Pattern formation and force generation by cell ensembles in a filamentous matrix

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Abstract Adhesion-dependent soft tissue cells both create and sense tension in the extracellular matrix. Therefore cells can actively interact through the mechanics of the surrounding matrix. An intracellular positive feedback loop upregulates cellular contractility in stiff or tensed environments. Here we theoretically address the resulting pattern formation and force generation for the case of a filamentous matrix, which we model as a two-dimensional cable network. Cells are modeled as anisotropic contraction dipoles which move in favor of tensed directions in the matrix. Our Monte Carlo simulations suggest that at small densities, cells align in strings, while at high densities, they form interconnected meshworks. Cellular activation both by biochemical factors and by tension leads to a hyperbolic increase in tissue tension. We also discuss the effect of cell density on tissue tension and shape.

1 Introduction

During recent years, mechanical tension has emerged as an essential and unifying organizing principle spanning both cell and tissue scales [1]. All adhesion-dependent cell types from soft tissue are contractile and thus create tension in the surrounding extracellular matrix. The molecular basis of cellular contractility is the actomyosin system, which can be inhibited by e.g. blebbistatin, a specific inhibitor for the myosin II motor. In a typical cell culture experiment, the actin cytoskeleton tends to organize into contractile bundles, so-called stress fibers, which terminate at mature cell-matrix adhesions, so-called focal adhesions. This system can be con-

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sidered to act as a little muscle specifically assembled to deform the environment. In some cases, this deformation serves a specific physiological purpose, like e.g. wound closure after injury. However, a growing body of evidence shows that the system of stress fibers and focal adhesions also functions as a force sensing apparatus [2]. The exact details of this sensing mechanism are still unclear, although most likely there are several mechanisms acting in parallel, including mechanical unfolding of proteins, changes in the spatial coordination of enzymatic reactions and opening of stretch-sensitive ion channels [3]. Because cells both create and sense forces, they are able to actively sense the stiffness of their environment. By culturing cells on elastic substrates of varying stiffness, it has been shown that indeed many essential cellular processes depend on the actively sensed extracellular stiffness, including adhesion, migration, proliferation and differentiation [4]. An impressive demonstration of the importance of stiffness sensing is the fact that rigidity-dependent differentiation of mesenchymal stem cells is prevented by administering the myosin II inhibitor blebbistatin [5].

The combination of force generation and sensing common to adhesion-dependent soft tissue cells suggests that cells might interact mechanically through the mechanical properties of the extracellular matrix. In order to model the elastic interaction of cells, one can make use of concepts developed before for physical defects in deformable media [6, 7, 8]. In particular, it has been suggested that the minimal system representing a mechanically active cell, namely one stress fibers anchored at two focal adhesions, can be modeled as an anisotropic force contraction dipole [9]. In contrast to physical force dipoles, however, cells are characterized by a rich internal structure, including complex feedback loops coupling cytoskeletal mechanics and signal transduction [10]. Therefore the system of cells and matrix cannot be expected to minimize the total energy, as it is usually assumed for systems of passive defects in deformable media. Instead it has been suggested that most adherent tissue cells (including fibroblasts, smooth muscle cells and endothelial cells) effectively behave as if they minimized the elastic energy which they invest into the matrix deformation [11, 12]. This extremum principle provides a good starting point for a theoretical analysis and predicts that cells orientate in the direction of large stiffness or tension, exactly as observed experimentally [13, 14]. Using this extremum principle, one can explain many other observations made experimentally, including their parallel and perpendicular orientations close to free and clamped boundaries, respectively. The mechanical feedback loop leading to cell activation can be described theoretically using the concept of susceptibility tensors for polarization, which also allows one to include the effect of cell shape [15, 16]. In order to address dynamical situations like cell reorientation dynamics under cyclic substrate stretch, an overdamped dynamics has been formulated based on an energy functional which assumes that cells tend to maintain an optimal stress in the matrix [17, 18].

Given the importance of tension for single soft tissue cells, it is not surprising to find that tension is also a major regulator of tissue. Tension has long been implicated in cell growth in tissue, in particular during development [19]. Using microcontact printing to control tissue shape and tension, it has been shown that cell proliferation

is enhanced in regions of large tension [20]. It has been found that osteoblast growth depends on the curvature of the substrate, possibly through forces being developed in the tissue [21]. Mechanical feedback has been suggested as a possible explanation for the observed uniform growth in the Drosophila wing disc [22]. Mechanical tension has also been implicated as a major determinant of cancer progression. Tumors were found to correlate with upregulated contractility and a stiffer environment, with dramatic consequences for invasiveness [23, 24].

In order to reveal the underlying mechanisms, experimental models have to be developed for cell-matrix interactions in tissues. It has been shown early that collagen-cell mixtures result in tissue-like structures under large tension [25, 26]. In order to measure the tension developed in these systems, different versions of a cell force monitor have been developed [27, 28]. In these studies, it was found that tension always builds up in a hyperbolic fashion, similar to the build-up of force by single cells [29]. Recently it has been shown that the shape of spatially constrained model tissues of this kind provide evidence both for the presence of tension and the fibrous mechanics of the system [30]. It has also been argued that the response of the polymer matrix to cell traction is strongly determined by its non-linear properties [31].

Given the essential role of tension for the behaviour of single cells and for the homeostasis of tissue, one might expect that the tension developed in tissue can be explained by the tension-dependent activity of the dispersed cells. When going from the level of single cells to the tissue level, however, one has to take care to consider possible collective effects. Indeed it has been shown with elastic substrate work that a certain degree of substrate softness is required for cells to feel each other during tissue formation [32] and migration [33], possibly because elastic interaction through the substrate is the main mode of cellular interaction in this case. This interpretation is supported by the observation that for cell-collagen mixtures, a critical threshold in cell density is required for gel compactification to occur [31]. Theoretically it has been suggested before that collective effects of mechanically interacting cells will lead to phase transitions as a function of cell density and material parameters [34]. However, in this work the mechanical properties of the matrix have been described by isotropic linear elasticity, which is the simplest assumption possible, but far from the non-linear and anisotropic characteristics of the matrix in tissue models or real tissues. In this contribution, we theoretically address the role of filamentous matrix mechanics for pattern formation and force generation in ensembles of cells.

2 Model

Tissue cells are often polarized and thereby can be modeled as anisotropic force contraction dipoles with dipolar strength $P = Fl$. An extreme case would be a cell with one stress fiber of length l, where equal and oppositely directed forces F are exerted at each end for reasons of mechanical equilibrium. During the adhesion process,

cells spread out and contract against the external stiffness to achieve the force level given by F. If the matrix is modeled simply by a harmonic spring with spring constant K and if the build-up of force is reversible (no energy dissipation takes place), then the cell exactly invests an energy $W = F^2/2K$ into its surrounding. Therefore the stiffer the environmnent, the less energy the cell has to invest to achieve the force F. Experimentally it has been found that if confronted with a choice of different stiffnesses, e.g. corresponding to different directions in the matrix, then cells will prefer the direction of largest stiffness. Thus the cell behaviour formally corresponds to minimizing the elastic energy invested into the matrix [11, 12]. This theoretical framework allows to treat stiffness and tension on the same footing (both are favorable for migration and mature adhesion). In the following we will use these concepts as a starting point to study the interaction of cell ensembles in a fibrous matrix. Cellular dipoles are dispersed into a matrix and moved until the overall energy stored in the matrix is minimized. For simplicity we consider the same dipole strength for each cellular dipole in the ensemble.

The matrix is modeled as a two-dimensional cable network, which is a simple model for fibrous networks like the actin cytoskeleton or the extracellular matrix [35, 36, 30]. The joining points of the cables are called nodes and cables connecting two neighboring nodes are called edges. In order to ensure macroscopic isotropy and a finite Poisson ratio, we use a triangular network. The force acting on a node due to the deformation of a link to length l reads [35]

$$\mathbf{F} = \begin{cases} E_c A_c \, (\frac{l}{l_r} - 1) & \text{for } l > l_r \\ 0 & \text{for } l \leq l_r \end{cases} \tag{1}$$

Here E_c is the cable's Young modulus, A_c is its cross-sectional area and l_r is its resting length. Because the values given for collagen matrices differ widely in the literature, here we use only dimensionless parameters.

Cell dipoles are inserted between two neighboring nodes and are allowed to contract with equal and oppositely directed forces F. Equilibrium is established by iteratively solving force balance for every node until all of them are simultaneously satisfied. The iterations are terminated once the maximum force on every node becomes smaller than $F \times 10^{-4}$. In order to avoid collapse of the network in regions which are compressed, we introduce a critical length $l_c = 0.1\, l_r$ below which nodes are treated as repulsive. This means that if two nodes come closer to each other than the length l_c, they are glued such that they behave like a single node under any compressive load along their joining axis.

For studying the ordering kinetics of the cell dipole system, a completely disordered configuration is chosen initially by distributing dipoles randomly over the network. To mimic cellular motility, the dipole system is updated with a Monte Carlo (MC) algorithm. A dipole can jump into six possible places in the neighborhood of its original position. If ΔW is the energy difference between initial and equilibrated configurations, then a dipole move is accepted with probability

$$P(\Delta W) = \begin{cases} \exp(-\beta \Delta W) & \text{for } \Delta W \geq 0 \\ 1 & \text{for } \Delta W < 0, \end{cases} \tag{2}$$

where β is inverse thermal energy. After each update the entire system is mechanically equilibrated. A single Monte Carlo step (MCS) corresponds to attempted updates of N dipoles.

Build-up of force in tissue models has been experimentally studied before with so-called cell force monitors [27, 28]. To mimic the experimental situation we clamp two opposite sides of the network, leaving the other two sides free. The force measured with the cell force monitor usually starts in a linear fashion from zero and then crosses over to some saturation value. We study two possible mechanisms for the activation of cell contractility. In mechanism 1 (chemical activation), cells are switched on at random times, possibly due to some biochemical signals present in the system, and are not allowed to change configuration due to the mechanical input. In mechanism 2 (mechanical activation), in addition to the random activation by mechanism 1, there is also mechanical activation if locally some threshold in matrix strain is reached. In this mechanisms we also allow for cell reorientation (but not for repositioning).

3 Results

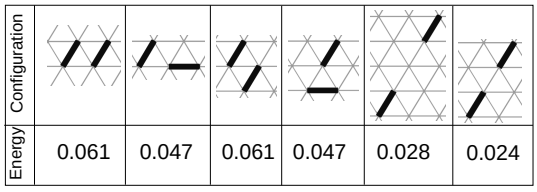

Fig. 1 Configuration versus energy for two cellular dipole on a triangular matrix of stiffness $E = 1$. Cellular dipoles are pulling on the matrix with a force 0.1 at each end. One clearly sees that the minimum energy configuration corresponds to the closest allowed proximity of cells along a line.

In Fig. 1 we show the energies of a two-dipole system on a lattice under periodic boundary conditions. Initially dipoles are randomly distributed on a $L_x \times L_y$ triangular lattice with periodic boundary conditions. We have kept the temperature $T = 0.001$ which is low enough to see the ordering effect. The system is then allowed to evolve under MC dynamics. The equilibrium is achieved when the average cluster configuration remain unchanged with time. Fig. 1 for two dipoles suggests that the cells tend to organize in lines. In Fig. 2 we show typical snapshots of the time development for $N = 60$ dipoles. Indeed one finds that they assemble into a network of strings.

In order to consider cell activation as it occurs in the cell force monitor, we turn to a triangular lattice of size $L_x \times L_y$ whose two vertical sides (L_y) are clamped and the horizontal ones (L_x) are kept free to move. Under this constraint, any internal contraction inside the lattice would cause inward curvature of the horizontal edges and

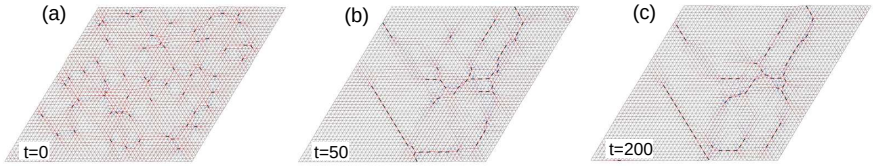

Fig. 2 Snapshots of cellular ordering on a 2d triangular lattice in a system of size $L_x = L_y = 50$ at times t =0, 50 and 200. There are 60 dipoles in the system and temperature $T = 0.001$ is kept constant. Color code: thick blue line segments are cellular dipoles, grey represents the matrix and faded red lines refers to the intensity of the strains produced by the dipoles.

no displacement of the vertical edges. In the beginning, all inactive cellular dipoles at a fixed density are uniformly randomly seeded into the matrix. The system is then allowed to evolve under two different mechanisms proposed earlier. Mechanism 1 assumes that each cell is activated at random time steps and does not change its configuration after activation. To simulate this we assign a randomly picked cell an activation time chosen from a Poissonian distribution starting at zero. As time progresses more and more cells get activated until a large simulation time $t_{max} \sim 500$ is reached when there is almost no inactive cell left in the system. Snapshot of the randomly oriented dipoles in the matrix at time $t_{max} = 500$ and the force $F(t)$ generated by the active cells as a function of time t are shown in Fig. 3. After $t \sim 300$ there is hardly any activities going on in the system, leading to a saturated force level F_{sat}. For the same number of cellular dipoles F_{sat} depends upon the elasticity of the matrix.

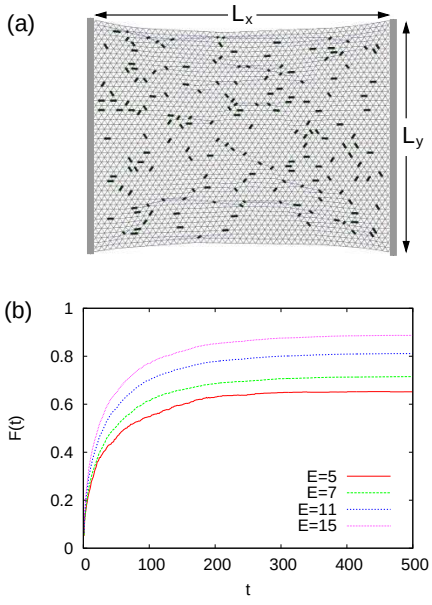

Fig. 3 (a) Snapshot of the cellular patterning after they are activated randomly according to the mechanism 1. Color code: thick blue line segments are cellular dipoles, grey represents the matrix and two thick vertical lines on opposite sides of the lattice refers to the clamped boundaries. It is evident from the figure, that cells are oriented randomly within the lattice. (b) Force $F(t)$ generated by the randomly activated cells. Different elastic constants E of the matrix gives rise to different force level after saturation.

In mechanism 2, an activated cell may mechanically influence an inactive cell to bring it into the active state. During the simulation, at each time step, the total tensile deformation of the matrix is calculated where the inactive cells are located. If the normalized resultant deformation exceeds a threshold value D_{th} (e.g.,0.2), the latter gets activated and pulls on the matrix. The final evolution pattern and force developed in this way are shown in Fig. 4. Unlike the previous case, in this case cells are allowed to rotate and find the minimal energy configuration. Since minimum energy of the cell corresponds to maximal effective stiffness of the matrix, cells adjust their orientations satisfying this criteria. Unlike the random cellular morphologies obtained in the previous case (mechanism 1), pattern obtained in the present case in the asymptotic time regime are ordered. We find that most of the cells prefer to orient perpendicular to the clamped boundaries and form chain-like structures. In this case the saturated force F_{sat} does not depend upon the stiffness of the matrix.

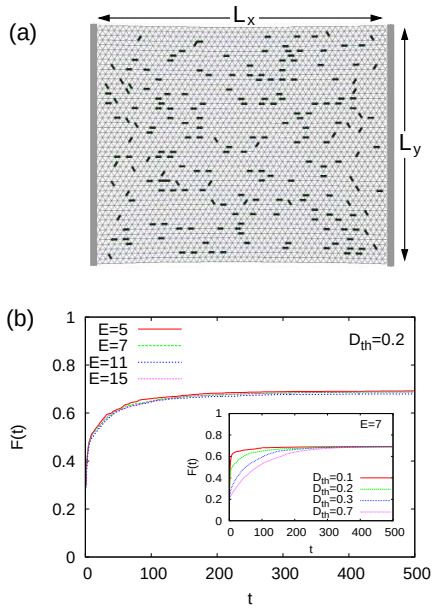

Fig. 4 (a) Pattern formed by the cellular assembly according to mechanism 2. Color code: same as in Fig. 3. It is observed that most of the cells are aligned perpendicular to the vertical clamped boundaries. (b) Force generated by randomly placed cellular dipoles. Cells are activated randomly and also mechanically when they are pulled strongly by other active cells. Data are collected for different E with fixed threshold activation deformation D_{th}=0.2. Inset: $F(t)$ for different D_{th} with fixed $E = 7$.

In order to investigate the effect of cell density on tissue tension, we finally considered a configuration in which tissues of different shapes are pinned at discrete points of adhesion. Experimentally it has been found before that such setups lead to inward curved tissue shapes with the arc radii given a quantitative measure of tissue tension [26, 30]. We considered triangular and hexagonal model tissues pinned at their corners. Cells are randomly seeded in the matrix at certain densities ρ. After the equilibrium is achieved, the radius of curvature R of the matrix is measured between two neighboring vertices. The snapshots and average radii of curvature of the cell seeded matrix are plotted as function of ρ in Fig. 5. Comparing snapshots of Fig. 5(a,b) with (d,e) it is clear that the inward curvature of the matrix is caused

by the contractile force of the cells. Fig. 5(c,f) demonstrate that radius R and cell density ρ scale inversely with each other.

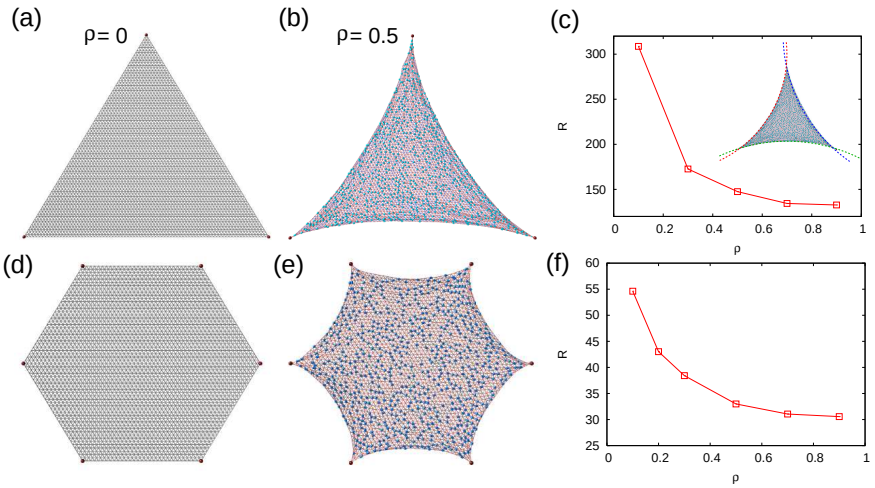

Fig. 5 Subplots (a) and (d) represents triangular and hexagonal elastic matrix without cell pinned at the 3 and 6 vertices respectively. (b) and (e) are the corresponding figures with cell dipoles seeded at density $\rho = 0.5$. Due to the contractile force of the seeded cells one can see the inward curvature of the matrix edges between successive pinned vertices. In subplot (c) and (f) we plot the average radius of curvature R as a function of the cell density for triangular and hexagonal matrix respectively.

4 Discussion

In this contribution we have used Monte Carlo simulations of contractile cells (modeled as force dipoles) in a fibrous matrix (modeled as cable network) to study pattern formation and force generation in tissues. In such simulations, temperature is a measure for cell activity, namely the tendency of cells to change position and orientation [34]. Our model focuses on mechanical effects, but includes internal regulation by introducing a Poisson process for cell activation. Because temperature is fixed at a relatively low value, pattern formation occurs. The higher matrix stiffness, the higher the ordering temperature and the slower the dynamics. In general we observed very slow dynamics because for cell ensembles many metastable states exist in which the system becomes traped. Regarding pattern formation, we observe that cells assemble into strings. At higher densities, these strings form interconnected networks running through the matrix. Due to the increasing activation of cells, tissue tension builds up in a hyperbolic way. However, the saturation level depends on the exact details of the switching mechanism. Only if the cells are responsive

to mechanical cues does one get a tissue tension which is independent of matrix stiffness, as has been reported before from experiments with a cell force monitor for fibroblast contraction of a collagen-GAG matrix [28]. With increasing cell density, the effective tissue tension rises and the shape of pinned tissue becomes more invaginated. A simple Laplace law would predict $R = \lambda/\sigma$, where the tension in the tissue is decomposed into a line tension acting at the periphery and a surface tension acting in the bulk [30]. Assuming $\sigma \sim \rho$, one exactly arrives at the inverse relation revealed by Fig. 5.

In summary, if going from the level of single cells to tissues, collective effects are essential and introduce many additional phenomena such as density thresholds, structural transitions and slowed-down dynamics due to metastable states. Although simple models are essential to gain a fundamental understanding, in the future more realistic models are required, in particular in view of possible applications in tissue engineering. Here we have used cable networks as a first step towards more realistic models for the matrix and a simple Poisson-type activation of cells as a first step towards modelling the biochemical aspects of mechanotransduction. In the future, this approach might be extended in several regards. Anisotropic force contraction dipoles are only the first order approximation for the complex mechanical activity of cells and might be extended to more general tensors for mechanical activity and susceptibility [15, 16]. Alternatively one might combine our approach with whole cell models incorporating the way focal adhesions and stress fibers grow as a function of the coupling to the matrix [37]. In the long run, such an approach should also include the details of force-modulated signals to the cytoskeleton of adherent cells [10]. Finally more detailed models have to be developed for the dynamics of the matrix, which might collapse locally under cell traction [31].

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