



Universiteit
Leiden
The Netherlands

Multiscale mathematical biology of cell-extracellular matrix interactions during morphogenesis

Rens, E.G.

Citation

Rens, E. G. (2018, June 27). *Multiscale mathematical biology of cell-extracellular matrix interactions during morphogenesis*. Retrieved from <https://hdl.handle.net/1887/62863>

Version: Not Applicable (or Unknown)

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/62863>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/62863> holds various files of this Leiden University dissertation

Author: Rens, Lisanne

Title: Multiscale mathematical biology of cell-extracellular matrix interactions during morphogenesis

Date: 2018-06-27



7

Discussion

In this thesis we aimed to better understand how interactions between cells and the extracellular matrix can drive morphogenesis by making use of multiscale computational models. Mathematical modeling is a powerful tool that is often used alongside of experimental approaches. Mathematical models can test hypotheses on the driving mechanisms behind morphogenesis. Furthermore, it enables us to better understand a biological system, by studying the necessary mechanisms and the complex interplay between different mechanisms involved. In this thesis, we mainly focused on modeling of mechanical cell-matrix interactions at a cellular and molecular level and predicting how this influences the dynamics at the tissue scale. In the past, many models have neglected the interplay between cells and the extracellular matrix. Most models that did consider mechanical cell-matrix interactions mainly focused on matrix fiber remodeling as a result of stresses but not on the direct response of cells to stresses. Our model is based on experimental observations that show that cells communicate by sensing traction stresses in the substrate [33]. Our model couples the cellular Potts model (CPM) that describes cells as a collection of lattice sites, to a finite element model (FEM) that calculates substrate deformations. We assumed that cells apply a traction force to the substrate, inducing substrate strains, and that cells respond to this strain. With these models we studied how cells respond to matrix stiffness and how cell-cell communication through matrix stresses drives tissue patterning. Besides mechanical signaling, cells also use chemical signaling through the extracellular matrix to communicate. So, we also studied how chemical signaling can pattern tissues. The models described in this work pave the way to study the complex interplay between cells and mechanical and chemical forces in the extracellular matrix.

7.1 Summarizing discussion

In chapter 2, we showed that if cells preferentially extend pseudopods along substrate strains, cells are able to form network-like patterns, that resemble *in vitro* vascular networks. In accordance with experimental observations, cells only formed networks in simulations with substrates of intermediate stiffness. Furthermore, this model also reproduced cells sprouting from a cell aggregate. This model suggests a alternate mechanism for network formation and sprouting, among many existing ones which are often based on chemical signaling [78, 115].

In chapter 3, we showed that our model also explains cell alignment to static uniaxial stretch. The model suggests that by contracting the matrix, cells locally amplify substrate strains in the orientation of the global stretch cue, allowing the cell to elongate along the strain. By applying forces on the matrix, the simulated cells locally align with other cells, so that the cells forms string like patterns along the uniaxial strain. This string formation depended on cell-cell adhesivity and cell density, which could explain why tightly packed endothelial cells do orient [95] but do not form strings, while fibroblasts form strings [96].

To explain cell orientation to substrate strains, other mathematical models based

on homeostatic principles were employed: these assumed that cells reorient in order to optimize a certain physical quantity [46, 181], such as local stresses [181] or the applied work [46]. Our model suggests a cellular mechanism at a level of protrusions, that allows a cell to migrate and reorient by reshaping itself. Our multiscale models thus show how a relatively simple mechanism at the subcellular scale can induce complex tissue patterns. The complexity in our multiscale model presents itself not in the individual models or their assumptions, which are kept relatively simple, but in the coupling of different scales in our model. By introducing multiple scales in a model, we are able to translate lower scale dynamics to dynamics at a tissue level.

In chapter 4, we introduced dynamics at the scale of cell-matrix adhesions in our model, to understand how force based focal adhesion dynamics regulate cell response to matrix stiffness. We modeled focal adhesions as clusters of integrin bonds, which grow according to catch-slip bond dynamics [232]. Cells apply a force on these clusters, which builds up faster on stiffer matrices [97], so that the focal adhesions grow on stiffer matrices. We coupled focal adhesion model to the CPM by assuming that cell-matrix retractions are less probable for larger focal adhesions. This relatively simple model for focal adhesions and cell-matrix adhesion sufficed to explain cell spreading on compliant matrices. If we introduced that matrix stresses strengthen cell-matrix adhesion, we could also reproduce cell elongation on substrates of intermediate stiffness. Finally, in our model, cells movement up a stiffness gradient (durotaxis) is an emergent phenomenon. By introducing these focal adhesion dynamics in our multiscale model, we were able to better understand the response of cells to substrate stiffness.

Although physical forces can drive morphogenesis, chemical signaling is also important in tissue patterning. In chapter 5, we studied how chemical signaling through the extracellular matrix can regulate morphogen gradient formation. We developed a partial differential model of gradient formation of the Nodal protein that is involved in left-right patterning of embryos. Based on experimental data [98], we assumed that Nodal signaling induced intercellular Nodal production, and is cleaved into a mature form by the convertase FurinA. Once matured, it is able to be secreted into the extracellular space and diffuse. A combination of *in vitro* and *in silico* experiments indicate that FurinA is able to regulate speed of Nodal gradient formation and its signaling range.

Chapter 6 studies how chemical signaling can drive branching morphogenesis. Here, we introduce a CPM to explain branching morphogenesis. We base our model on *in vitro* observations of mammary epithelial cells that suggested that the autocrine growth factor TGF- β acts as a inhibitor of cell movement [99]. Due to a curvature effect of autocrine signal accumulation at concave tissue boundaries, extensions are more favorable at convex areas. This mechanism is reinforced, which allows the extensions to branch out. Thus, our model suggests that inhibition of cell movement by an autocrine signal suffices to reproduce branching morphogenesis.

Altogether, we have used multiscale models to study the role of cell-matrix interactions in morphogenesis. These models described components at different tissue scales

(such as growth factors, focal adhesions, cells and the substrate) and how these scales interact with each other. We studied how single cells respond to matrix stiffness and how matrix stiffness can regulate vascular network formation and sprouting. We also studied how matrix stresses regulate cell alignment. Furthermore, we investigated how chemical signaling through the matrix can drive branching morphogenesis. The multi-scale models presented in this thesis pave the way to a more complete understanding of how cells can organize into tissues.

7.2 Model validation

A mathematical model is useful in itself because it gives a better understanding of a biological system, but to drive science even further, complementary experimental studies should be performed. Experimental data allows us to validate, or invalidate, a mechanism proposed by the model. Because a model is just a representation of reality, we can never prove concepts with our model, we can only provide clues. If an experimental system behaves the same way as the model system in response to varying a specific parameter, it becomes more likely that the mechanism proposed by the model is valid. For instance, in chapter 4, we propose that the range of substrate stiffness on which cells elongates depends on the velocity of the molecular motor proteins. This could be validated by either using different cell types that express different types of motor proteins with different velocities or by increasing motor protein velocity by affecting ATP levels. In chapter 6, we propose that an epithelial tissue can branch by secreting TGF- β which inhibits cell movement. Our model predicts that the extent of branching depends on the strength of the autocrine signal. Experiments in which TGF- β signaling is increased and decreased can help validate this mechanism.

If the experimental data does not coincide with the mathematical model, the effort has not been futile. Experimental data can provide insights into what is truly happening, provide a new hypothesis, which can then be further explored by the mathematical model. Maybe the model is wrong, but because models are typically a simple representation of reality, it is possible that the model just misses crucial elements. Experimental studies can provide clues into what aspects are missing. By iteratively extending our model and validating our model with *in vitro* experiments, we can obtain a better understanding of the mechanisms that drive the biological system. For instance, in chapter 2, we could explain why cells form networks of substrates of intermediate stiffness, but not why the range of substrate stiffness on which networks are formed changes when the matrix density changes [37]. Since cells respond to matrix mechanics through focal adhesions, it might be possible to explain this observation with our extended model that includes focal adhesion dynamics of chapter 4. *in vitro* experiments indicate that focal adhesion growth and thus cell spreading depends on matrix density. As a preliminary result, we show that our focal adhesion model (chapter 4) can already reproduce vascular network formation (Figure 7.1), which makes it promising to further study the relation between matrix properties and network formation.

Experimental approaches have made tremendous progress in tracking cell movement [332] and also the dynamics at a molecular level, such as cell traction forces [333, 334] and the imaging the molecules in focal adhesions [335]. This makes it possible to test multiscale models at multiple tissue levels. Doing complementary experimental studies to mathematical modeling has other benefits as well. It can help us to formulate our model. For instance, for more focal adhesion molecules it is being unraveled how forces affect their structure and as a result their binding, so that qualitative models can be developed [336]. Experimental data can also provide us with realistic parameter values in our model. Unfortunately, this is not always possible, as some parameters can not directly be coupled to experimental quantities. In this case, we can tune parameters values qualitatively. For instance, cell-cell and cell-matrix adhesive energies in our model can be tuned so that they have the same relative relation as the adhesive forces of cell-cell adhesions and cell-matrix adhesions [337]. Or, we could tune the unknown parameter values so that the model best reproduces a particular system, enabling us to study deviations from that system. For instance, we could tune the unknown parameters in our focal adhesion model to a specific cell type that is spreading on a compliant substrate. Then, we can change the measurable parameters to fit to other cell types and use our model to understand why different cell types spread differently.

Mathematical models should also be validated by other modeling frameworks. Since the model behavior and thus its results may also depend on the model framework that is used [338], it is crucial to employ other modeling frameworks and implement similar dynamics and investigate the resulting model behavior. So, in order to be sure that our results are not an artifact of the particular framework that was used, ideally we should implement our assumptions into other cell-based modeling frameworks, to check if assumptions specific to the cellular Potts model, such as the lattice and definition of cell protrusions, do not greatly affect the model behavior. We do not suspect major effects, because we have applied sufficient noise and investigated various assumptions.

To gain a better idea of how all model parameters interact and affect our model output, we could perform a global sensitivity analysis [339]. This would be instead of the local sensitivity analysis as presented in this thesis, where we typically vary only one or two parameters while keeping others fixed. Sensitivity analysis requires many costly and time consuming simulations. Alternatively, it may be possible to formulate our model assumptions in a analytical framework. This can then be used to determine under which conditions the model obtains a certain configuration [340]. For instance, for which parameter values does a cell elongate? How does durotactic speed depend on the model parameters?

7.3 Refinement of cell-matrix coupling

To better be able to understand the the biological system of interest and to be able to validate a model, the model should represent the system well. However, because we have made various simplifying assumptions in our model, we did not perfectly describe

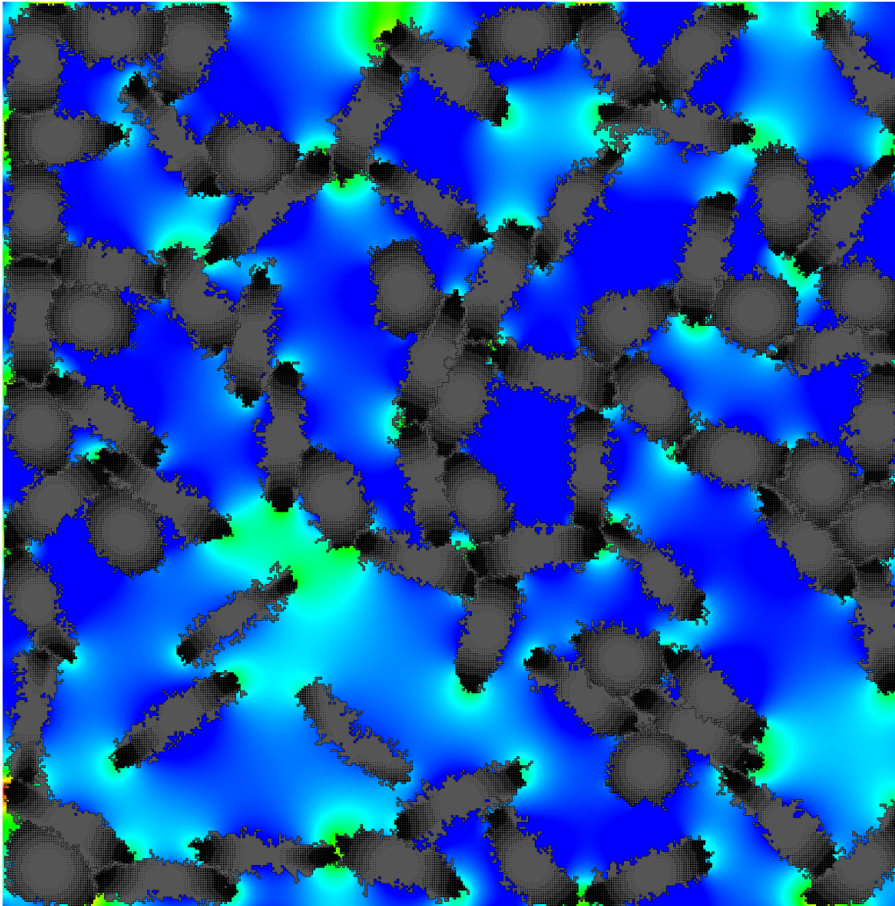


Figure 7.1: Network formation in the hybrid CPM-FEM model including focal adhesion dynamics, as presented in chapter 4. Cells apply a force on the matrix, which lets integrin clusters grow according to catch-slip bond dynamics. Matrix stresses generated by neighbouring cells stabilize these adhesions, so that the net movement of cells is towards each other and as cells elongate, they locally align and thus are able to form networks. Colors: hydrostatic stress.

the system. For instance, in chapter 2 and 3, we assumed that cells perceive a strain stiffening of the substrate. However, we did not let the matrix actually stiffen over time. After every timestep in the CPM, we apply a FEM to calculate the substrate strains, but assumed a homogenous stiffness and initially undeformed matrix every time. So, there was no memory of previous displacements in the FEM. However, as a first step, making the model as simple as possible allows us to better understand its behavior.

Another computational simplification was the decoupling of the cellular forces in the Hamiltonian of the CPM from the cellular forces in the FEM. Ideally, these forces should be equivalent in both compartments of the model. In our model, this is not the case as the cellular forces are described by a CPM Hamiltonian while the forces in the FEM are described using the FMA model. Albert & Schwarz [193] who also coupled the CPM with a FEM for the substrate, did use the same force descriptions in their CPM and FEM. They assumed that cells have a surface and line tension, which they described in a CPM Hamiltonian. For a surface and line tension, the corresponding forces in the FEM was formulated as follows. The surface tension results in a force normal to the cell membrane and the line tension generates an additional normal force proportional to the curvature of the membrane. In the FEM, the normal force on the CPM cell boundary was approximated using a marching square algorithm. Furthermore, the resulting force was smoothed by a kernel around the cell membrane. We could do a similar thing, but we chose not to do so in this thesis, since we wanted to maintain the description of the line tension as in the usual CPM formulation. Here, the adhesive energy between cells and the matrix describes a line tension, if the neighbourhood is large enough [256]. The benefit of this formulation, is that it also describes cell-cell adhesion and is very useful to study cell-cell interactions.

In order to test if it would improve our model, we developed a method to calculate forces from a generic Hamiltonian formulation in the CPM. The method is described in the Appendix of this dissertation. This method is based on that the energy difference ΔH describes a force in the direction of the copy movement. Figure 7.2 shows a force field for $H = \lambda A + \sum J$ and the corresponding substrate stress field, which we compare to the FMA model for cell traction forces. The downside of this method is, that because CPM movements are always horizontal, vertical or diagonal, the forces align to the lattice as well. So, a grid effect occurs. We could alleviate this by considering a larger neighborhood order for copy attempts in the CPM. Furthermore, since CPM movements only occur at the cell boundary, the forces are only described on the cell membrane (Figure 7.2). We could alleviate this issue by smoothing the forces to a region around the cell membrane, using a kernel, similar to what Albert & Schwarz did. Another option, would be to formulate our forces in the FEM (the FMA model) and then translate that to a Hamiltonian. However, this formulation does not separate a surface and line tension. In conclusion, there are currently too many unresolved issues with this method, so we decided to stick with the FMA model in this thesis. In order to have the same formulation for cell forces in the CPM and the FEM, more work should be done and we should outweigh the advantages and disadvantages of the different

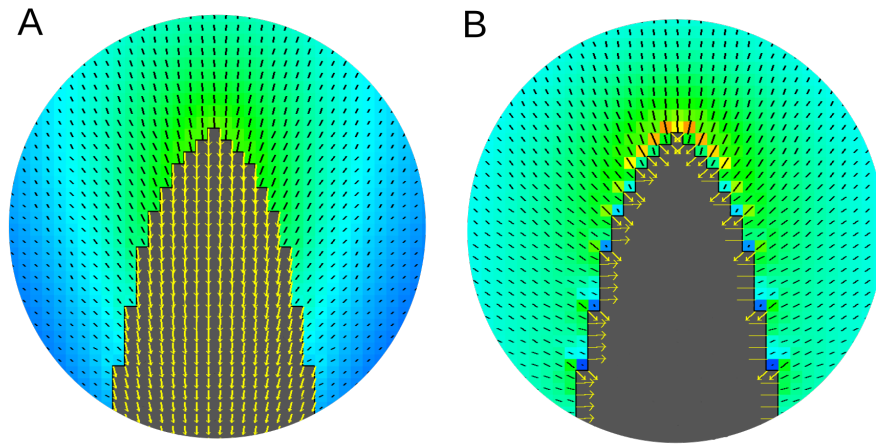


Figure 7.2: Cell forces around the tip of an elongated cell. (A) The FMA model as used in this thesis; (B) The traction forces derived from the Hamiltonian as presented in the Appendix of this dissertation. Colors: principal stress; orientation and length of black line pieces: orientation and magnitude of principal stress, yellow arrows: cell traction forces.

formulations that are possible.

7.4 Possible model extensions

In our modeling efforts we did not only make computational simplifications but also neglected various important known driving mechanisms of morphogenesis. An advantage of mathematical modeling is that we can focus on one specific mechanism, for instance matrix stiffness, while neglecting others. In this way, we can better understand how a specific factor influences the biological system. The disadvantage of such an incomplete model is that we can not explain all experimental observations, because we are missing important factors. Including more dynamics and interactions between cells, the substrate, cell-matrix adhesions and signaling factors can increase our understanding of a biological system. Here, we will give an overview of interesting aspects that can be included in our model and what it can be used for.

7.4.1 Matrix type and density

Because cell spreading does not only depend on matrix stiffness but also on other matrix properties, such as matrix type and density, it would be good to describe substrate mechanics in more detail. Cell spreading is typically increased on matrices with a higher density [124], or depends biphasically on matrix density [201]. To deal with

matrix density, we could model this phenomenologically, by relating model parameters, such as the growth rate of focal adhesions, to matrix density. We could also model this explicitly by including more detail in our substrate/FEM model. More detailed finite element models for the substrate have been coupled to circular discrete cells [89]. Coupling such models to a CPM will allow us to better understand how tissue respond to matrix mechanics on both a cellular level and tissue level. Cell spreading also depends on the type of matrix protein (*e.g.* fibronectin, collagen, laminin) that cells interact with [341]. Cells bind to different matrix proteins with different types of integrins. We could fit the catch and slip bond parameters of integrins in our model to those experimentally identified for the different integrins, to study how matrix type affects cell spreading.

7.4.2 Fibrous matrices

As the orientation of matrix fibers regulates cell migration [28], it is important to also take matrix fibers into consideration. Cells not only respond to matrix fibers, but also actively remodel matrix fibers. For instance, by applying forces on the matrix, cells can reorient matrix fibers [175]. Cells also deposit and degrade matrix fibers and even cross-link them [54]. Matrix fibers affect matrix stiffness and the extent of strain-stiffening [342], which in turn affects cell migration. So, intricate interplays between cells, fibers and matrix stiffness occur during morphogenesis. To study this, we should extend our model to include matrix fibers. More refined models of the extracellular matrix including matrix fibers are being developed. Matrix fibers can for instance be incorporated in finite element models [88, 343]. As fibers have the same spatial scale as cells, it might be more appropriate to model fibers using discrete approaches. For instance, fibers can be described by springs and the reorientation and cross-linking of fibers can be modeled [344]. Previous models including matrix fiber descriptions did not include cell shape [88, 90] or are computationally expensive for a larger number of cells [91]. By coupling matrix fiber models to our CPM framework, we can study the effect of long-range communication between cells through the matrix fibers on the tissue level. Furthermore, we could study how the interactions between cells, fibers and matrix stiffness regulate morphogenesis.

7.4.3 Chemical-mechanical interactions

Another interesting extension would be to combine models for chemical and mechanical signaling in cells. For instance, signaling of Rho-family GTPases in the cytoskeleton, which is involved in cell migration and cell polarization, regulates cell contractility and cell-matrix forces in turn feed back on Rho-signaling. It would be interesting to implement existing models of Rho-family GTPases signaling [148] and cell-matrix interactions [345]. Coupling such models to our multiscale model including focal adhesion, could give new insights on the molecular mechanisms behind cells polarization and migration as a result of mechanical and chemical cues [346]. There also exists

feedback between mechanical forces and growth factors in the extracellular matrix. For instance, the growth factor TGF- β is secreted by epithelial cells in an inactive form that subsequently can bind to the matrix [325]. Stretching of this bound molecule allows it to be released from the matrix in an active form, that can diffuse freely through the matrix [326]. On stiffer matrices, the matrix-bound TGF- β experiences more stress, which induces its release [326]. The release from TGF- β is of particular interest to fibrotic diseases [347] and branching morphogenesis. We could couple our CPM for branching morphogenesis to a FEM and include binding of TGF- β to the matrix and its release from the matrix [348] and study how matrix stresses and matrix stiffness influences branching morphogenesis. Activation of TGF- β can also occur in other ways than matrix stress [321]. The activation of TGF- β is for instance mediated by integrin binding [349]. Upon binding with integrin, the matrix stress activation of TGF- β is further promoted [350]. In turn, TGF- β feeds back on cell contractility [349]. It would be interesting to investigate such feedback dynamics with our model. Furthermore, it is possible that other growth factors can also be released from the matrix by mechanical forces [351]. So, modeling such dynamics can help explore the possible effects matrix-bound growth factors have on morphogenesis.

Another important aspect in tissue patterning is cell-cell signaling mediated by membrane bound signaling molecules. Interestingly, such signaling also depends on physical forces. For instance, internalization of the VEGF-R2 receptors and VEGF signaling is enhanced on stiffer matrices, most likely due to increased contraction [352]. Because the CPM allows to describe cell-cell membrane contact, our model may be used to study how such dynamics [352] affects the formation of vascular networks on compliant matrices. Furthermore, it has been shown that cell-cell cadherin adhesions, that mediate cell-cell signaling, can behave as catch-bonds [353]. Through cadherins, cells apply forces on each other and such forces can be transmitted through the whole tissue via actin stress fibers [27]. Actin stress fibers remodel and reorient in response to forces [177, 354]. The orientation of stress fibers regulates cell shape [355] and the direction of force application [356]. We could implement a model for stress fiber remodeling (see *e.g.* [357]) into our CPM. Due to our the multiscale nature of our model, such an approach may give insights into how feedback between forces, cell shape and stress fibers regulates cell response to matrix mechanics. On a tissue level scale, we could study how long-range intercellular cell-cell communication via forces drives morphogenesis.

7.5 Long term prospects

The type of multiscale modeling approaches as presented in this thesis may in the long run assist in developing new treatments. Here we will provide examples of some of the possibilities. For instance, our modeling approach could be used to help design tissue engineering approaches. By mechanically loading tissues, it is possible to align tissues [55]. Our model could help to investigate what experimental condition brings about

the desired tissue pattern. For instance, van der Schaft *et al.* [95] showed that a tissue consisting of a mix of muscle cells and endothelial cells aligned to uniaxial stretch and that the endothelial cells started to form lumen. Models can be used to better understand the effect of a mixture of different cell types on the tissue patterning. Furthermore, we could use our modeling approach to help understand how cell alignment depends on the type of stretching. Cells typically align parallel to static uniaxial stretch but perpendicular to cyclic stretch. Interestingly, cell alignment to cyclic stretch depends on the amplitude [358] and frequency of the stretch [359] and the stiffness of the substrate [177]. Other future directions are the response of tissues to biaxial stretch [360] and the effect of tissue geometry on tissue patterning [361].

Mechanical forces also play a crucial role in cancer. Physical forces on different scales regulate cancer progression. Changes in the mechanics of the extracellular matrix are associated with cancer metastasis [54]. Cancer cells are able to excessively remodel the matrix, inducing high matrix alignment and matrix stiffening, which directs cancer cell migration. Furthermore, matrix properties, like for instance matrix stiffness, regulates the malignant phenotype of cells *in vitro* [362]. Mechanical cell-cell interactions through cadherins have also been shown to drive collective cell migration *in vitro*, suggesting an involvement in cancer invasion [363]. The modeling methods presented in this thesis may be ultimately used to give insights into how cancer growth and metastatic potential are associated with cell and tissue specific parameters derived from tissue samples. Models can also be used to help develop new targets for cancer treatment. For instance, models that relate focal adhesion dynamics to tissue level dynamics can provide clues into how focal adhesions may be targeted to inhibit cancer progression. Furthermore, models can give insights into how cancer progression could be inhibited by manipulating the extracellular matrix.

In conclusion, due to the multiscale nature of our model, it is possible to include many interesting dynamics and due to its generic set up, it is possible to study many different experimental systems. These examples range from tissue engineering, cancer migration/invasion and various morphogenetic processes like cell sorting [337, 364], angiogenesis, branching, left-right patterning [365] and somitogenesis [366].

