attractive and repulsive interactions.

Many models of endothelial cell aggregation contain relatively coarse-grained descriptions of chemotaxis. They assume that cell velocity relates linearly to the chemoattractant gradients and sometimes saturates at higher chemoattractant concentrations. Microfluidics experiments (see [9], reviewed in Section 3.2) that have quantitatively measured endothelial cell chemotactic response suggest that the interdependencies between gradients of the chemoattractant VEGF, its concentration and the chemotactic response are more complicated. For example, Chen *et al.* [9] found a

minimum number of VEGF molecules needed for a chemotactic response and a minimum steepness of the VEGF gradients. Also they observed a saturation concentration in their experiments, above which the endothelial cells do not respond to gradients. Thus this experimental system enables modelers to employ quantitative descriptions of endothelial cell responses to VEGF.

As discussed in Section 3, new experimental techniques, including traction force microscopy and atomic force microscopy, are now quantifying the forces mammalian cells exert on the matrix in ever more detail. Although the cellular Potts model does not describe cellular forces explicitly, several authors [29, 16, 26] have observed that ΔH—*i.e.* the energy gradient determining the probability of pseudopod extension and retraction—can be interpreted as a force applied locally to the cell membrane if movements obey the overdamped force-velocity relation, *i.e.* $\vec{v} \propto \vec{F}$, a realistic assumption for the highly viscous protein matrix mammalian cells live in. The wealth of data produced by traction force microsopy and microfluidics techniques will soon allow us to attach physical units of force to the effective energy changes ΔH.

4.3 Linking subcellular mechanics to cell motility

A particular attractive property of the CPM in comparison to cell-based models describing cells as point particles or ellipsoids, is the way it models eukaryotic cell movement. Continuous, random displacements of the cell boundaries qualitatively reproduce the continuous, random extension and retraction of pseudopods, which is the primary driving force of eukaryotic cell movement. Biologically, pseudopod extension and retraction probabilities depend on internal factors (*e.g.* cell volume, cell polarity) and on external factors (*e.g.* chemoattractants, ECM mechanics). As briefly discussed in Section 4.1 the extension and retraction probabilities are easily made qualitatively dependent on a range of internal and external factors, simply by adding extra terms to the Hamiltonian. However, now that experiments can precisely quantify the cell surface ruffling and its dependence on the microenvironment, the next challenge will be to represent these measurements correctly in the CPM.

In the CPM, the precise extension and retraction probabilities of pseudopods, $P(\Delta H)$, depend on at least two factors: 1) the effective energy change ΔH associated with the extension or retraction of a pseudopod, as given by the Hamiltonian (Eq. 4.1), and 2) the function $P(\Delta H)$ that translates the effective energy change to the probability the movement is actually performed (Eq. 4.2). Typically CPM models use a Boltzmann probability function (Eq. 4.2), a relic of its descent from statistical physics, where a system temperature T determines the overall "cell motility", *i.e.* the extent to which energetically unfavorable extensions or retraction are accepted. Thus, we could improve on the biophysical realism of the CPM by 1) substituting the Boltzmann probability function for one better matching experimental rates of membrane ruffling [16], or 2) developing more precise Hamiltonians producing experimentally correct membrane ruffling rates. Apart from the phenomenological approaches outlined here, which would obtain pseudopod dynamics from empirical data, pseudopod dynamics can also be derived from the underlying subcellular dynamics. Marée and coworkers [28] derived pseudopod extension and retraction from mesoscale descriptions of the actin cytoskeleton in keratocytes, predicting qualitatively correct cell shapes.

Ultimately we hope to mechanistically link gene network dynamics to quantitative descriptions

of cell behavior, which will help us link genetic defects to alterations of cell behavior. Although such complete, dynamic models of cell behavior are still far ahead (but as recent work suggests, certainly realistic [22]), it is already possible to use phenomenological approaches. Tsukada and coworkers [53] could relate activity of the Rho-family GTPases Rac1, Cdc42, and RhoA to displacements of the cell membrane. Bakal and coworkers [4] automatically classified the morphology of Drosophila knock-out cell lines based on similarities to reference morphologies. Both these papers linked specific gene and protein activity to aspects of cell behavior including protrusion and retraction of lamellipodia, adhesion, and cortical tension, in wild-type and knock-out cell lines. We hope that eventually quantifications of cell behavior and the underlying gene networks—either using the empirical approaches discussed in Section 3 or using more detailed computational and experimental analyses of subcellular biomechanics [55]—will pave the way for a quantitative CPM: the qCPM.

5 Conclusion

Understanding the mechanisms of biological development requires insight into the intricate interactions between multiple levels of organization: the genome, the molecular scale, the cytoskeleton, the cell, the tissues and the whole organism. We have argued previously that of these levels of organization, the central, natural level of abstraction is the cell [29]: the gene and protein networks regulate the cells' biophysical properties and a limited set of cellular behaviors and responses. Cell-based modeling then helps unravel the biophysical mechanisms by which relatively simple cellular behavior of single cells produces the staggering complexity of multicellular organisms.

Unraveling complex developmental mechanisms starts with a detailed analysis of a simple system consisting of only a few interacting elements of the full system. In developmental biology, a typical simplification is to isolate organ systems or to isolate one or a few cell types into a cell culture model. *In silico* models will then help reproduce the essential cell behaviors responsible for the patterning seen in the cell culture models. Then, after identifying the differences between the computer models and the cell cultures, a more detailed model of cell behavior will refine the computer model. Thus going back and forth between *in vitro* and *in silico* models yields an ever more detailed understanding of how the collective behavior of individual cells drives tissue morphogenesis.

A major problem with this approach is that in many cases, several alternative single cell mechanisms seem to produce similar outcomes at the tissue scale. For example, both reaction-diffusion mechanisms [10, 23] and a haptotactic mechanism [60] plausibly reproduce *in vitro* chondrogenesis micromass cultures, while a multitude of mechanisms, including those involving chemotaxis, mechanical traction or preferential adhesion to elongated structures, correctly reproduce *in vitro* blood vessel growth.

Although the different mechanisms will differ in their kinetic properties and stability [31] distinguishing them based on an analysis of the *in vitro* cell culture will not always be realistic. The reason is that the descriptions of single cell behavior in many cell-based models are relatively coarse-grained, so we cannot be sure to what extent observed differences between the *in silico* and

in vitro models are due to incorrect assumptions on cell behavior (*e.g.*, cell movement could be actually due to mechanical traction instead of the chemotactic movement the model described) or simply due an imprecise, but otherwise correct description of cell behavior.

We have argued that recent experimental techniques, including microfluidics and traction force microscopy, allow for much more detailed, quantitative *in vitro* measurements of single cell behaviors. Thus, the time has now come to build cell-based models using precise, parameterized descriptions of single-cell behavior, instead of the qualitative sets of assumptions that are commonly used now. The resulting quantitative cell-based models will describe the behavior of cell cultures more accurately and quantitatively predict the effects of experimental manipulations. At the same time, advanced image characterization techniques will distinguish subtle differences between patterns formed *in vitro* and those formed in ranges of alternative *in silico* models. Thus quantitative cell-based modeling will make it possible to experimentally validate or reject the hypotheses represented by alternative, dynamic *in silico* models of cell cultures.

Eventually, a combination of cell-based modeling and *in vitro* experiments could yield detailed, quantitative data-sets describing the behavior of a wide range of cell types, including cell lines or primary cell isolations widely used in cell cultures (*e.g.* HeLA cells or HUVEC) or embryonic cell lines. Such sets of "cell specifications" could culminate into a range of "off-the-shelf" *in silico* cell lines to be used for rapidly setting-up new simulations with cell-based modeling packages (*e.g. CompuCell3D* [11]). This would enormously speed up both computational and experimental research in developmental biology.

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