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Multiscale mathematical biology of cell-extracellular matrix interactions during morphogenesis

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Introduction

This chapter is in part based on

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Encyclopedia of Cell Biology, Academic Press/Elsevier,

Hirashima T, **Rens EG**, and Merks RMH (2017) *Cellular Potts Modeling Of Complex Multicellular Behaviors In Tissue Morphogenesis*. Development Growth and Differentiation 59(5):329-339.

and

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1.1 Morphogenesis

Every multi-cellular organism originates from a single fertilized egg cell. This cell multiplies and the resulting clump of cells undergoes complex shape changes to form different tissues and organs, resulting in a full functioning organism. This process is called morphogenesis and involves a seemingly fixed choreography of cells, which move collectively while differentiating and interacting with each other. Much like in a ballet choreography, slight missteps of a member of the *corps de ballet*, will not result in a complete failure of the formation. Indeed, the mechanisms behind morphogenesis are quite robust. However, if many members fail to do their part, and errors are not compensated, the resulting formation will not be right. Indeed, failure of migration and differentiation of cells during embryonic growth leads to numerous birth defects, such as cardiac diseases.

Morphogenesis is not only important during embryonic growth, it also plays a crucial role throughout development. During the lifetime of an organism, cells reorganize and reshape tissues to maintain optimal functioning of organs. For example, capillaries continuously reorganize in order to keep properly distributing the oxygen in the body and comply to the changing demands of the surrounding tissues [1]. The ability of tissues to adapt can be very helpful. Continuing on the example of blood vessels, the growth of new blood vessels during wound healing supplies the new tissue with oxygen. Blood vessel formation, however, may also be harmful. Excessive growth of blood vessels induced by a tumor can further progress growth of the tumor and metastases [2, 3]. Thus, morphogenesis is involved in tissue homeostasis, healing and disease. In order to better treat diseases and heal organs, we need a good understanding of the mechanisms behind morphogenesis.

Due to great progress in genetic sciences since the 1970s, developmental biology has largely focused on gene regulation of developmental growth and disease. It was, and is, often investigated how the knock-out or over-expression of genes affects the shape and state of the organism [4, 5]. Such studies provided us with insights into which genes control which developmental process and which diseases are associated with them [4, 5]. Furthermore, it is even possible to visualize the activity of multiple genes concurrently [6]. This enables the association of spatial and temporal patterns of gene expressions with the development of organs and disease [7]. However, such data does not fully explain how and what cell behavior, that is regulated by gene expressions, drives the growth and form of multicellular organisms. Signaling molecules that affect the transcription rate of genes, either directly or indirectly through a gene regulatory network, are often considered to regulate morphogenesis. Signaling molecules are produced by cells and diffuse through the tissue. If cell behavior depends on the local level of the signaling molecule, a cell can “read out” its position based on the distribution/gradient of the signaling molecule [8]. In 1952, Alan Turing showed that two diffusive chemicals that react with one another, can produce patterns such as stripes and spots, depending on their diffusibility [9]. Such chemicals were termed “morphogens” and their concentration gradients have since often been postulated to drive

various morphogenetic processes. For instance, reducing the binding rate of the signaling molecule FGF10, involved in epithelial branching, to the substrate lowered its diffusion rate and as a result the tissue branched instead of elongated [10]. Gradients of morphogens are thought to provide tissues with global positional information [8] by regulating cell proliferation, differentiation and motility through gene transcription. Non-diffusive membrane-bound signaling molecules can also regulate morphogenesis by inducing cell-cell communication. For instance, in the Delta-Notch signaling pathway [11], Delta production of one cell activates Notch in a neighbouring cell, inhibiting the production of Delta. This mechanism results in a salt-pepper pattern of two cell types in a tissue [12]. Thus, both global and local chemical signaling can drive morphogenesis.

1.1.1 Physical forces in development

It has become increasingly clear that not only chemical signals but also mechanical forces, originating from, for instance, tissue movements, greatly impact morphogenesis. As early as 1874, Wilhelm His proposed that not only heritable traits but also mechanical forces determine embryonic growth [13]. In 1917, D'Arcy Thompson published his famous book "On Growth and Form" [14], where he argued how physical forces and environmental constraints can shape biological forms, such as cells and whole organisms. If a tissue is subject to forces, it undergoes shape changes, similar to non-biological materials. A recent example of this phenomenon is a physical model resembling the gut, made of silicone rubber, attached to a latex sheet resembling the dorsal mesenteric sheet, which showed that differential growth of the two tissues may drive looping of the gut [15]. Experimental data seems to confirm this mechanism, as the gut tube experiences strain due to tension of the mesentery and only loops when attached to it [15]. Theoretical modeling of elastic tissues has given further insight into how physical forces and environmental constraints can mediate shape changes, such as bending, buckling and extensions of tissues [16]. Besides such "passive" responses of tissues to forces, the cells in tissues also change their activity in response to force. Physical forces can change gene expression and other intracellular activities. For instance, mechanical loading of bone stimulates osteocytic cells to produce bone and also inhibits osteoclasts to break down bone [17]. Intricate interplays between osteocytes and osteoclasts is thought to regulate the formation of bone in response to mechanical loading [18, 19]. Because of the increasing evidence of the ability of forces to drive development, physical forces are thought to play an equally important role as chemical signaling in morphogenesis.

1.1.2 The cell as a contractile apparatus

Similar to chemical signaling, mechanical forces can originate from surrounding tissues or at a local level. In 1980, Harris and coworkers showed that mechanical forces can originate locally from cells itself [20]. By placing a cell on a silicone rubber

substrata, the substrate started to wrinkle, indicating that cells apply forces to their environment [20]. Indeed, it has become clear that the cytoskeleton is able to resist and generate forces, making the cell a mechanically active material. As a result, the cell has been modeled by a tensegrity model, which is originally a structural principle coined by the architect Buckminster Fuller and first applied to describe cell structure by Donald Ingber [21]. In this model, cells are thought to stabilize in shape by a balance of forces between tensed actin filaments, intermediate filaments, compressed microtubules within the cytoskeleton and adherence to its environment (Figure 1.1).

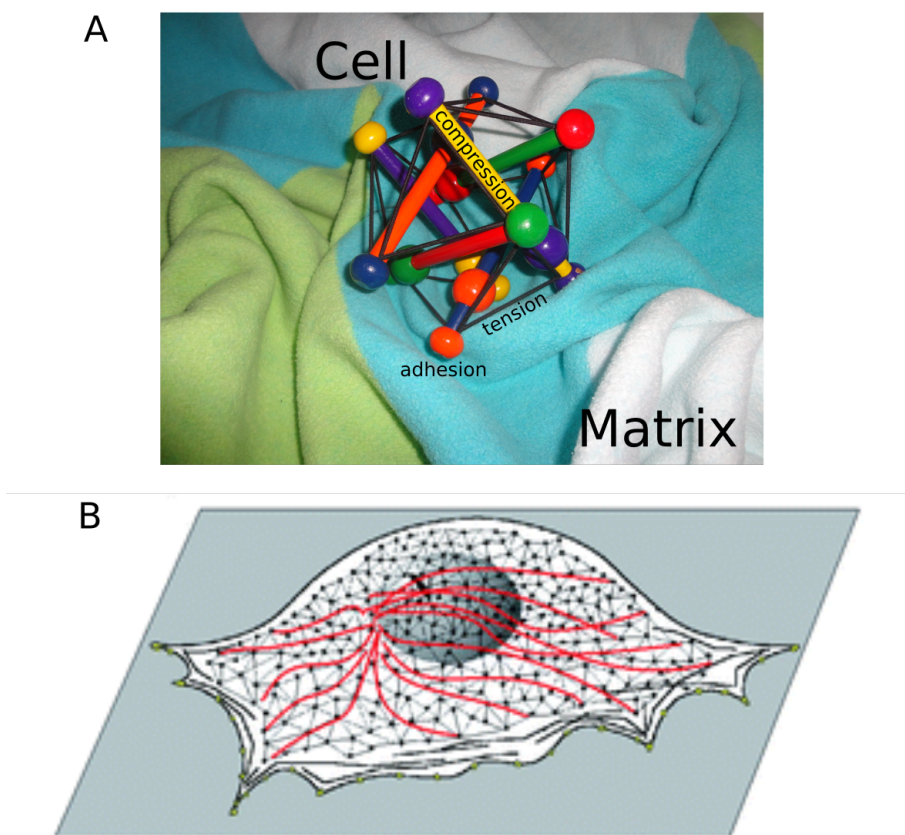


Figure 1.1: Tensegrity model of the cell and its cytoskeleton. (A) A tensegrity toy illustrates the principle of a self-stabilizing network of compressed struts and tensed cables, courtesy of Daniel Lont; (B) A schematic representation of a cell adherent to the extracellular matrix. Red shows radially oriented microtubules that oppose the inward-directed forces of the actomyosin network depicted in black. Panel B was reproduced in part from [22] with permission of The Royal Society of Chemistry.

Actin filaments can assemble in large load bearing structures called stress fibers, which are actin filaments bundled by non-muscle myosin II molecules. Through hydrolysis of ATP, myosin motors convert chemical energy into mechanical energy and walk along the connected actin filaments, which makes the stress fiber become tensed [23]. This allows a cell to generate significant forces (locally up to tens of nano Newtons [24]), and since stress fibers attach to cell adhesion molecules at the membrane, these forces are transmitted to what the cell adheres to.

Cells in tissues adhere to each other through the Cadherin adhesion molecules, that link to the cytoskeleton [25], which allows for force transmission between cells in tissues. Long range force transmission has been shown to regulate tissue shaping. For instance, dorsal closure of *Drosophila melanogaster* (fruitfly) is mediated by the propagation of stresses, originating from collective cell contractions at various places of origin in the embryo (reviewed in [26]). Cell contraction via actin-myosin has been implicated in many other developmental processes, such as tissue elongation and collective cell migration (reviewed in [27]).

1.1.3 Mechanical interactions with the extracellular matrix

Cells in tissues are surrounded by the *extracellular matrix* (ECM), an interconnected network of fibers and proteins that supports tissues. By adhering to and applying forces on the ECM, cells are able to sense and respond to the mechanical cues in the ECM. The architecture of the ECM mediates cell migration [28]; cells move up ECM density gradients (haptotaxis), matrix stiffness gradients (durotaxis) and along fibers (contact guidance). Not only do cells respond to the structure of the ECM, but they can also actively change its local architecture. Cells deposit matrix fibers, reorient, degrade and link the fibers in the ECM. Also, cells locally stiffen the matrix either by contracting it, or by depositing matrix fibers. Because cells sense matrix deformations generated by adjacent cells, matrix remodeling allows cells to communicate via the ECM.

Advances in *in vitro* modeling of cells and the extracellular matrix has given more insight into mechanical cell-cell communication [29]. In such studies, synthetic gels or naturally-derived gels are used to mimic the extracellular matrix. Matrigel is a natural gel that contains a mixture of extracellular matrix proteins, such as growth factors and collagen fibers. Although Matrigel mimics the complex environment well, it is difficult to tune separate effects, such as fiber density or matrix stiffness or exclude the effects of unknown components of the gel. Instead, synthetic gels may be used, of which the components are controlled and its mechanical properties are tunable [30, 31]. An example of synthetic gels are polyacrylamide (PA) gels, which are flexible substrates with tunable stiffness. These gels are derivatized with RGD peptides or coated with matrix fibers, to allow cell-substrate binding.

PA gels have been used to reconstruct cell traction forces based on matrix deformations [32]. Similar experimental set-ups were used to show that these traction forces can mediate cell-cell communication (*e.g.* [33], Figure 1.2A). In this study [33], it was shown that cell-cell contact depends on the stiffness of the substrate. On the softest

substrates, cells adhere to one another while on substrates of intermediate stiffness cells repeatedly touch and break contact. On the stiffest substrates, the cells first make contact but then migrate away. The cells were able to apply sufficient force to create substrate deformations under nearby cells and the range of substrate deformation was correlated with the separation distance between cells. This study showed that cells communicate short range ($\approx 25 \mu\text{m}$) through matrix stresses on PA gels. The range of communication is much longer on collagen gels, and can even be up to $450 \mu\text{m}$, depending on cell type and gel stiffness [34]. Long range communication has been attributed to strain-stiffening of fibrous gels [34, 35]. Indeed, cells can apply sufficient force to strain-stiffen the matrix (Figure 1.2B). This allows cells to elongate and align with one another, forming network-like structures [34]. Network formation has often been observed and depends on the magnitude of cell force, substrate stiffness and substrate density [36, 37]. Besides network formation, local substrate deformations also regulate other processes such as collective cell migration [38, 39].

Cells can also steer the migration of other cells by means of traction force induced realignment of matrix fibers [40, 41], to which cells migrate along by contact guidance. Since fibers are quite long and can span multiple cells, they allow for long range communication. Based on experimental images of cells and matrix fibers, a computational study was performed to that suggests that fiber alignment is crucial long range stress propagation (Figure 1.2C).

Theoretical models

Many theoretical models were developed to understand how cells respond to mechanical cues in the matrix [42, 43]. Theoretical models were often based on homeostatic principles, motivated by experimental observations of cells that maintain local stresses or strains in the substrate [44, 45]. For instance, a theoretical model of cells represented as contractile dipole forces suggested that cells can reorient to matrix stress if it attempts to maintain either local stresses or strains. Furthermore, either stress or strain optimization allowed cells to move towards neighboring cells. Using a similar theoretical model, it was proposed that cells minimize the amount of work needed to contract the matrix [46]. This allows cells to align to each other and form networks-like structures [47]. However, it is still poorly understood how and why a cell would maintain local stresses or strains or minimize the amount of work. Cytoskeleton remodeling is governed by the dynamics of stress fibers and cell-matrix adhesions, which are in turn influenced by matrix stresses and thus are considered to regulate the response of cells to matrix mechanics. Theoretical models have suggested that cells can respond to matrix stresses by aligning stress fibers, upregulating traction forces [48] and stabilizing cell-matrix adhesions [49, 50]. Although such models have provided further insight into how cells can respond to the ECM, it is still poorly understood how this affects tissue organization.

Cell-cell communication through matrix stresses are very relevant to many morphogenetic processes. For instance, sprouting cells from an aggregate has been shown to be

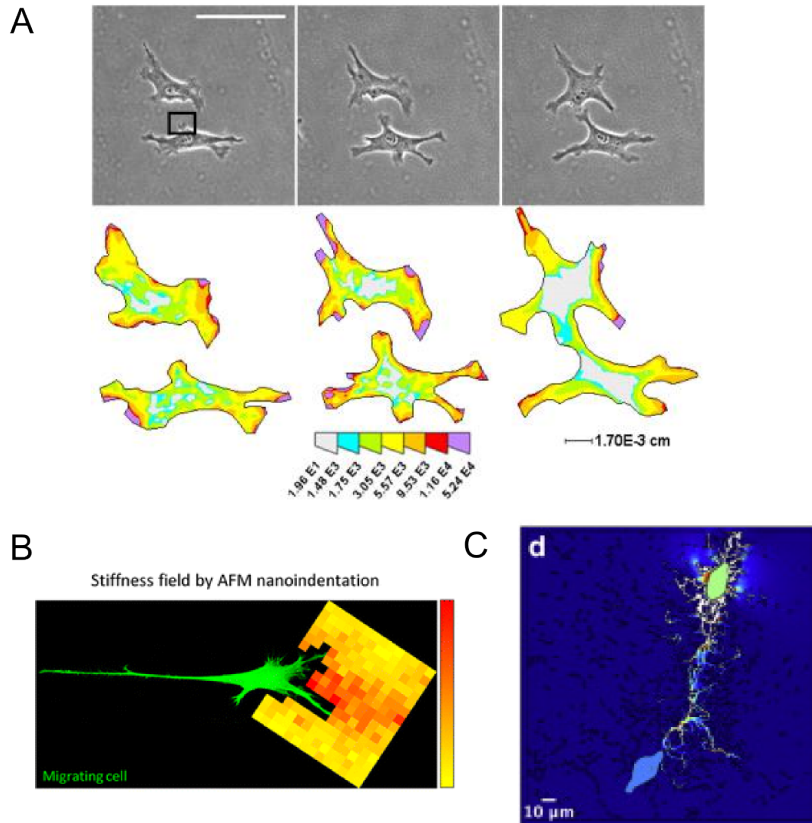


Figure 1.2: Experimental observations of matrix remodeling and mechanical cell-cell communication. (A) Two adjacent cells on polyacrylamide gels with traction stress distributions. The cell at the bottom extends a small pseudopod towards the top cell while it was moving away, revealing that the cell senses traction stresses of the adjacent cell. Reprinted from [33] with permission from Elsevier; (B) Heatmap of the matrix stiffness around a migration fibroblast, indicating that the cell locally stiffens the matrix. Adapted from [38] with permission from American Chemical Society; (C) Heatmap of stresses between two adjacent cells on a fibrous matrix. Stresses were calculated using a finite element model of a nonlinear strain-hardening material based on the experimental images of the cells and fibers. This shows that stresses propagate between cells via matrix fibers. Adapted from [51] with permission from Elsevier.

mediated by traction force induced reorientation of matrix fibers, which guides the directional outgrowth of cells [41, 52]. Furthermore, during *Drosophila* egg development, collagen deposition of cells generate a stiffness gradient, which instructs elongation of the follicle [53]. However, excessive matrix remodeling, which may occur during wound healing and is associated with fibrotic diseases, can lead to organ dysfunction [54]. Also, cancerous cells can significantly reorient and stiffen the matrix, which allows a tumor to grow and metastasize [54]. In conclusion, cell-matrix interactions have been implicated in morphogenesis, homeostasis and disease.

A better understanding of how cell-matrix interactions shape tissues, can greatly benefit medicine. For instance, cancer therapy would benefit from a better understanding on how the mechanical properties of cells and the matrix may drive cancer progression [54]. Cancer metastasis is associated with increased remodeling of the matrix [54]. By targeting, for instance, enzymes involved in matrix remodeling, we could obtain new therapeutic agents for cancer therapy [54]. Furthermore, mechanical loading of artificial tissues regulates the orientation and structural integrity of the tissue [55]. Artificial tissues may be implemented to promote healing of a damaged organ or even replace it and the efficiency of tissue engineered constructs depends on how well the artificial tissue resembles the present *in vivo* tissue structure [56]. So, a better understanding of how forces regulate tissue formation can benefit the design of such constructs [57].

1.2 Mathematical modeling of morphogenesis

Because of the complexity of biology, it is very challenging to study morphogenesis by solely doing experimental studies. Mathematical modeling has become a useful tool to study morphogenesis and has many benefits. In contrast to experimental approaches, in a mathematical model, every variable can be tracked, and it is relatively easy to knock out specific mechanisms or variables and interactions, while maintaining others. Hypotheses from experimental observations can be formulated in mathematical equations. Solving or simulating mathematical models can thus help us understand how certain mechanisms influence the behavior of the system [58]. Furthermore, models can be used to investigate how different mechanisms interact in a system. A mathematical model can also be used to make a prediction about the experimental system [58].

Mathematical models can be used to describe and study different scales of the biological system. The microscale of cell biology, like for instance protein and gene interactive networks within cells, has been described by mathematical models [59]. Although we can measure how proteins and genes interact, we can not grasp what the consequence is of all interactions between different genes and proteins. Mathematical modeling can provide insights into how changes in such large interactions networks affect the expression of the cell [59]. However, such models do not translate to the impact on the tissue level. Other mathematical models focused on dynamics at the tissue level by modeling the tissue as a continuum by averaging out individual cells. These models give insight how laws of motions for the cells results in tissue patterning. Such

models have been used to study pattern formation in a wide variety of developmental processes [60]. However, continuum models do not provide sufficient detail of cellular mechanisms, such as cell shape changes, that are relevant to morphogenesis. It has been proposed that mathematical biology should take a cell-centered approach [61]. More specifically, it was argued that cells should be considered as black boxes that behave a certain way, while neglecting the intercellular mechanisms that drive this behavior. The individual cell behavior can be inferred from experimental observations and be put into a cell-based model. Then, we can use this cell-based model to understand how a specific input (*i.e. cell behavior*) leads to a certain output (tissue formation). By comparing the model output to experimental observations, we can determine if this cell behavior suffices to explain the experimental data. If not, the model can be further adapted and subsequently tested with experiments to find the cell behavior that suffices to reproduce certain experimental observations.

As morphogenesis is an inherently multi-scale problem we believe that we should take a multi-scale mathematical modeling approach. Organisms consist of tissues, which consist of cells, the extracellular matrix and their molecules. During development, all different scales in biology interact and feed back on one another. For instance, gene expressions change cell behavior, which change tissue forces, which in turn change gene expressions. So, a better understanding of how forces can drive morphogenesis should take into account the forces acting and interacting at all different scales. A multi-scale model consists of separate models that describe the scales of interest, from the molecular scale, to the cellular scale to the tissue scale [62]. We believe that we should start with modeling only the scales and interactions of interest, like for instance cells and the extracellular matrix and their interactions. Then, if the model is not able to explain the experimental observations, we can further build up the model, by adding more mechanisms, scales and interactions between scales. Doing complementary biological experiments allows us to validate model predictions but also to investigate what aspects are missing in our model. Such a feedback loop between experimental biologists and mathematical modelers allows for a systematic exploration of the necessary mechanisms in a biological system and thus increases our understanding of the system.

1.2.1 Cell based modeling

Because cells are the building blocks of tissues, we aim to develop multi-scale models that explicitly describe cells. Discrete cell-based models describe cells as individual elements, and thus, in contrast to continuum models, provide sufficient detail on the cellular level. We can divide cell based models in single and multi-particle methods. Single-particle models represent cells as point particles or ellipsoids. Multiple-particle-based models use a collection of particles to represent each cell, allowing for a more detailed description of cell shape [63]. A further distinction is made between representations on regular lattices, which can be computationally more efficient, and off-lattice representations, which allows more flexibility in cell shape. An overview of cell-based

methodology is given in Figure 1.3. In single particle-based models cell migration is described by differential equations of motion for each particle. These differential equations usually include active, random or directed cell migrations and external forces applied to the cell, which can be either cell-cell interactive forces or forces from the environment [see, *e.g.* 64]. Alternatively, particles comply to a set of behavioral or migratory rules [see, *e.g.* 65], as in an agent-based model.

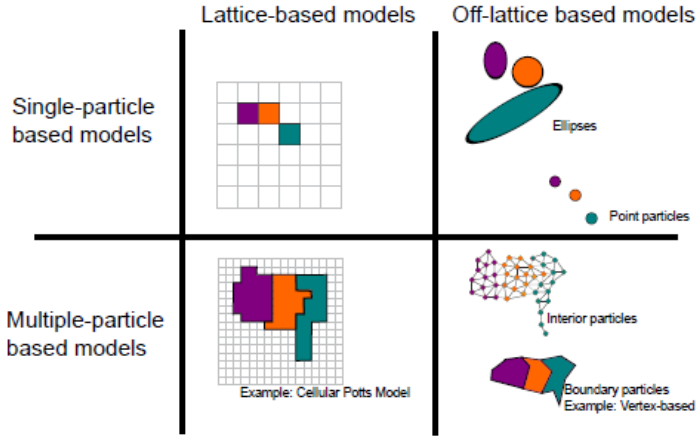


Figure 1.3: An overview of cell-based modeling methods. Reprinted [66] with permission from Elsevier.

Using particle-based methods, various cell migration behaviors have been modeled. For instance, Szabo *et al.* [67] included diffusion, directional persistence and an attraction to anisotropic structures to model cell organization into network-like patterns. Cell-cell interactions, such as pressure and velocity adaption to neighbors, were added by Sepulveda *et al.* [68] and Byrne *et al.* [65] respectively. Dallon *et al.* [69, 70] simulated contact guidance, haptokinesis and chemotaxis in a particle-based model.

While particle-based methods make it possible to simulate how individual cell behaviour is responsible for collective cell motility during morphogenesis, more detail may be needed. The shape of the cell is key to many developmental processes. The shape of the cell determines the extent of cell-cell interactions, *i.e.* cell-cell signalling or cell-cell adhesion. To account for differential cell shape, multiple particle methods describe cells as a collection of connected particles, so that the boundary or interior of a cell is defined (Figure 1.3). Thus, cell shape is explicit and can change in response to external forces and interactions with adjacent cells. The subcellular elements method [71] is an example of an off-lattice multiple particle method, where cells are divided into subcellular elements (for instance, points in space), which can locally interact with elements of the same cell and elements of other cells via equations of motion.

These equations describe both the internal rheology of individual cells, as well as the adhesive and repulsive forces with adjacent cells. Mechanotactic and chemotactic cell migration can be included in such models [see *e.g.*, 72]. Alternative off-lattice and lattice-based multi-particle methods are the vertex-based models and the cellular Potts model (CPM). The CPM describes cell shape and cell movement on the level of protrusions. It can be easily extended to describe various cell behaviors and other scales such as intercellular dynamics and the extracellular matrix. For this reason, the research presented in this thesis has employed the CPM, so we will describe the CPM in more detail here.

The CPM [73, 74] represents cells as a set of connected lattice sites (Figure 1.4) on a 2D square lattice $\Lambda \subset \mathbb{Z}^2$. Each lattice site, $\vec{x} \in \Lambda$, has a state $\sigma(\vec{x}) \in \{0, 1, \dots, n\}$ that identifies the individual cell the lattice site belongs to, where $\sigma = 0$ represents the surrounding medium, and n is the total number of cells in the lattice. Forces acting on the cells are described in the Hamiltonian,

$$H = \sum_{(\vec{x}, \vec{x}')} J(\tau(\sigma(\vec{x})), \tau(\sigma(\vec{x}')))(1 - \delta(\sigma(\vec{x}), \sigma(\vec{x}'))) + \lambda \sum_{\sigma \in [1, n]} (a_\sigma - A_\sigma)^2, \quad (1.1)$$

where J is an adhesive energy between two adjacent sites $\vec{x}$ and $\vec{x}'$ and δ is the Kronecker delta function: $\delta(x, y) = 1$ if $x = y$ and $\delta(x, y) = 0$ otherwise, such that the first term counts the total adhesive energy across cell-cell and cell-medium interfaces (Figure 1.4). The second term is a cellular volume conservation term, with a_σ the cell area, A_σ , the resting area of cell σ , and λ a compressibility parameter. To mimic cell motility and membrane fluctuations, the cellular Potts model iteratively attempts to copy the state $\sigma(\vec{x})$ of a randomly selected lattice site $\vec{x}$, into a randomly selected, adjacent lattice site $\vec{x}'$; If such a copy reduces the value of the Hamiltonian ($\Delta H \leq 0$), it is accepted. If the attempt would increase the value of the Hamiltonian ($\Delta H > 0$) it is accepted with a Boltzmann probability, $P(\Delta H) = \exp(-\Delta H/T)$. These copy steps account for the intrinsic random motility of cells, with large values of T corresponding with more random motility. During one time step, N copy attempts are made, with N the total number of lattice sites in the lattice.

The CPM has often been extended to account for additional cell behaviours, which are typically described by additional terms in the Hamiltonian (Eq. 1.1). Hybrid Cellular Potts models, in which the CPM is combined with discretized continuum models (PDEs) to account for secreted chemical signals [76] were developed to study how chemical signaling affects tissue patterning. Other extensions include anisotropic cell adhesion [77], cell elongation [78] and persistent cell motility [79].

1.2.2 Modeling of mechanical cell-matrix interactions

Various modeling techniques can be used to model the extracellular matrix. Similar to cells, depending on the context and objective, the ECM can be modeled using continuum or discrete approaches. Within continuum models we can make a further distinction between models excluding and including matrix fibers. In this section, we

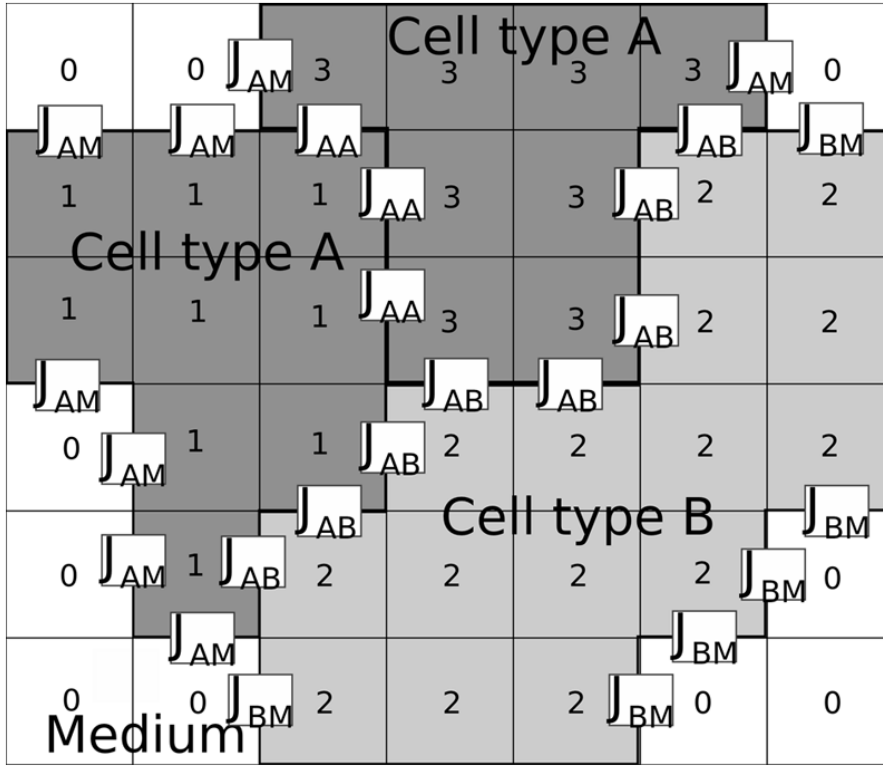


Figure 1.4: An overview of the cellular Potts model. Three cells with two cell types interact via adhesive energies and the surrounding medium. Reprinted from [75].

will give a overview of matrix models that were coupled to cell models in order to investigate how cell-matrix interactions can influence morphogenesis.

Murray and Oster [80] developed a continuum model for the extracellular matrix. Here, the ECM was modeled as an viscoelastic material that deforms in response to external forces and the laws of motions are described using partial differential equations (PDEs). To study cell-matrix interactions, PDEs that describe cell movement can be coupled to the PDEs that describe the matrix. In the model by Murray and Oster, cells are assumed to contract the matrix so that the matrix deforms. Cells then are pulled passively along substrate deformations. This causes an initially random dispersed cells to accumulate the matrix underneath them so that cells form aggregates. Then, tension lines between aggregates enable the aggregates to connect and form patterns. This model has been extended many times to include active cell-matrix interactions. For instance, models that included active cell movement along matrix strains, chemotaxis and haptotaxis were used to study cellular network formation [81–83].

Continuum modeling approaches have also been used to describe matrix fibers. For

instance, the ECM has been modeled by a vector field that describes the orientation of fibers [69]. The reorientation of the vector field is modeled by an ordinary differential equation, where it is assumed that the vector field reorients to an external force field with a rate proportional to the magnitude of the external force. As the ECM is anisotropic and not unidirectional it was proposed that the ECM should be modeled as a tensor field instead [84]. These vector or tensor fields of matrix fibers were coupled to discrete models of cells and applied to study wound healing [69, 84].

Continuum models that describe both matrix elasticity and fiber orientation have also been developed. Barocas and Tranquillo developed a biphasic theory for tissues, where the tissue is described as a mixture of the matrix and cells [85]. This model defines the fraction of volume that contains either cells or the matrix and describes how the two phases deform and interact through forces. An additional tensor field is introduced that describes the fiber orientation which rotates in response to cell forces and affects cell migration [86]. Recently, a similar multiphase modeling framework was adopted that in addition describes how the fiber orientation affects the stress in the matrix. Using this model, it was studied how matrix anisotropy affects cellular pattern formation [87]. Matrix fibers can also be included in elastic material models by considering fiber-reinforced elastic materials. Checa *et al.* [88] modeled an elastic material where the orientation of fibers determines the stress in the material [88]. It was assumed that the fibers rotate towards the principal stress orientation of the tension field generated by discrete cells. This model was used to study the effect of boundary conditions on cellular self-organization and fiber alignment [88]. Yang *et al.* [89] modeled how the orientation of the fibers but also that the density of the fibers affects matrix stresses. This model was coupled to a discrete model of cells to study the effect of matrix fiber realignment during wound healing (Figure 1.5A).

Because fibers can be of the same length or even longer than cells, it may not be appropriate to model a fibrous matrix as a continuum. Instead, models were developed that describe both the cell and matrix fibers as discrete objects. Schluter *et al.* modeled fibers as thin cylinders that rotate as a lever as a cell pulls on it [90]. Discrete cells then move along the orientation of the fibers. Fibers and cells have also been represented as a set of connected nodes and springs [91, 92]. In such models, fibers are multiple springs connected to each other by particles that can be anchored to the cell. The sum of all forces between cells and the matrix deforms the elements, effectively resulting in cell migration. Typically, discrete models are computationally more expensive and thus have focused on single cell migration or the migration of two interacting cells (Figure 1.5B) [90–92]. In the CPM, the architecture of the ECM has been modeled by including ECM fibers as immovable lattice sites in the medium surrounding the cells in the CPM (Figure 1.5C) [93, 94]. By allowing more lattice sites to be of type fiber, higher densities of matrix fibers can be modeled. Note however that the fibers in this model are static.

In the models described in this section, cell-matrix interactions were often based on the alignment of fibers in the matrix. However, experimental observations indicate that

1. Introduction

cells can also communicate through matrix stresses in the absence of fibers [33]. Some continuum models [81–83] have focused on how matrix stresses influence pattern formation. In this thesis, we take a cell-based modeling approach to study how matrix stresses affects morphogenesis.

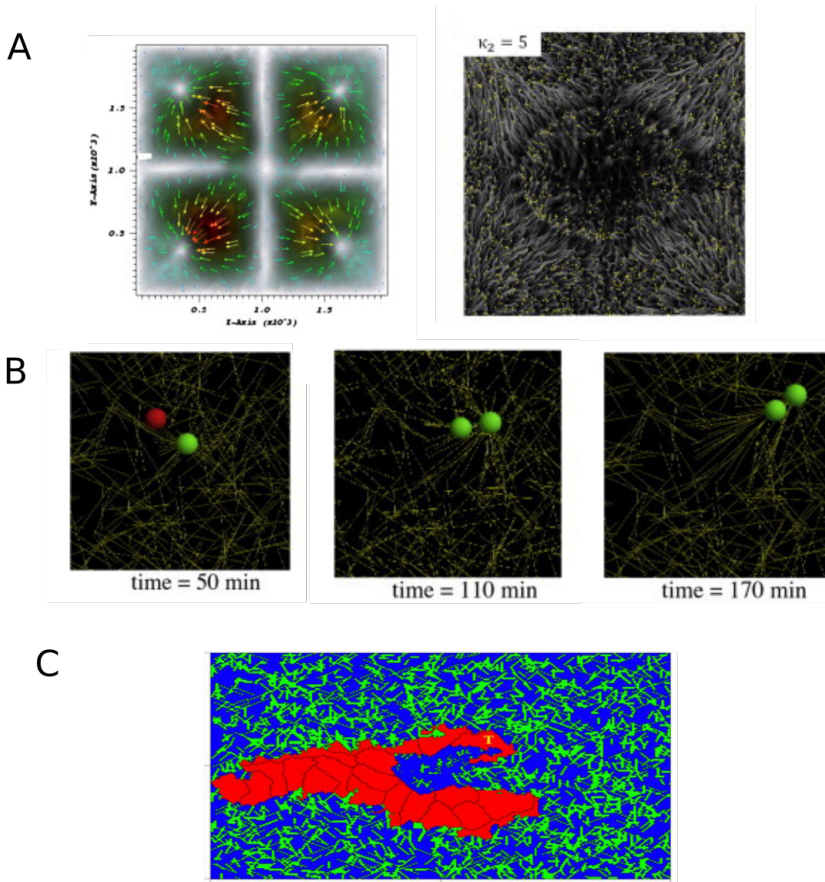


Figure 1.5: Examples of multiscale models of cell-extracellular matrix interactions. (A) Substrate deformation given by natural tension lines around the wound (Left), collagen fibers (grey) align with tension field, discrete cells are indicated by yellow spots and invade the wound (Right). Adapted from [89] with permission from Elsevier; (B) Two discrete circular cells pulling on and migrating in a fibrous matrix, one cell follows the other by contact guidance along the track of fibers that were aligned by the leader cell. Adapted from [90] with permission from Elsevier; (C) cellular Potts model (red cells) coupled to discrete matrix fibers (green), cells form sprout that branches. Adapt from [94].

1.3 Thesis outline

In this thesis, we address the overarching question “How do cell-matrix interactions drive morphogenesis?”. We mainly focus on mechanical cell-interactions through matrix stresses but also study chemical signaling via the matrix. We take a multiscale computational modeling approach to attempt to answer our research question. We have developed a multiscale model, by extending the Cellular Potts Model with a finite element model of the substrate. In our model, the cell and the ECM interact through a feedback-loop. By pulling on the matrix, the cell adapts the matrix, and in turn, the cells sense the matrix and respond to it, a process called *dynamic reciprocity*. By testing different hypotheses on cell-matrix interactions, we can use our model to understand how this affects morphogenesis. Since our model is generic it does not specify a certain cell type or other tissue-specific constraints, so we can use our model to study different systems.

In chapter 2, we use our model to explain how matrix stiffness regulates vasculogenesis. Experimental observations have shown that endothelial cells on compliant polyacrylamide gels only form vascular network-like patterns on substrates of intermediate stiffness [37]. In our model, we assume that by straining the matrix, the matrix strain-stiffens. Furthermore, the cells respond to the matrix by preferentially extending protrusions on stiffer matrix sites, based on the observation that cell-matrix adhesions are larger on stiff matrices, and in the direction of strain. This minimal assumption already reproduces observed single cell behavior; cells elongate on substrates of intermediate stiffness. When simulating a group of cells, the cells elongate and locally align with each other because they respond to the matrix strains induced by neighbouring cells. This local cell-cell alignment then results in global cellular network formation. We furthermore show that this cell behavior enables cells to sprout from a circular blob of cells, suggesting that the proposed mechanical cell-matrix interactions might also drive sprouting angiogenesis.

In chapter 3, we dive a little deeper into cell alignment. Here, we aim to understand how cells and tissues can align along matrix strains. *In vitro* experiments where a tissue is uniaxially loaded show that many cell types elongate along the orientation of static matrix strain. Furthermore, whole tissues can align to strain [95] or cells organize into stripes oriented along the strain [96]. Our model suggests that cell alignment to static strain is promoted by cellular forces. Cells respond to the static strain by slightly elongating. If a cell then applies a force to the matrix, it locally increases the strain of the matrix at the tip of its body. This increased strain then allows cells to elongate even further, compared to a cell that would not have strained the matrix. So, a positive feedback loop between cell extensions and cell contractility enables a cell to align along the strain. Simulations of multiple cells suggest that this mechanism enables cell-cell alignment so that cells form stripes of cells along strain. Increasing cell-cell adhesions and cell density makes the stripes disappear, while cells still elongate, suggesting that tissue level alignment depends on cell specific parameters.

In chapter 4, we present a hybrid model that includes focal adhesion dynamics, in

order to gain a molecular level understanding of how cells respond to matrix rigidity. Focal adhesions are mechanosensitive molecular structures that bind the cell to the ECM. In our model, the cells apply a force to the focal adhesions and the rate of force build-up depends on matrix stiffness and the velocity of motor proteins [97]. We assume that the likelihood of cell-matrix deadhesion decreases with focal adhesion size. Our model suggests that on stiff matrices, the cells build up enough force so that focal adhesions grow and the cell is able to spread. If we included that matrix stresses induces adhesion strengthening, the simulated cells elongated on matrices of intermediate stiffness. We show that the range on which cells elongate depends on the velocity of the motor protein. Finally, we show that cells in our model durotact: move up a stiffness gradient. So, with a more detailed model of focal adhesions we can now explain cell spreading, elongation and durotaxis on a molecular level.

In chapter 5 and 6, we will focus on chemical signaling through the extracellular matrix. In chapter 5, we introduce a model that describes the formation of a Nodal signaling gradient. Nodal is one of the signaling molecules that is involved in left-right patterning of embryos. Experimental observations in zebrafish show that the protein FurinA is able to cleave the Nodal protein Southpaw to a mature form, so that it can be secreted by cells [98]. The experiments suggest that FurinA regulates the signaling range of Nodal [98]. To better understand the dynamics, we introduce a PDE model that assumes that the rate of maturation of Southpaw depends on the level of FurinA. The model shows that the speed of extracellular Southpaw gradient formation and the range of this gradient increases with FurinA levels, which was confirmed by our experimental data.

In chapter 6, we introduce a multiscale cell based model for epithelial branching. Here, we show that gradients of an autocrine signaling factor can drive branching morphogenesis. Experimental observations of mammary epithelial cells [99] indicated that branching sites are regulated by TGF- β , an inhibitory autocrine signal. Based on these observations, we assume that cell extensions at the tissue boundary negatively depend on the local level of the autocrine signal. In this cellular Potts model, the tissue secretes the autocrine that accumulates at concave tissue boundaries. This curvature effect allows the simulated tissue to branch.

