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COMMENTARY

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Modeling cell shape and dynamics on micropatterns

Philipp J. Albert and Ulrich S. Schwarz

Institute for Theoretical Physics and BioQuant, Heidelberg University, Heidelberg, Germany

ABSTRACT

Adhesive micropatterns have become a standard tool to study cells under defined conditions. Applications range from controlling the differentiation and fate of single cells to guiding the collective migration of cell sheets. In long-term experiments, single cell normalization is challenged by cell division. For all of these setups, mathematical models predicting cell shape and dynamics can guide pattern design. Here we review recent advances in predicting and explaining cell shape, traction forces and dynamics on micropatterns. Starting with contour models as the simplest approach to explain concave cell shapes, we move on to network and continuum descriptions as examples for static models. To describe dynamic processes, cellular Potts, vertex and phase field models can be used. Different types of model are appropriate to address different biological questions and together, they provide a versatile tool box to predict cell behavior on micropatterns.

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Introduction

Over the last 2 decades, physical features like cell shape and forces have emerged as determinants of cell fate that are equally important as are biochemical and genetic factors. It has been shown that cells do not need only nutrition and growth factors to survive and grow, but also a certain level of spatial extension and force generation. [1-7]

Most cells in animal tissue grow in an environment in which matrix and neighboring cells provide defined boundary conditions that result in a regular cell shape.[8,9] To study animal cells in detail, however, they are usually grown in cell culture on a 2-dimensional substrate. The lower dimensionality in cell culture compared to tissue makes it easier to image cells and many quantitative experiments would not be possible without the simplification that a 2 dimensional environment provides. As in physics, reducing degrees of freedom and studying systems under controlled conditions became an integral part of cell biology after the first cell culture systems have been established.

On the other side, however, cells can react very sensitively to changes in their environment. Removing the confinement and boundary conditions imposed by matrix and neighboring cells in 3-dimensional tissue and studying them in cell culture often results in cells which display a large variety of sizes and shapes. Without appropriate markers it is often very challenging to identify boundaries between cell or to determine their sizes. Experiments with structure-related readouts, such as the spatial organization of the actin cytoskeleton or the spatial distribution of cell organelles, are hard to quantify due to the inherent variability of cell shape in cell culture. The initial simplification by studying cells extracted from the complex tissue environment turns out to be a drawback when it comes to shape- and structure-related readouts.

Controlling cell adhesion through the extracellular matrix (ECM) by micropatterns (MP) allows to impose boundary conditions in cell culture and therefore brings back the ability to control their shape and structure. Different fabrication techniques exist to manufacture MP, e.g. in two and 3 dimensions, for dynamically adjustable MP and for MP on elastic substrates.[10-12]

Micropatterns allow precise control of where and how cells adhere. Pioneering work with MP has demonstrated that besides the amount of ECM ligand offered to a cell also its spatial distribution is important for cell survival.[1] For example, it is more beneficial for cell survival to deposit the same amount of ECM ligand as distributed small spots rather than as one connected island. The precise control of cell shapes allows to minimize variations inherent to biological systems as illustrated in Fig. 1. Seeding cells on an array of MP (Fig. 1A) results in a very uniform cell shape (Fig. 1B). In this example, cells are stained for actin. Aligning and averaging all cells reveals a strong localization of actin to the cell periphery,

CONTACT Ulrich S. Schwarz Ulrich.Schwarz@bioquant.uni-heidelberg.de Heidelberg University, Institute for Theoretical Physics, Philosophenweg 19, 69120 Heidelberg, Germany.

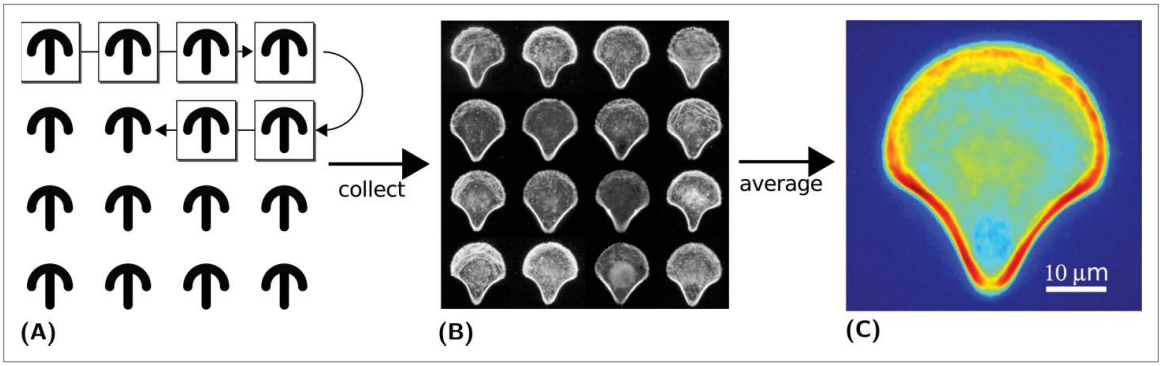

Figure 1. Cell normalization by micropattern. (A) Cells are grown on an array of isolated crossbow patterns. (B) Images of the single cells are collected (here HeLa-cells stained for actin). (C) By averaging the different images, a normalized or standard cell is obtained with prominent actin features at concave segments of the cell contour. The fluorescent intensities are converted to colors for better visibility. Image courtesy of Dr. Starkuviene-Erfle.

especially to the concave regions above non-adhesive areas (Fig. 1C). This approach resembles the mean field description often employed in physics to describe the average in a fluctuating system. Once this reference system is established, one can start to measure deviations from the mean.

MP are very versatile and have found many applications[13-15] relating form and function[16] of cells. They are also very helpful tools to study the growth of microtubules and actin in defined environments.[11,14] Of special interest with respect to modeling approaches are the effects of the adhesive geometry on the cytoskeleton,[17,18] cell polarization,[19-21] endomembrane organization[22,23] and traction forces.[24-27] They have also been used to study how the adhesive geometry influences the orientation of the mitotic spindle during cell division.[28-30] Cell migration on MP has also been studied extensively, including the bias of single cell migration by ratchet[31-36] geometries and the persistent rotational movement of small multicellular systems.[37-39] The influence of geometry on the collective behavior of larger multicellular systems such as cell monolayers has also be investigated.[40] Cells were found to propagate alignment to pattern edges over several hundred of micrometer into the pattern.[41] Removable barriers were used in wound healing assays to study the formation of leader cells[42] which was found to be increased in regions of high curvature.[43] The removable barrier approach has also been combined with defined adhesive geometries to study cell migration into channels of different width,[44] bridge formation[45] and wound closure above non-adhesive regions.[46,115]

These examples show that studying cells on micropatterns leads to a wealth of quantitative data. However, in order to make sense out of these data, one needs to develop appropriate concepts and mathematical models. During the last decades a lot of attention was devoted to understanding the interaction between cell adhesion, shape, function and dynamics through models.[47,48] This review focuses on how models can benefit from the simplification that MP provide and how they contribute to our understanding of cell mechanics.

Static cell shape models

Single cells on MP tend to become very flat, with only the nucleus sticking out into the third dimension. As long as the nucleus does not play an important role for the biological questions being addressed, it is therefore appropriate to start with a 2-dimensional description of cell shape. The simplest approach is to focus on the shape of its contour. We first discuss this approach using cell spreading above non-adhesive regions of concave MP (as shown in Fig. 1B) as an instructive example. This invaginated shape persists on dot patterns as shown in Fig. 2A where actin again strongly localizes to the invaginated contour.

The invaginated shape is a consequence of the contractility generated in the actin cytoskeleton. The actin cortex beneath the membrane pulls the contour inwards while stress fibers forming in the cell periphery resist the contraction and pull the contour outwards. The resulting arcs are circular as shown in the bottom part of Fig. 2A. The exactly circular shape can be understood in terms of the different tensions.[49-51] This is similar to the spherical shape of soap bubbles explained by the Laplace law. For soap bubbles a surface tension acting in the membrane tries to minimize its surface area and is balanced by the internal pressure. The Laplace law $R = 2\sigma/p$ describes how surface tension σ, soap bubble radius R and pressure p are related. When this concept is applied in 2 dimensions, the surface tension is replaced by a line tension λ and the pressure by a surface tension σ as illustrated in Fig. 2B. For a constant line tension the radius is then given by $R = \lambda/\sigma$.[49] Note that the factor of 2 vanishes

because we work in 2 rather than 3 dimensions now and that in contrast to the soap bubble, this situation now is only stable as long as we have the adhesion sites being present so that the 2 tensions can work against each other. This simple tension model suggests that circular arcs appear because both line and surface tensions are constant. A non-circular shape can only be achieved by spatially variable tension or if the surface tension does not act normal to the contour.

Detailed quantitative analysis of the arc radius revealed a correlation with the distance d between the anchoring points (see Fig. 2C) which cannot be explained with the simple tension model. However, it can be explained by an additional contribution to the line tension that arises from elastic stretch in the contour (tension-elasticity model).[49] Such a line tension depends on d through the contour length, but is constant for any given d, thus leading again to circular arcs. Arcs with attachment points spaced further apart have a higher line tension, making their radius larger, as seen in Fig. 2A.

Surface and line tension are antagonists when it comes to cell area of concave cells. Reducing them by myosin inhibition revealed that arc radius can either decrease (line tension relaxes stronger)[49] or increase (surface tension relaxes stronger)[52] depending on the experimental details. The simple tension and tension-elasticity model cannot only explain cell shapes but also traction forces.[53] Combining the MP approach with traction force measurements allows to quantify surface and line tension during myosin inhibition and supports the idea of an elastic contribution to the line tension.[52]

The simple tension and tension-elasticity models are the simplest type of models as they only consider the contour of the cell. At the next higher level of description, one would like to include details of the force-generating cell interior. This is achieved e.g., by contractile network models[54-56] with examples shown in Fig. 2D. The links in these networks are assumed to be contractile bundles of actin filaments. To obtain cells with circular arcs the links must fulfill 2 conditions. Firstly, they need to respond asymmetrically to length changes. When compressed, they need to buckle and when extended, they need to behave like Hookean springs. Secondly, actomyosin contractility needs to be described by a constant pull between neighbors.[49,56] Then the predictions of the tension-elasticity model agree well with this active

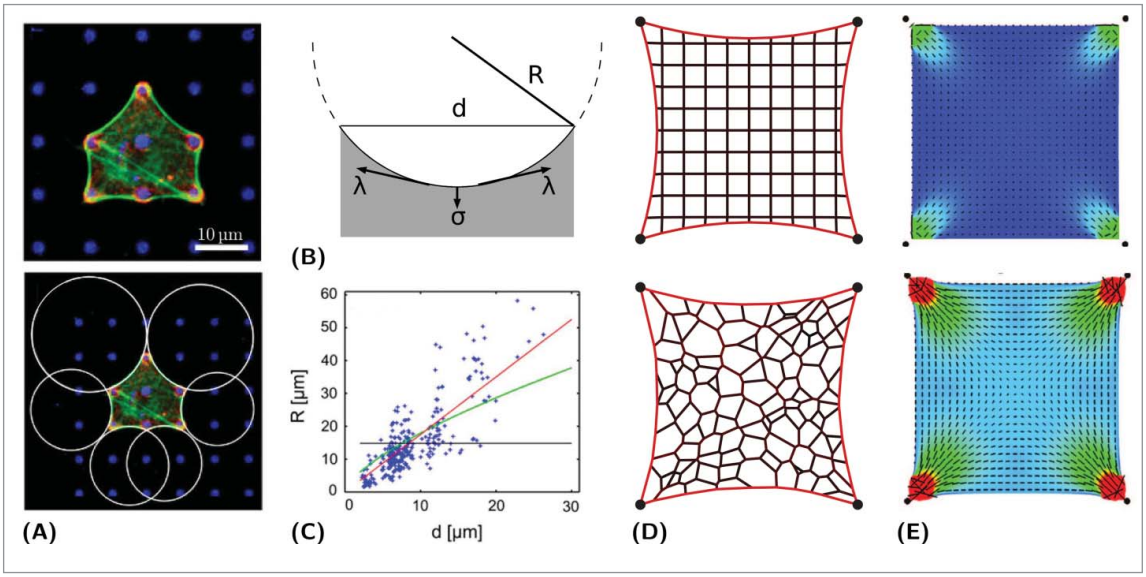

Figure 2. Cell shape on flat substrates with pinned adhesions. (A) Cell on fibronectin dot pattern (blue) stained for actin (green). Prominent stress fibers are visible at the edge of the cell. The same cell is shown in the bottom part with circles fitted to the arcs. Reprinted from ref. 49 with permission from Elsevier. (B) Part of the cell contour (gray) pinned between 2 points with distance d. The surface tension σ and line tension λ result in a circular contour with radius R. (C) The radius R increases with spanning distance d. The solid lines are different predictions by the simple tension and tension elasticity models. Reprinted from ref. 49 with permission from Elsevier. (D) Cable network model representations with rectangular (top) and irregular (bottom) networks. The cells are pinned at the 4 corner and the network links represent contractile actin bundles. For active contractile cable models the cell contour is circular. Reprinted with permission from ref. 56, © 2012 by the American Physical Society. (E) Cells represented in a thermoelastic model. The cells are elastically connected to the substrate at the 4 corners. In the top and bottom parts the elastic anchorage is soft and stiff, respectively. The color code and the lines indicate actin stress fiber activity and direction, respectively. Reprinted with permission from ref. 71, © 2006 National Academy of Sciences, U.S.A.

contracting cable model. Surprisingly, the exact network architecture is not relevant for these results. [56]

An alternative to describe contractility by a constant pull is to treat the links as Hookean springs with a reduced resting length.[54,55] However, these passive cable networks result in flat non-circular contours.[56] If the links are treated as Hookean springs under compression, compression energy is accumulated in the bulk and again circular contours do not emerge.[49,56] Network models are not restricted to MP, they can also be applied to cells on continuously adhesive substrates, but this requires knowledge of the spatial arrangement of adhesion sites and stress fibers.[57-59] In the future, these models might be combined with more detailed models for contractile networks.[60-63]

An alternative approach to network models are continuum models which lack discrete elements and are often implemented with finite element methods. A method commonly used to describe contractile cell monolayers is thermoelasticity.[64-66] Cells are treated as passive elastic material in this approach and contractility is introduced by lowering its temperature. This approach can be justified more rigorously by active gel theory that leads to the same negative pressure term in the force balance as in thermoelasticity.[67] In its simplest variant, this model does not describe the anisotropy of contraction and therefore is most appropriate for large scale systems, such as cell monolayers in which polarization of the single cells is averaged out. It has been used to predict traction forces for multicellular systems[64,65] and single cells[68,69] on MP.

The thermoelastic approach can be extended to account for anisotropic contraction generated by stress fibers.[70,71] Stress fibers are treated with a continuum description meaning that at every point of the cell a value for the contractile strength in dependence on the contraction direction exists. Examples are shown in Fig. 2E where the color encodes the strength of stress fibers and the lines indicate the main contraction direction. The strength of contraction depends through a positive feedback loop on how much tension the fibers can build up in a specific direction. This means that stress fibers along directions with no resistance (e.g. in horizontal or vertical direction for cells pinned at corners as in Fig. 2E) disassemble because the contour retracts offering no resistance. In diagonal direction the contour cannot retract, stress fibers can build up tension and become stronger due to the positive feedback. Likewise, a mechanosensitive response to soft substrates is included in this model. The pinning points in the corners of Fig. 2E are anchored to the substrate by springs of different stiffness. With soft coupling as in the top part of Fig. 2E stress fibers have no resistance and cannot build up tension. As a consequence they disassemble. Only with a stiffer anchoring foundation as in the bottom part of Fig. 2E appreciable levels of stress fiber activity are reached. This model allows to predict both traction forces and distribution of stress fibers for cells on MP. Extensions allow to predict the assembly of focal adhesions with a similar feedback mechanism[72] on MP and traction forces on pillar arrays.[73]

Thermoelastic models usually assume a linear elastic material law, meaning that cells react in the same way to compression and extension. For a tissue this is a reasonable assumption because the volume of cells is essentially conserved and compression is equally important as is extension. However, for single cells adhering to a substrate this constraint is not as strict. Isolated cells can change their attachment area by exchanging material with the third dimension. This means that they offer little resistance to compression (e.g., generated by a increased contractility in the contour) because they can just become smaller and thicker. In fact, this reflects the idea behind contractile cable networks which buckle, slide or disassemble under compression. A linear elastic material is analogous to a Hookean spring network in this regard and results in non-circular flat cell contours as shown in Fig. 2E. A circular shape can be obtained by adding a cortical tension[74,75] which predicts correctly the observed correlation between arc radius and spanning distance[49] or by introducing a bending for cortical actin bundles reflecting their resistance to curvature changes.[76]

Dynamic cell shape models

The static nature of the above models prevents them from describing dynamic processes such as cell spreading,[17,77] migration,[32,31] division[28-30] or the movement of cell pairs[37,38] which have been investigated in great detail on MP. They are also not able to predict cell shapes if the final attachment points are not known, e.g. on MP which are too large for a single cell to be fully covered. The cortical tension described in the previous section responsible for the cell shape is also important for tissue growth. By using 3-dimensional scaffolds it has been demonstrated that tissue growth is faster in concave regions where the cortical tension results in an outward directed force compared to convex regions.[78,79]

Many models describe the effects of different tension and other cell properties by an effective energy function which depends on cell shape. Standard approaches to address cell dynamics and shape in 2 dimensions are cellular Potts models (CPM), vertex models and phase field models. The most common choice for the energy

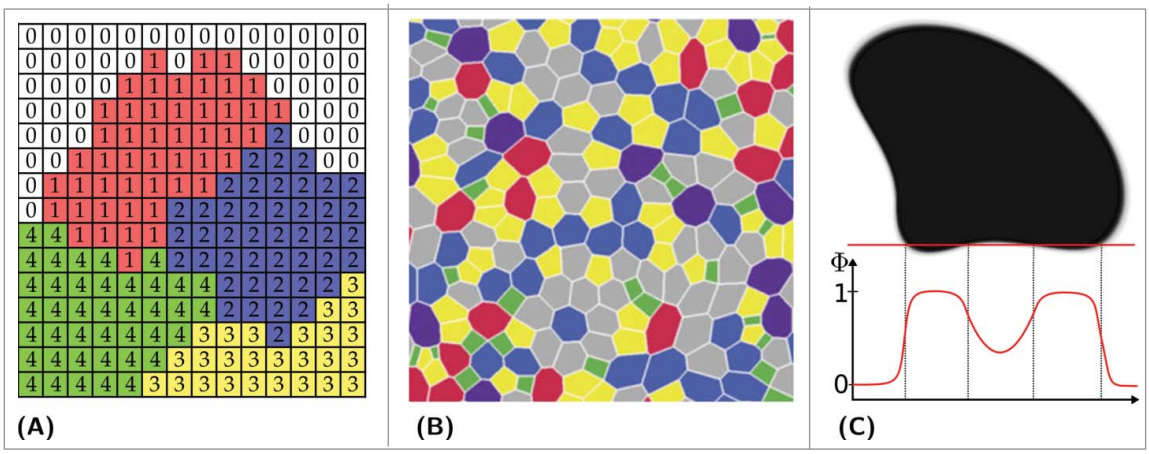

Figure 3. Dynamic cell shape models. (A) Two dimensional lattice based cell representation in a cellular Potts model. All lattice sites with the same spin label (indicated by a number and color) belong to one cell. The medium surrounding cells is labeled with 0. (B) Vertex model representation of tissue. Each face surrounded by edges represents a cell. The edges usually meet in 3-fold vertices. The color of the cells encodes the number of neighbors. Reprinted from ref. 81, © 2007, with permission from Elsevier. (C) Phase field model representation of a keratocyte. The phase field Φ describes an evolving contour. Inside the cell the field is equal to $\Phi = 1$ and outside it is $\Phi = 0$ with the cell boundary at $\Phi = 1/2$. A cut through the cell is visualized in the lower part of the figure.

function used in this context is

$$E = \frac{1}{2}\alpha(A - A_0)^2 + \frac{1}{2}J(P - P_0)^2 \qquad (1)$$

Here, E denotes the energy of a single cell, A its area and P its perimeter. If the cell area deviates from the target area A_0 the energy is increased. Due to the quadratic form it does not matter if the cell area is smaller or larger than the target area for the energy increase. The same holds if the perimeter deviates from the target perimeter P_0. The elastic constants α and J control how strongly deviations are penalized. Using quadratic terms in Eq. 1 essentially describes the cell as an elastic solid, because forces associated with area or perimeter changes scale linearly with the change (forces are proportional to the derivative of Eq. 1 with respect to the area or perimeter). CPM and especially vertex models where developed for multicellular systems where each cell contributes with an energy as defined in Eq. 1. The total energy is the sum over all single cell energies plus interaction terms discussed below. It is now assumed that the optimal cell shape and dynamics is found by minimizing this

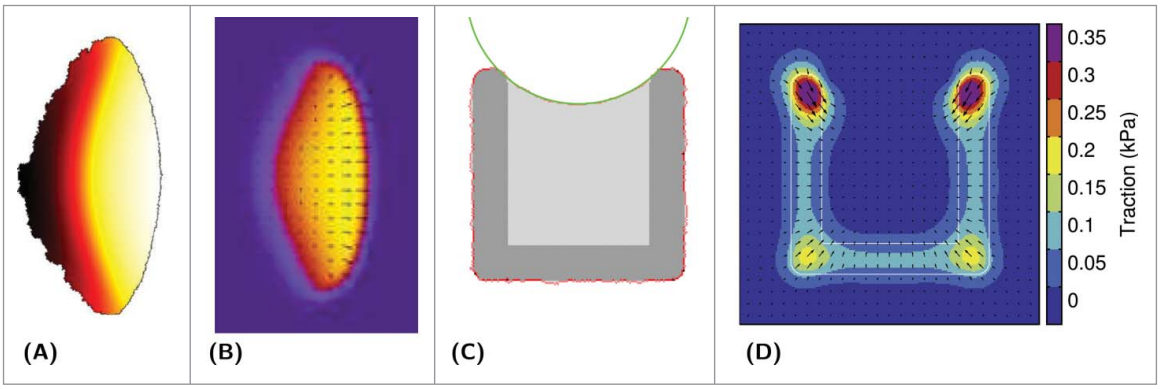

Figure 4. Dynamic models in action. (A) Keratocyte crawling to the right described by a CPM. Migration is driven by a reaction diffusion system of the small G-proteins solved in the domain defined by the cell. The color indicates Arp2/3 concentration which is at a maximum at the leading edge. Reprinted from ref. 100, © 2006, with permission from Springer. (B) Keratocyte crawling to the right described by a phase field model. The color indicates the value of the phase field ranging from 1 inside the cell to 0 outside. The arrows indicate the actin orientation field. Reprinted from ref. 118, © 2014, with permission from Springer. (C) Cell on a U-shaped pattern represented with a CPM.[109] The circular contour at the concave edge is indicated by a circle. (D) Traction force predicted with a CPM on a U-shaped pattern. The force is higher at the top corners where the actin stress fiber spanning across the non-adhesive area adheres to the substrate and increases the traction force.

energy. How this minimization is performed is one of the main differences between CPM, vertex and phase field models.

CPMs originate from the Potts model which in turn is a generalized Ising model. The Ising model describes interacting magnetic spins arranged on a lattice. The spins can point up or down and an energy is assigned to each spin which depends on the relative orientations of the spins. For example, for a ferromagnet 2 neighboring magnetic spins want to point in the same direction. The system is usually coupled to a heat bath meaning that the spins can be flipped by thermal energy and the energy landscape can be explored by Metropolis dynamics. In a CPM cells are represented on a lattice as illustrated in Fig. 3A. Each lattice site belonging to a cell is labeled by a number. The area used in Eq. 1 is then proportional to the number of lattice sites belonging to one cell. There are several methods to define a perimeter on a lattice which go beyond the scope of this review. They usually involve summing of the neighborhood of a lattice site[116] and are similar to anti-aliasing in image processing. With an appropriate lattice resolution arbitrary shaped cells can be represented. Shape changes are achieved by changing the indices of lattice sites.

Vertex models represent cells as polygons with straight edges as illustrated in Fig. 3B. Shape changes are achieved by moving one of the vertices. They cannot account for arbitrarily shaped cells because of the straight contour of the edges and are therefore of little interest to describe single cells on MP. They are usually employed on a larger scale of several hundred of cells where the detailed shape of single cells is of little interest. They are equivalent to a CPM when the cell contour has a infinite bending rigidity (except for points where 3 or more cells meet). CPM and vertex models predict the same cellular organization for a two dimensional tissue which is determined by the mechanical properties of individual cells.[116]

The phase field approach originates from the fields of crystal growth and solidification. Solidification problems

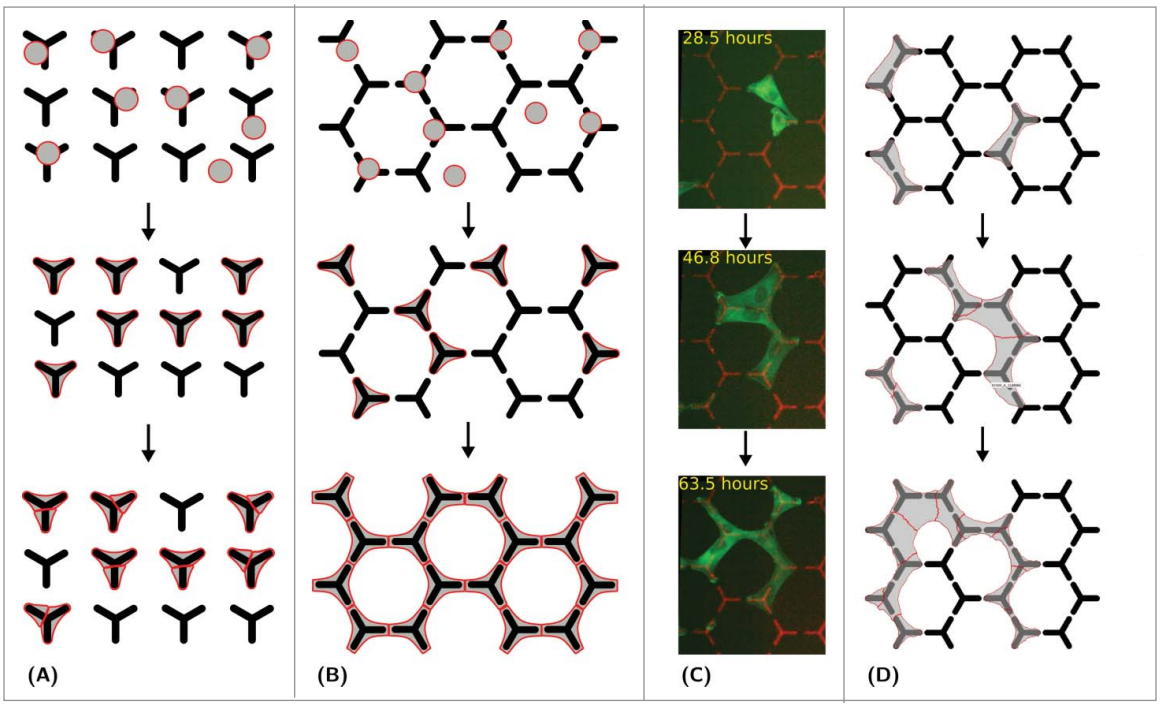

Figure 5. Cell shape on micropatterned networks. (A) Schematics for isolated micropatterns. Spherical cells initially suspended in a medium make contact with the substrate and attach to adhesive micropatterns (top). Cells without contact to patterns die and cells with contact spread over the pattern. The micropatterns normalize the cell shape (middle). Cells start to divide and with neighboring patterns to far away 2 cells are stuck on one island (bottom). The normalization ceases, the cell pair might start to rotate or oscillate, and the cells might be in an unfavorable condition because they have too little area to spread. (B) Schematics of a micropatterned network (here a honeycomb network). In this illustration cells make contact and adhere (top) and their shape gets normalized (middle). After cell division the daughter cells can migrate to neighboring patterns (bottom). The cells adhere to each other but stay normalized even after cell division. (C) Experimental realization of HeLa cells on honeycomb pattern. Cells spread across several patterns and cells in contact can bridge large nonadhesive areas. The cells are not normalized by the honeycomb layout. Image courtesy of Sebastien Degot and Yoran Margaron. (D) CPM-simulation on honeycomb network. As in experiments cells initially spread over several patterns and migrate to neighboring patterns. Cells in contact spread above non-adhesive areas. Thus the cell shape is indeed not normalized by the honeycomb layout.

deal with the interface between 2 phases which, when applied to cells, is the interface between cell and surrounding medium. Solidification problems usually require the solution of a diffusion equation for particles or heat in an evolving domain which is difficult because the domain boundary needs to be tracked. Phase field models soften the sharp domain, replacing it by a continuous field $\Phi(x,y)$ depending on the spatial position as illustrated in Fig. 3C. It separates the interior of a cell (at $\Phi = 1$) from the exterior (at $\Phi = 0$) with the cell boundary at $\Phi = 1/2$. The cell shape and mechanics are determined by a free energy which is a function of the phase field. To drive the system into 2 separated phases a double well potential with minimum at $\Phi = 1$ and at $\Phi = 0$ are part of the free energy. It can also contain terms to constrain the cell area similar to Eq. 1. A phase field model has the advantage that reaction diffusion dynamics, e.g., for proteins involved in cell migration, can be easily incorporated. Evolution of the cell shape is usually carried out with overdamped dynamics.

The shape of cells described by Eq. 1 is determined by the relative values of the target area and perimeter and the elastic constants. The perimeter term wants to make the cell circular since this is the shape with minimal perimeter to area ratio. For a cell monolayer, the optimal shapes are hexagonal having a minimal perimeter to area while the whole surface is covered. However, given a target area A_0 there is only one perimeter P_c matching the corresponding circle. If the target perimeter P_0 is now chosen larger than P_c the shapes cannot be circular any more. In the case of a CPM the cell contour would roughen to increase its perimeter. This is not possible in a vertex model due to the straight contour and cells become elongated and more ellipsoidal. Cells with a perimeter smaller than the target perimeter can have a negative line tension meaning that the cortex at cell-cell contacts pushes outwards to elongate the cell perimeter. The biological mechanism behind that is not clear because cell-cell contacts are usually assumed to be contractile. Pushing outwards should result in buckling of the contour which is not possible due to the infinite bending rigidity in a vertex model. Therefore, care has to be taken when models claim that morphological changes are observed when parameters are changed as this can be due to a negative line tension. It is interesting to note that Eq. 1 is often written in the form

$$E = \frac{1}{2}\alpha(A - A_0)^2 - JP_0P + \frac{1}{2}JP^2 \qquad (2)$$

where an irrelevant constant term has been dropped. The line tension is the derivative with respect to P. Thus it scales like $(P\text{-}P_0)$ and becomes negative if the perimeter P is smaller than the target perimeter P_0 (P_0 can be negative, in this case the line tension is always positive).

Vertex models have been used to investigate how cell growth is controlled in the *Drosophila* wing imaginal disc[80] and how cell sheet packing is influenced by cell elasticity, interaction and division.[81-83] They have also been used to study cortical tension in surface and bulk cells[84] and to quantify tension from images of cell sheets.[85] However, due to their coarse shape description with straight contours they cannot be used to describe cells on MP.

CPMs can account for arbitrary shapes and therefore are very well suited for modeling cells on MP. They have been originally developed to study cell sorting as proposed by the differential adhesion hypothesis.[86,87] The original CPM used a energy function Eq. 2 with only a linear term in the cell perimeter. The perimeter term controls then the interaction strength between different cell types and drives cell sorting. The quadratic perimeter term as used in Eq. 2 was introduced later to allow for negative interaction energies between cells.[88] There are also variants without area constraint.[89] CPMs have found a wide range of applications[90] due to their flexibility in including different types of cell-cell interactions or stimulus by external cues.[91] They have been used to study gastrulation of zebrafish embryos,[92] cell packing in the *Drosophila* retina[93] or cell mitosis.[94] To study collective migration of cell sheets CPMs often use a velocity alignment model which takes memory effects and polarity alignment of interacting cells into account. This variant has been successfully used to study cell-ECM invasion,[95] T-cell migration into lymph nodes,[96] tumor growth and invasion,[117] and the transition to collective motion[97,98] matching experimental observations for the formation of swirls on circular MP.[99] Velocity alignment is a coarse grained mechanism to describe cell motility and CPMs allow for a more detailed description of the motility mechanism.

The next more detailed level of modeling is the representation of the actin machinery driving motility by a spatially dependent field inside the cell. This field is influenced by other cells mimicking contact inhibition and past movements of the cells. With the assumption that breaking existing cell-cell contacts is dissipative, this model successfully reproduces the dependence of the rotational persistence time on colony size for cells on circular MP.[39] CPMs also allow a very detailed description of the migrating machinery as demonstrated for keratocytes.[100,101] The coupling of the acting machinery to the cell shape works by introducing an energy bias in regions with high actin polymerization in favor of membrane extension. Actin dynamic is driven by a reaction diffusion system of the small G-proteins where Cdc42

activates Rac which then activates Rho. Cdc42 and Rho inhibit each other. The mutual inhibition of Cdc42 and Rho can lead to a front back polarity in a cell as shown in Fig. 4A with actin polymerization being promoted by high Cdc42 concentrations at the front while contraction at the back is promoted by Rac. Cell shape, polarity, speed and reactions to external gradients are correctly predicted by this model. As the reaction diffusion system is solved in the domain predicted by the CPM simulations, the interaction between shape and small G-Protein dynamics can be studied, e.g. how shape changes help cells to react to external stimuli or how cells interact with obstacles.[101] A similar level of detail can be achieved with phase field models because they are particularly suited to describe a moving contour driven by internal reactions. Keratocyte movement,[102] (with an example shown in Fig. 4B), traction forces,[103] and the effects of collisions[104] and contact inhibition for cell movement on MP[106] have been studied with them.

The migration of single cells and cell pairs on MP has also been addressed with particle-based models where cells are represented by a point-like object. On ratchet-shaped MP a persistent random walk model successfully reproduced the biased movement into one direction.[32,31] Coupling 2 cells performing a persistent random walk demonstrated that rotational motion of cell pairs depends on the coupling strength and persistence time of the individual cells.[37] An active Brownian particle model has demonstrated that the persistence of cells is increased on stripe-shaped MP.[105] Particle-based models can also address cell shapes when a cell consists of multiple particles.[107]

On a single cell level, CPMs where also applied to study the shape of cells on MP.[108] The interaction with the pattern is achieved by lowering the cell energy in Eq. 1 when the cell is in contact with the pattern mimicking the formation of adhesion contacts. Recently, a CPM has been combined with the tension elasticity model to predict traction forces and spreading dynamics on MP.[109] In this implementation of the CPM the cell area is not constraint as strictly as in Eq. 1. Cells can increase their area by taking new material from the third dimension. Stress fibers forming at the cell periphery are treated explicitly with an example shown in Fig. 4C resulting in the correct dependence of arc radius on spanning distance predicted by the tension elasticity model. In contrast to the tension elasticity model the final cell shape can be predicted on arbitrary MP with this approach. An example of the traction forces predicted by this model are shown in Fig. 4D. Traction forces can also be predicted for multicellular systems in combination with feedback from substrate deformations. Here, cells prefer to extend into directions where the substrate is stiffer which can correctly describe network formation of endothelial cells.[110]

The energy based approach used for CPM vertex and phase field model usually neglects any internal cell organization. Hybrid models exist which explicitly describe internal stress fibers, lamellipodia, fillopodia and detailed focal adhesion assembly.[111-113] However, due to their detailed character those models require many parameters which can often only be adjusted phenomenologically.

Toward rational MP design

The combination of MP and modeling is a very powerful method to obtain quantitative results about the underlying cell mechanics. MP have also strongly contributed to our understanding of cell migration, especially in combination with removable barriers. Yet, when it comes to the construction of a normalized cell as described in Fig. 1, cell division poses a large challenge for the MP approach. Many experiments require cells to remain on a substrate for a long time, e.g., for RNAi-screens.[23,22] The time required to transfect the cells with siRNA is usually longer than the cell division cycle. As they start to divide, special pattern layout is required to ensure shape normalization. Seeded on isolated MP island the cells would loose normalization as illustrated in Fig. 5A. To maintain cell normalization even after several rounds of cell divisions, networks of MPs as shown in Fig. 5B are required. One cannot know if the cells divide in the right orientation such that one daughter cell is able to move on to the next pattern. Similarly, it is not known in advance if 2 cells positioned on the same island are stable or start to migrate or even if a single cell already migrates to the next pattern. In fact, placing HeLa cells on honeycomb layouts reveals that they provide no normalization effect as shown in Fig. 5C. The cells migrate from one island to another and they form cell-cell junctions allowing them to spread into the non-adhesive regions. Experimentally going through many different network layouts to find a working one would take too much time and resources. The models described above can help to predict the normalization effect that a given layout has on cells as illustrated in Fig. 5D. Here, a CPM was used in combination with a velocity alignment model to drive cell migration resulting in cells behaving very similar to experiments. In combination with insights for optimal seeding strategies[114] and appropriate optimization techniques, pattern layouts can be designed which ensure cell normalization after several rounds of cell divisions. Thus the combination of MP and modeling can not only lead to new and important insights into the underlying biological mechanisms, but also to useful applications.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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