A minimal model for nematic cell ordering in tissue

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Recent advances mean that samples of artificial tissue can now be grown. Hence there is a need for theoretical understanding of these tissues commensurate with the size of experimental systems. We present a minimal model for predicting cell orientations and modification of tissue shape resulting from the active forces in cells. The extracellular matrix (ECM), a biopolymer network found between cells, is represented by an elastic network. Cells in the model induce tension in this network according to their symmetries. This in turn influences nearby cells leading to nematic order and change of tissue shape. Simulated annealing solutions of the model show close agreement with experimental results for artificial neural tissue. Thus, the subtle interplay between forces generated by cells and the ECM that leads to the ordering of tissues is reproduced. Applications of the model are discussed.

Recent advances in artificial tissue have led to a need for theoretical understanding of the way that cells order in tissue samples, which could lead to computational design of such tissues. Tissues are active materials that can show types of order usually associated with liquid crystals such as nematic phases [1]. Glial cells that support nerve tissue order can be grown artificially into structures that show cellular alignment of this type [2, 14], indicating that the kinds of microscopic models typical in statistical physics could describe this order. Cells in organisms do not exist in isolation and grow within a biopolymer network known as the extracellular matrix (ECM), which has viscoelastic properties and can distort due to the actions of cells, and in turn influence the arrangement of the cells. The goal of this letter is to develop a minimal model that is sufficiently simple to describe the physics of large numbers of cells in tissue structures, while sufficiently complex to capture the results of the subtle interplay between the extracellular matrix and active forces in cells (and hence amenable to detailed large scale simulations of order in real tissues).

Force dipoles are a popular approach for describing the interactions between cells and the ECM [5]. The interactions of dipolar and multipolar cells with external strain fields and with each other have been investigated [6–10], with further studies of many-body effects between small numbers of cells with fixed positions using Monte Carlo simulations [11] and applications to the self-polarization of cells in elongated gels [12] and the onset of order in muscle tissue [13]. The force-dipole approach has provided insight into the reasons that cells orient, especially in response to external stresses. While these models are undoubtedly sophisticated, repulsive terms leading to finite compressibility of the viscoelastic ECM are not present. To stop collapse, cells in these models are constrained either to fixed positions, or small movements about such fixed positions. Cells in tissues are not constrained in this way. Thus these models do not capture the feedback between cells and the tension and shape of the ECM that are found in real tissues.

On the other hand, tissues can distort according to the stresses upon them. These distortions can be effectively modeled using contractile network models, which have been used to describe the shapes of both single tethered cells and bulk tissue shapes [13, 10]. However, contractile network models have not been used to describe the interplay between forces originating from cell ordering and tissue shapes and structures.

In this letter, a model that can handle the subtle interplay between tension induced by active forces in cells and remodeling of the ECM due to cellular ordering is developed. The model goes beyond previous work by combining models of a force dipole type with contractile network models to investigate cellular ordering in tissues. The model presented in this letter is based on a network of springs representing the ECM, with the interactions between cells and ECM dictated by symmetry considerations. The cells in these structures are well separated, such that there is no direct interaction between cells, rather interaction is mediated via the ECM. In the absence of cells, the energy of the network is given by a sum over Hooke’s law for each individual filament, \( E = \sum_{ij} \frac{k}{2} (|l_{ij} - l_{eq}|)^2 \) where \( l_{ij} \) is a vector between cells at vertices \( i \) and \( j \), \( l_{eq} \) is the equilibrium length between springs and \( k \) is the spring constant.

For simplicity, cells are positioned at the vertices between springs. Cells are able to remodel, pull on and respond to the extracellular matrix [17], and as such the tissue sample, which is a material comprising both ECM and cells is active and can generate its own stresses. The effect of the active forces generated by cells is modeled as an effective modification of the equilibrium length of the surrounding springs, \( l_{eq} = l_0 - f(\Theta) \) where \( l_0 \) is the equilibrium length of the bond in the absence of cells, \( \Theta \) is the angle between bond and cell orientations. It is noted that this modification represents the end result of the active processes, rather than the time-dependent details of such processes.
The cells considered here are elongated with approximately order 2 rotational symmetry corresponding to glial (and many other) cell types, so \( f \) must be an even function depending on the orientation of the cell relative to the spring representing the ECM. This modification is consistent with a force dipole. Thus to leading order, the distance between cells is

\[
E = \sum_{ij} \frac{\kappa_{ij}}{2} \left( |l_{ij}| - l_{0,ij} \left( 1 - \Delta_{ij} \left( 2 - |l_{ij} \cdot s_i| - |l_{ij} \cdot s_j|^2 \right) \right)^2 \right)
\]

where \( \kappa_{ij} \) characterizes range. A combination of updates with different \( \Delta \theta \) of up to 2 orders of magnitude smaller are also used. Updates made for fixed / variable update ranges. The cellular arrangement is consistent with a force dipole. Thus to leading order, tension is found using a simulated annealing approach \[19, 20\].

The spring network has a regular lattice structure, which is selected for its physical properties. Simulations are carried out for both 2D and 3D networks. Spring networks with square or simple cubic lattices have zero shear modulus \[15\]. Thus 2D simulations are performed on triangular lattices and 3D simulations on face centered cubic (FCC) lattices, since the triangular and tetrahedral structures in these lattices ensure shear modulus. A schematic of our model can be seen in Fig. 1.

Bischofs and Schwarz have proposed that cells find an orientation consistent with the minimum work needed to obtain that configuration \[4\], i.e. the ground state, which is found using a simulated annealing approach \[19, 20\]. A set of Monte Carlo updates are made, in this case single cells or groups of cells can be moved, reoriented, or both. Cell positions and orientations are characterized using azimuthal and polar angles, \( \theta \) and \( \phi \) and their coordinates, \( x, y \) and \( z \). During reorientation, both angles are updated either from a Lorenzian distribution \[20\], or a stepped distribution. For the Lorenzian distribution, taking orientations as an example, angles are updated according to \( \theta \to \theta' = \theta + \Delta \theta \tan (\frac{\pi}{2} (r - 1/2)) \). The alternative step function update is \( \theta \to \theta' = \theta + \Delta \theta (r - 1/2) \), where \( \Delta \theta \) characterizes range. A combination of updates with different \( \Delta \theta \) of up to 2 orders of magnitude smaller are also used. Updates are made for fixed / variable update ranges. The cellular arrangement with the lowest energy is selected. At this point, tensions in the springs are calculated.

Figure 2 shows experimental data for a system of glial cells. Data were gathered using a confocal microscope and analyzed using Velocity (PerkinElmer, Waltham, MA, USA). The artificial extracellular matrix starts as a rectangular type I collagen gel seeded with glial cells tethered by a set of four pillars. The system is not under the influence of external tension. Tension arises solely due to the interactions between cells and the matrix. Experimental details for the growth of glial cells can be found in Refs. \[22\,24\]. In Fig. 2(b), yellow cells are aligned along the short axis, and blue cells along the long axis.
The main features of the experimental arrangement of cells are a triangular (Delta) region just to the right of the region between the pillars, a region aligned along the short axis between the pillars and a region of glial cells aligned along the long axis towards the center of the sample. The sample is also narrower at its center. Approximately 10,000 cells are identified in the confocal micrograph image.

The results of simulations for a similar pillar-like system can be seen in Fig. 3. The positions of cells are fixed around the pillar, but permitted to change angle. 2D results are shown for three different values of $\Delta = 0.25$, $\Delta = 0.3$, and $\Delta = 0.35$ for $l_0 = 100\mu m$ (panels (a)-(c)). Results are also shown for $\Delta = 0.35$ and a shorter lattice spacing $l_0 = 50\mu m$, which leads to a broadly similar arrangement of cells (panel (d)). Key features of the experimental data can be seen. Nematic regions can be seen both along the pillars and along the long axis towards the center of the sample. Where the nematic regions with different orientations meet, there is a region of mixed orientations. As $\Delta$ is increased, the waist at the middle of the simulated sample decreases. A similar waist size to the experimental data is achieved for $\Delta \sim 0.35$. Panel (e) shows the results for a 3D simulation with $\Delta = 0.35$ and $l_0 = 100\mu m$. Similar structures are seen in 2D and 3D cases.

The magnitude of the tension, $T$, within each spring of the network is shown in Fig. 4. Tension in NN and NNN
bonds are shown. The color represents the dimensionless value $T/\kappa_{NN}/l_0$, with red representing regions of highest positive tension, blue regions of highest negative tension and light gray regions of lowest tension. The strongest tension can be found in the small region between the pillars, extending into a roughly triangular shape along the long axis.

Comparison of these simulated arrangements with experimental models of artificial glial tissue indicates that the model produces broadly similar cell arrangements to those in artificial engineered tissue, comprising a triangular mixed region and regions with nematic alignment. The model presented here has some key strengths: (1) It is simple (with similarities to the Heisenberg model of spin alignments) and can therefore be used to simulate large numbers of cells. (2) The glial cells modeled in this paper have approximate two fold symmetry. The model explicitly includes an elastic network representing the extra-cellular matrix and is therefore able to predict the shapes of tissues. (3) The model produces broadly similar cell arrangements to those in artificial engineered tissue, comprising a triangular mixed region and regions with nematic alignment.

For balance, some limitations of the current model are noted. (1) The model is applicable for situations where the cells are not very tightly packed. Alternative approaches describe tissues as active continuum materials [1]. (2) It is not applicable to situations in which there is no extracellular matrix, e.g. growth of bacteria within nutrient molds, for which models such as the cellular Pott’s model would be more appropriate (e.g. [23, 26]). (3) It is also static, and can not describe situations where there are oscillatory strain stimuli (e.g. [27, 29]).

Cell ordering is particularly important in artificial tissues. The use of artificial or engineered tissue for regenerative medicine has the potential to significantly improve human health [30] and provide new biological models for drug testing [31]. However, there are challenges involved in growing artificial tissue with suitable cellular organization for application in this area. The cells in real tissues are typically arranged in ways that may be difficult to reproduce artificially, and without that order the resultant tissue may be scar like (the cells in scars tend to be oriented along multiple directions rather than correlated along a single direction as in healthy tissue). This is a particular problem for nerve tissue, which must be well ordered to function properly. In the central nervous system (comprising the brain and spinal cord), neurons are typically guided by glial cells, so well ordered glial cells in artificial tissue could lead to well directed neuron growth for e.g. nerve repair. The desire is that these regions are as well oriented as possible along a single direction. Simulations of the model described here could be fast enough to use for the design of experimental systems for better artificial tissue growth. Particularly relevant aspects would include cell density and distribution (e.g. the results of uneven distributions or gradients of cells) and ECM density and stiffness. All these parameters are expensive and time-consuming to investigate experimentally so the ability to run simulations to predict outcomes would be extremely valuable.

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