Analysis of Cellular Organization in Soft Environments: A review on Cell organization in active media due to active mechanosensing [1]

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1 Introduction

The behavior of adhering cells, particularly their response to mechanical stimuli, holds insights into their dynamic interaction with the surrounding extracellular matrix (ECM). As cells attach to their substrate, they actively interact with their mechanical environment, and through that interaction, they gain information that results in repositioning and orientation. Recent research has revealed that cells not only exert force upon their surroundings through contractile motions but also gain crucial information about its mechanical properties.

A principle governing this phenomenon is the tendency of cells to reinforce their contacts and cytoskeletal structures in regions of heightened mechanical stiffness. This concept explains a wide array of experimental observations. One such observation belongs to Harris et al. who saw large tension fields for fibroblasts on elastic substrates, which induce mechanical activity of other cells, even when located at a considerable distance. When plated on elastic substrates of increased rigidity, many cell types show increased spreading and better-developed stress fibers and focal adhesions. Fibroblasts on elastic substrates orient in the direction of tensile strain and locomote in favor of regions of larger rigidity or tensile strain.

The mechanical signature of adherent cells extends beyond their immediate physiological roles, to processes such as tissue development, wound healing and metastasis. Recent strides in understanding the molecular mechanisms of mechanotransduction have highlighted the pivotal role played by focal adhesions. These mechanosensitive complexes connect the ECM and the actin cytoskeleton and serve as receptors for extracellular cues, triggering a series of cellular responses that result in shape remodeling and spatial reorientation.

In this study, the authors employ elasticity theory to model the ECM and find the optimal organization of cells for different scenarios. They propose an optimization model that perfectly justifies the cell's preference for large effective stiffness and is in agreement with a lot of experimental data.

2 Theory

Backed by recent experiments and observations of elastic substrates, an optimization principle can be proposed to describe the behavior of adherent cells: cells position and orient themselves to maximize the effective stiffness of their environment. This principle depends on the elasticity of the surrounding medium, which serves as the primary input for cellular decision-making. To account for this, we model the extracellular matrix using isotropic linear elasticity theory and describe its stiffness using two elastic moduli: the Young modulus (E) and the Poisson ratio (ν).

Optimal cell position and orientation are identified by minimizing the quantity W, reflecting a cellular preference for maximal effective stiffness in its local elastic environment. W serves as a measure of the information a cell

can extract through active mechanosensing. The agreement of this model with experimental data will ultimately validate its utility. The origin of the optimization principle comes from observing that cells tend to favor environments with maximal effective stiffness. While this observation has been successful in explaining experimental results, researchers also seek to propose a mechanism supporting this assumption.

We could consider the cellular reorientation phenomena as a result of cells aiming to displace the surrounding material, and therefore preferring effective softness. However, this would imply the existence of additional signaling mechanisms. A more realistic scenario is that force triggers cellular responses by displacing elastic components inside the cell, linearly related to force through an internal spring constant.

Adhering cells probe their environment by pulling at multiple contacts simultaneously using actin stress fibers. Each contact encounters a different elastic environment, represented by varying spring constants. In an isotropic environment, where all spring constants are equal, cells exhibit a round or stellate morphology, depending on the number of contacts. However, in an anisotropic environment, force buildup is more efficient in one direction, leading to directional growth. Here, the anisotropic properties provide orientation cues, with cells aligning along the direction of maximal stiffness. This orientation may be followed by cell locomotion, depending on factors like motility.

2.1 optimal position and orientation [2]

The forces that adhering cells exert on their surrounding environment are mainly due to actomyosin contractility, so cellular forces are continuously generated by the cell and include non-equilibrium processes. By pulling on the environment, the cells extract enough information from the elastic environment and we want to express this information in terms of a scalar quantity. One characteristic of the cell-medium interaction is the work the cell has to perform in order to build up a force F with which it can pull on the surrounding environment, and experimental observations suggest that we can simply think of this process as cells trying to minimize this energy by preferring the direction with large effective stiffness.

Let's simplify this process and imagine the extracellular environment as a spring with a certain stiffness (K), and cells exert force by pulling on it through a single cell-matrix contact. Recent experiments suggest that the growth of these contacts is linked to reaching a specific force threshold (F). To achieve this force, cells must invest energy $(W = F^2/2K)$ into stretching the spring. Therefore, the stiffer the spring (larger K), the less work is required, making force buildup more efficient. Alternatively, we can view this process as cells investing power (L) to stretch the spring, taking time (t = $F^2/2KL$) to reach the force. This suggests that contacts encountering greater stiffness grow faster than others. The cell probes its surroundings through multiple stress fibers, and the different stiffnesses at each point of contact eventually lead to cells orienting in the direction with the biggest stiffness where the build-up of force is most efficient.

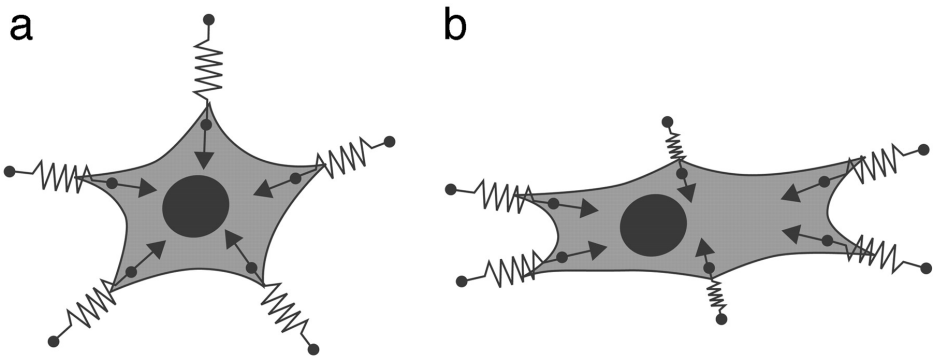

Figure 1: (a) If spring constants are similar in all sides of the cell (isotropic elasticity) the growth is homogenous (b) If spring constants are largest in one specific direction, the corresponding contacts outgrow the others, and the cell orients in the direction of maximal stiffness of the environment.

2.2 Extranction of information through active mechanosensing

To describe this behavior more efficiently, we model the extracellular matrix using the continuum elasticity theory. We introduce the concept of **local effective stiffness** of the environment as an analog to the spring constant K. The work W required to build up a force F in the environment and deform the substrate in an isotropic medium with no strain, is expressed as:

$$W_0 = \frac{1}{2}\int d^3 r C_{ijkl} u^{\mathrm{c}}_{ij}(\vec{r}) u^{\mathrm{c}}_{kl}(\vec{r}) = \frac{1}{2}\vec{F}\cdot\vec{u}_{\mathrm{c}}\left(\vec{r}_{\mathrm{c}}\right), \tag{1}$$

where u_e is the displacement caused by the cell, u^c_{ij} is the strain tensor and C_{ijkl} is the elastic constant tensor based on E and ν, The young modulus(describing rigidity) and the Poisson ratio(describing the relevant weight of compression to shear modes). In a homogeneous medium, the elastic constants do not change and W_0 is a constant. Here cells have a round or stellar shape, depending on the number of contacts. However, in an anisotropic environment, force buildup is more efficient in one direction, leading to directional growth. Here, the anisotropic properties provide orientation cues, with cells aligning along the direction of maximal stiffness. This orientation may be followed by cell locomotion, depending on factors like motility.

2.3 homogenous external strain

If we place the cell in a prestained homogenous medium, It needs to perform a work ΔW^e to generate force in the presence of an externally imposed strain tensor field.

$$\Delta W^e = \int d^3 r C_{ijkl} u^{\mathrm{c}}_{ij}(\vec{r}) u^{\mathrm{e}}_{kl}(\vec{r}) \tag{2}$$

we can expand the above equation in terms of the force multi-poles. Contact points are coupled through the actin cytoskeleton to ensure force balance, setting a constraint on the system and resulting in a force pattern known as an anisotropic force contraction dipole,P_{ij}.

$$\Delta W^e = P_{ij} u^e_{ij} \tag{3}$$

Where $P_{ij} = P n_i n_j$ in which P is the dipole strength, the product of force magnitude and force separation, and n is its orientation. For contractile forces, the tensor P_{ij} has negative eigenvalues. Therefore the favorable state would be to have tensile strain ($u_{ij} > 0$), which corresponds to an increase in effective stiffness.

2.4 Boundaries

Cells in structures such as tissue or organ surfaces, since the effects of a boundary. When cells exert force, these boundaries modify the tension compared to when cells are in a spacious, uniform environment. This boundary-induced tension, known as image strain, mimics the effect of external strain. Consequently, cells can detect not only the presence of nearby surfaces but also their shape and boundary characteristics. A sample case studied in the paper by Bischofs et al. [1] is for a semiinfinite space with a planar surface. We consider two different scenarios, one in which we have a clamped boundary, for which displacement vanishes, and the other is a free boundary on which normal stress vanishes. The change in work due to the boundary effect is found to be

$$\Delta W^b = \frac{P^2}{E d^3}\left(a_\nu + b_\nu \cos^2\theta + c_\nu \cos^4\theta\right) \tag{4}$$

where the coefficients a_ν, b_ν, and c_ν are complicated functions of the poison ratio ν. For free surface, the coefficients are positive, therefore the optimal orientation is $\theta = \frac{\pi}{2}$, with cells being parallel to the boundary. In a clamped surface, the coefficients are negative, therefore the optimal orientation is $\theta = 0$, with cells being normal to the boundary.

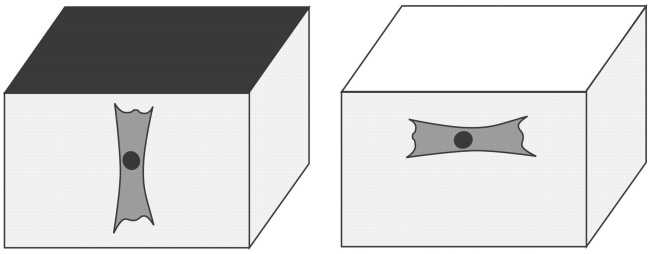

Figure 2: Cells prefer the direction of maximal effective stiffness. Therefore, they orient perpendicular to a clamped surface(left) and parallel to the free surface (right))

2.5 cooperative effects

The traction of cells also produces strain fields that may be large enough to be discoverable by other cells, which leads to elastic interactions. We consider the simple case of the interaction of two cells, for which the change in stiffness is characterized by the term:

$$\Delta W^{P_1 P_2} = \frac{P_1 P_2}{E r^3} g_\nu \left(\theta_1, \theta_2, \theta\right) \tag{5}$$

with r being the distance between the force dipoles, and g_ν is a function with three degrees of freedom θ, θ_1, and θ_2. The minimum for this function is independent of ν, and happens at θ = θ_1 = $\theta_2 = 0$ for which cells are completely aligned. One interesting aspect of this interactions is that there's a positive feedback loop for cell alignment, and as more cells align in a particular direction, the signal for other cells to follow the same alignment grows stronger.

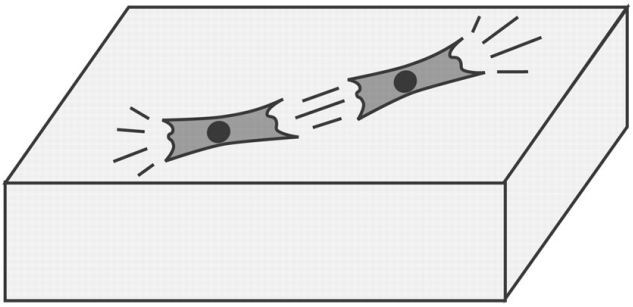

Figure 3: The maximal strain stiffening occurs along the axis of contraction,where cells form a string-like pattern along the axis

3 Discussion and conclusions

The optimization model introduced in this paper allows us to predict cell organization in soft media using elasticity theory and is in excellent agreement with a large body of experiments. It presents a mechanism that links the cellular preference for large effective stiffness to the growth of cell-matrix contacts. Since cell response to the local rigidity of their surrounding matrix affects the large-scale organization of the tissue, understanding the mechanism behind this phenomenon could have invaluable applications in tissue engineering, regenerative medicine and cancer therapy.

References

[1]I. Bischofs and U. Schwarz, “Cell organization in soft media due to active mechanosensing”, Proceedings of the National Academy of Sciences of the United States of America **100**, 9274–9 (2003).

[2]I. B. Bischofs, S. A. Safran, and U. S. Schwarz, “Elastic interactions of active cells with soft materials”, Phys. Rev. E **69**, 021911 (2004).