Multiscale mathematical biology of cell-extracellular matrix interactions during morphogenesis

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Summary

During embryogenesis (the growth of an organism from a fertilized egg cell) cells proliferate, differentiate, and collectively migrate to form different tissues at the right position and time in the body. Throughout the lifetime of an organism, tissues reshape in response to disease, tissue damage, and changes in environmental conditions. In order to develop effective treatments for birth defects and diseases, we need a good understanding of the mechanisms behind morphogenesis (the shaping of tissues). An important contributor to morphogenesis is the extracellular matrix, a gel-like material containing an intricate network of fibers that surrounds cells in tissues. Cells can sense the mechanical properties of the matrix, such as its stiffness, by applying forces on the matrix. In response to matrix stiffness, cells change their shape and migratory behavior. Cells communicate with neighbouring cells by applying forces on the matrix that locally stiffen the matrix. Hence, tissue stiffness affects the organization of cells and tissues. A different mechanism in which the extracellular matrix support cell-cell communications is via the establishment of chemical gradients, because chemicals affect the intercellular signaling and the behavior of cells. In this thesis, I aim to understand the response of cells and tissues to matrix stiffness and matrix stresses. In chapters 2 to 4, I develop multiscale mathematical models that describe cells applying forces on and responding to the extracellular matrix. Such models help to understand how cell-matrix interactions on the mesoscopic level affects the behavior on a cellular or tissue level. Chapters 5 and 6 focus on cell-cell communication through gradients of extracellular proteins.

In chapter 2, I introduce a cellular Potts model, that describes cell shape and couple this to a finite element model describing the extracellular matrix. Using the cell shape, I model the force field that the cells apply on the matrix and the resulting strains in the matrix are calculated using the finite element model. The strain in the matrix then feeds back into the cellular Potts model since I assume that cell extend towards high matrix strains. I use this model to explain *in vitro* observations of angiogenesis on compliant gels. My model suggests that by communicating through strains in the matrix (1) cells form vascular-like networks on matrices of intermediate stiffness with dynamics that mimic endothelial cells on compliant collagen gels *in vitro*, and (2) cells sprout from a round aggregate, mimicking sprouting angiogenesis. Thus, by responding to strains in the matrix generated by other cells, simulated cells align to each other and can organize into networks or start sprouting from a blob.

In chapter 3, I extend this model to study how cells respond to a static uniaxial stress in the matrix. In vitro observations have shown that cells elongate in parallel to a unaxial strain. My model suggests that when cells apply a force on the matrix they can better elongate along the matrix strain compared to cells that do not strain the matrix. Also, simulated cells form strings of cells in parallel to uniaxial stretch if they communicate with each other by applying forces on the matrix. Furthermore, string formation in our model depends on cell density and cell-cell adhesion. This possibly explains why fibroblasts do organize into strings oriented along uniaxial strain while less-adhesive endothelial cells, seeded at a higher cell density, do not form strings. My model suggests that by applying forces, the cell locally amplify the uniaxial stretch, which results in string formation along the uniaxial strain because the local strains allow the cells to align to each other.

In chapter 4, I add dynamics at a molecular scale to our hybrid cellular Potts - finite element model to better understand how individual cells respond to matrix stiffness. I aim to explain three generic cell behaviors: (1) cell area increases with matrix stiffness, (2) cells elongate on matrices of intermediate stiffness, and (3) cell migration towards areas of higher stiffness (durotaxis). Cells are able to sense substrate stiffness through focal adhesions (large bundles of cell-matrix adhesion molecules), which are larger and more stable on stiff matrices. I model focal adhesions as clusters of cell-matrix integrin bonds, that have been observed to behave as catch-bonds: bonds of which the lifetime increases under force. I assume that (1) cells apply a force to the focal adhesions and that (2) the rate of force build-up becomes faster as matrix stiffness increases, (3) the likelihood of release of the focal adhesion bond from the matrix decreases with the size of the focal adhesion. My model suggests that on stiffer matrices, a more rapid build up of forces allows focal adhesions to grow, which increases cell-matrix adhesion and this allows a cell to spread. Based on that stretching of certain focal adhesion molecules exposes binding sites for other focal adhesion molecules that stabilizes cell-matrix binding, I furthermore assume that matrix strains enhances cell-matrix bonds. This additional mechanism allows cells to elongate on matrices of intermediate stiffness. Besides accurately reproducing the cell shape as a function of ECM stiffness, this model also reproduces durotaxis. Thus, this highly detailed model can explain three generic cell responses to matrix stiffness based on dynamics at a molecular level.

In chapter 5, I shift my focus to tissue patterning by chemical signaling in the extracellular matrix. I study the extracellular gradient of the Nodal protein Spaw which is known to regulate left-right patterning in zebrafish. A mutant with a lack of protein FurinA is observed to have defects in left-right patterning. To understand how FurinA regulates the formation of the Spaw gradient, I develop a 1D model that describes the dynamics of Spaw in a set of partial differential equations based on experimental observations. In this model, extracellular Spaw activates intercellular production of Spaw. Furthermore, FurinA promotes the cleavage of Spaw into its mature form, that can be secreted by cells into the extracellular space. The model reproduces the experimental observations that higher levels of FurinA promotes the propogation speed

and signaling range of Spaw. Thus, it validates the *in vivo* hypothesis that by cleaving Spaw, FurinA can regulate the extracellular gradient of Spaw.

In chapter 6, I study how a chemical signaling factor can drive tissue branching. I extend a cellular Potts model with a partial differential equation that describes secretion of an inhibitory signal and its diffusion and decay in the extracellular matrix. Based on experimental observations, I assume that the signal inhibits cell extensions. I propose that this allows a tissue to branch by means of the following curvature dependent mechanism. The level of the inhibitory signal is higher at parts of the tissue boundary that are concave. Therefore, extensions are made preferientially at convex sites. This onsets a positive feedback loop where cell extensions make convex sites even more convex, which allows for further extensions, allowing the tissue to fully branch out. The model thus suggests that autocrine inhibition of cell movement suffices to explain branching morphogenesis.

In this thesis, I aim to understand how interactions between cells and the extracellular matrix can drive morphogenesis. I study how cells and tissues can shape in response to matrix stiffness and matrix stretch by applying forces to the matrix. I find that when cells communicate via strains in the matrix can align to each other and form patterns like networks or strings. With a model that describes cell-matrix interactions on a molecular level, I am able to predict how individual cells respond to matrix stiffness. Furthermore, I study how cell-cell communication through chemical signals in the extracellular matrix can drive tissue patterning. Altogether, I use multiscale mathematical modeling approaches to better understand how cells and tissue obtain their shapes in response to physical or chemical signals in the extracellular matrix.