Optimizing multicellular network formation on elastic substrates

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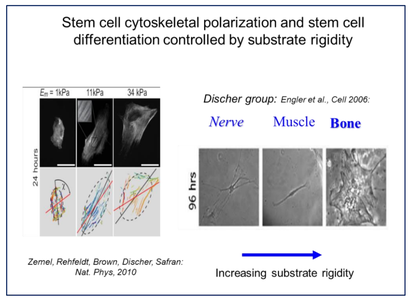
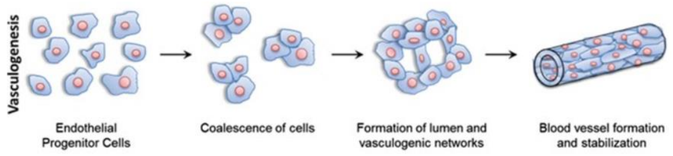
1. Motivation

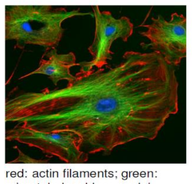
Cells are sensitive to the mechanical properties of their environment. Zemel et al. shows that cells orient their mean fiber alignment along the long axis of their body most strongly on an intermediate substrate stiffness. Engler et al. found that cell fate itself is heavily influenced by the stiffness of the substrate on which stem cells are seeded. We are interested specifically in the role of mechanics in the biological process of vasculogenesis wherein a group of progenitor cells locomote, coalesce, and self-assemble into ordered network structures known as vascular networks which eventually give rise to more complicated three-dimensional structures such as blood vessels.





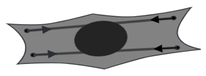
Peak et al., Microscale Technologies for Cell Engineering (2015).

2. Elasticity Theory



red: actin filaments; green: microtubules; blue: nuclei

Cells probe their mechanical environment by generating traction forces through a transmission of actin cytoskeleton tension induced by myosin motors via focal adhesions. These forces are contractile and dipolar.

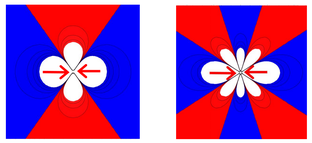


Polarized cell as an anisotropic contractile force dipole (Bischofs et al. Phys. Rev. E 69, (2004))

As such, we model cells as anisotropic contractile force dipoles which take the mathematical form of second order tensors that are equal to the product of the constituent force monopoles and separation vector. Additionally, we take these cells to be adhered to a surface such that displacements and forces in the normal direction are much smaller than their tangential counterparts. These assumptions yield strain plots shown below for two values of Poisson's ratio which produce qualitatively different strain maps.

$$P_{ij} = F_i a_j$$

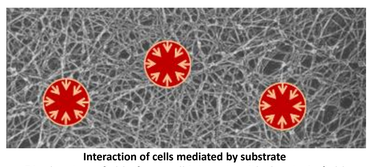
Deformation by single force dipole



Compressible: $\nu = 0$ Incompressible: $\nu = 0.5$

Red: compression, Blue: expansion

Cells interact via mutual deformations of the substrate. This pairwise interaction potential is the product of the local dipole stress and the resultant local strain field of a neighboring dipole. For a collection of interacting dipoles, the net interaction is the sum of pairwise interactions.



Interaction of cells mediated by substrate

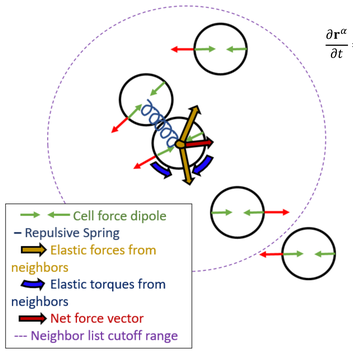
Dipolar stress field of one cell interacts with the strain field induced by another:

$$W_{12} = \int dx\, p_{ij}^{(1)}(x) u_{ij}^{(2)}(x)$$

$$\Delta W = \frac{P^2}{\bar{E} r^3} g_{\nu}(\theta_1, \theta_2, \theta)$$

The interaction of substrate mediated cell-cell elastic interactions is dependent on both the stiffness and compressibility of the substrate.

3. Brownian Dynamics Simulations



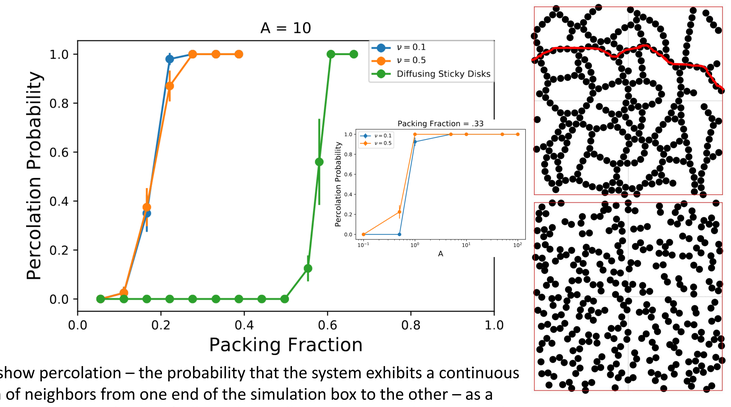
$$\frac{\partial \mathbf{r}^\alpha}{\partial t} = -\mu_T \left(\sum_\beta \frac{\partial W^{\alpha\beta}}{\partial \mathbf{r}^\alpha} + \sum_\xi \frac{\partial U^{\alpha\xi}}{\partial \mathbf{r}^\alpha} \right) + \eta^T, \quad \frac{\partial \mathbf{e}^\alpha}{\partial t} = -\mu_R \left(\sum_\beta \mathbf{e}^\alpha \times \frac{\partial W^{\alpha\beta}}{\partial e^\alpha} \right) + \eta^R$$

Elastic (first sum) Steric Spring (second sum) Elastic (second equation)

$$\langle \eta_i(t) \eta_j(t') \rangle = \delta(t - t') \delta_{ij}$$

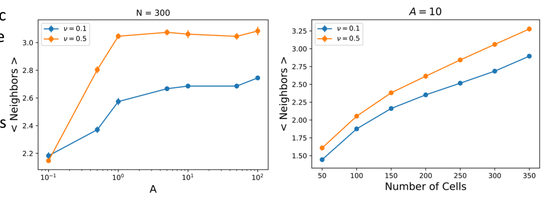
Our model considers cells as discrete agents which move and orient randomly and interact with one another through long-range elastic interactions via a force dipole strain field coupling and a short-range repulsive spring. The overdamped Langevin equations governing the position and orientation of a cell are shown above and solved numerically using forward Euler integration.

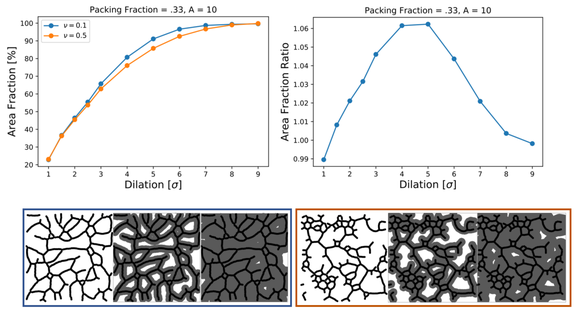
4. Transport Properties



We show percolation – the probability that the system exhibits a continuous path of neighbors from one end of the simulation box to the other – as a function of packing fraction/cell number. Cells interacting via long range elastic interactions require far fewer cells to percolate than purely diffusive sticky disks. The inset shows this result assumes a sufficient interaction strength – A.

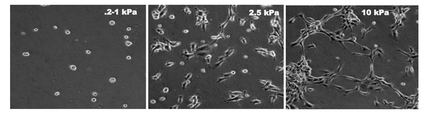
Neighbor counts infer characteristic topological features. The low v case saturates to an average neighbor count between two and three indicating an abundance of chains whereas the higher v case saturates to an average neighbor count greater than three indicating a prevalence of junctions.



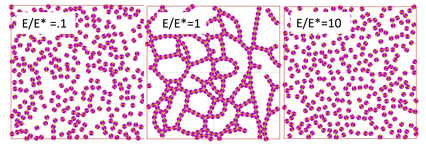


The ability of a biological network to efficiently cover space is crucial to deliver signals and materials. Assuming the drainage area of each cell to be a dilation factor times the cell size, we analyze how the filling area of our networks scale with this cell dilation. Lower v case increases area coverage as a function of dilation faster than higher v case.

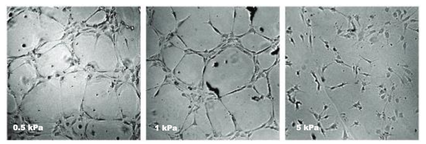
5. Network Morphology



Califano et al., Cel. Mol. Bioeng. (2008).

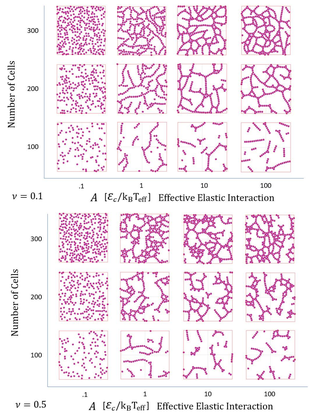


Where E is substrate stiffness and E* is the substrate stiffness at which a cell will exert maximal traction force.

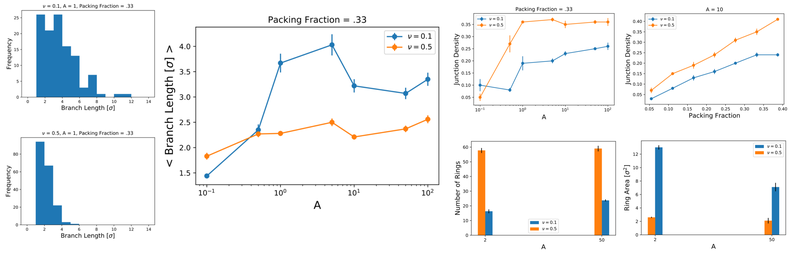


Rudiger et al., Cell Reports (2020).

Endothelial cells have been shown to remain isolated and not form networks when the surrounding substrate is too stiff or too soft. It is only within a range of substrate stiffnesses where cells will connect with one another and form networks. This behavior is recapitulated in our Brownian dynamics simulations.



At very low elastic interaction strength, cells are dominated by noise. At low cell densities, cells form into chains with many open ends. As packing fraction increases, cells form branched networks characterized by chains, junctions, and ring-like morphologies.



Low v cases exhibit a broader branch length distribution with a high sensitivity to noise, a low density of large rings, and a small junction density whereas the higher v case exhibits a narrow branch length distribution that is insensitive to noise, many small rings, and a large junction density.

6. Conclusion and Future Work

- Cells modeled as contractile force dipoles self-assemble into branched networks characterized by chains, junctions, and ring-like morphologies.
- Network formation is dependent on elastic substrate stiffness and cell number.
- Network morphology is highly dependent on substrate stiffness, compressibility, and cell number.
- Experimentally determined ranges of substrate stiffness which accommodate cell network assembly recreated in Brownian dynamics simulations.

- Study the role of more directed persistent activity by including self-propulsion in the model.
- Investigate the effect of confinement on network formation by introducing a closed repulsive spring-like boundary.
- Determine the mechanical properties of the cellular networks by imposing external stresses.

7. Acknowledgements

This work was funded by NSF-CREST: Center for Cellular and Biomolecular Machines at UC Merced (NSF-HRD-1527848)