Supplementary Information for

Mechanical interactions direct optimal multicellular network formation on elastic substrates

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This PDF file includes:

- Figs. S1 to S20 (not allowed for Brief Reports)
- Table S1 (not allowed for Brief Reports)
- SI References
Consistent with adherent cell behavior on soft substrates, we assume our model cells are elongated and exert contractile traction forces at the poles of their long body axis. It is by this behavior that we model our cells as contractile force dipoles. The mechanical interaction between a pair of force dipoles is illustrated by the schematic in Supp. Fig. S1 in the form of a 1D series of springs representing the effect of the elastic substrate. While the springs underlying the contractile dipoles are compressed, the springs between them are stretched. By moving to different positions in the medium for a given position of dipole A, the dipole B can reduce the net substrate deformation energy by compressing regions stretched by dipole A. this physical interaction between elastic dipoles considered here is analogous to the interaction of an electric dipole with the electric field induced by another dipole. A similar reciprocal force results on dipole A, since the interactions are based on an elastic free energy. The physical origin of this force is the tendency of the passive elastic medium to minimize its deformations in response to the active, contractile forces generated by the cells. We now assume these cells are on an isotropic, homogeneous, linear substrate in elastic halfspace of Young’s modulus and Poisson’s ratio $E$ and $\nu$, respectively, and derive the displacement field due to a coarse-graining of the traction forces on either side of the nucleus into single point like forces, $\mathbf{F}^1$ and $\mathbf{F}^2$ where $\mathbf{F}^1 = -\mathbf{F}^2$, separated by a distance $a$. Let the center of the force distribution lie at $r'$. Then, by elasticity theory, the displacement at position $r$ can be written

$$u_i(r) = G_{ij}(r - (r' - \frac{a}{2}))F^1_j + G_{ij}(r - (r' + \frac{a}{2}))F^2_j,$$  \hspace{1cm} (1)$$

where $G_{ij}$ is the Green’s function that captures the displacement in the elastic medium at the location of one cell (dipole) caused by the application of a point force at the location of the other (1) defined as

$$G_{ij}(r) = \frac{1 + \nu}{\pi Er}(1 - \nu)\frac{\delta_{ij}}{r} + \nu r\frac{\delta_{ij}}{r^3}.$$  \hspace{1cm} (2)$$

Replacing $\mathbf{F}^1 = -\mathbf{F}^2 = \mathbf{F}$ in eqn. 1 and performing a Taylor expansion about $r - r'$ to first order in $a$ gives

$$u_i(r) = \partial_k G_{ij}(r - r') F_j a_k = \partial_k G_{ij}(r - r') P_{jk},$$  \hspace{1cm} (3)$$

where $P_{jk} = F_j a_k$ is the force dipole representation of one of our cell’s force distribution, $\partial k$ is the partial derivative with respect to $x_k$, and terms of order $a^2$ and higher have been neglected. We now write the strain by the derivatives of the displacement field as

$$\varepsilon_{ij}(r) = \frac{1}{2}(\partial_i u_j(r) + \partial_j u_i(r)),$$  \hspace{1cm} (4)$$

where the $u_{xx}$ and $u_{yy}$ fields are shown in Supp. Fig. S2. Lastly, we note that that by coupling the strain field due to one cell in the proximity of another, we can write the work done by deforming the medium and thus an effective pairwise interaction potential energy given by

$$W_{\alpha\beta}(r_{\alpha\beta}) = P^\beta_i \partial_k \partial_l G_{ij}^{\alpha\beta}(r_{\alpha\beta}) P^{\alpha}_j,$$  \hspace{1cm} (5)$$

where $P_{\alpha}$ and $P_{\beta}$ are the magnitude of the contractile force dipole exerted by cell $\alpha$ and cell $\beta$, respectively. $E$ is the Young’s modulus of the elastic substrate, $\nu$ is Poisson’s ratio, and $r_{\alpha\beta} = r_{\beta} - r_{\alpha}$ is the separation vector connecting the positions of cell dipoles, $\alpha$ and $\beta$.

By transforming to the separation vector coordinate frame, the cell-cell elastic potential can be written as (2)

$$W_{\alpha\beta} = \frac{P_{\alpha} P_{\beta}}{E \pi^2}$$.  \hspace{1cm} (6)$$
When considering a culture of identical cells, the ratio is collected in the function,

\[ f(\nu; \theta_\alpha, \theta_\beta) = \frac{\nu(\nu + 1)}{2\pi} \left( 3(\cos^2 \theta_\alpha + \cos^2 \theta_\beta - 5 \cos^2 \theta_\alpha \cos^2 \theta_\beta - \frac{1}{3}) - (2 - \nu^{-1}) \cos^2 (\theta_\alpha - \theta_\beta) - 3(\nu^{-1} - 4) \cos \theta_\alpha \cos \theta_\beta \cos (\theta_\alpha - \theta_\beta) \right). \]  

[7]

For simplicity, we will assume the magnitude of all contractile cell force dipoles in our system are equal, so \( P_\alpha = P_\beta = P \), which is justified when considering a culture of identical cells.

Taking derivatives of eqn. 6 with respect to \( x_\beta \) and \( y_\beta \) to compute the \( x \) and \( y \) components of the force, respectively, on cell \( \alpha \) from cell \( \beta \) yields

\[
- \frac{dW_{\alpha,\beta}}{dx_\alpha} = \frac{dW_{\alpha,\beta}}{dx_\beta} = \frac{\partial W_{\alpha,\beta}}{\partial x_\alpha} \frac{\partial x_\alpha}{\partial x_\beta} + \frac{\partial W_{\alpha,\beta}}{\partial \theta_\alpha} \frac{\partial \theta_\alpha}{\partial x_\beta} + \frac{\partial W_{\alpha,\beta}}{\partial \theta_\beta} \frac{\partial \theta_\beta}{\partial x_\beta} \]  

[8]

and

\[
- \frac{dW_{\alpha,\beta}}{dy_\alpha} = \frac{dW_{\alpha,\beta}}{dy_\beta} = \frac{\partial W_{\alpha,\beta}}{\partial y_\alpha} \frac{\partial y_\alpha}{\partial y_\beta} + \frac{\partial W_{\alpha,\beta}}{\partial \theta_\alpha} \frac{\partial \theta_\alpha}{\partial y_\beta} + \frac{\partial W_{\alpha,\beta}}{\partial \theta_\beta} \frac{\partial \theta_\beta}{\partial y_\beta} \]  

[9]

where \( r_{\alpha,\beta,x} \equiv x_\beta - x_\alpha \) and \( r_{\alpha,\beta,y} \equiv y_\beta - y_\alpha \) are the \( x \) and \( y \) components of the separation vector \( r_{\alpha,\beta} \), respectively.

**Supp. Fig. S2.** \( u_{xx} \) (left) and \( u_{yy} \) (right) components of strain field due to a contractile force dipole oriented along the \( x \)-axis in elastic half-space of a linear, isotropic medium. \( u_{xx} \) component shows the \( \nu = 0.1 \) (top) map whose orientational distribution is that of an electric field from a quadrupole, while \( \nu = 0.5 \) (bottom) resembles an electric octupole. \( u_{xy} \) has a similar structure for both shown values of Poisson’s ratio, \( \nu \).
Supp. Fig. S3. Schematic of two interacting particles with all relevant angles and vectors labeled. \( \hat{n}_i \) are unit vectors of force dipoles. \( \theta'_i \) are angles of force dipoles with respect to the lab frame x-axis. \( \theta_\alpha \) and \( \theta_\beta \) are angles of force dipoles with respect to their separation vector \( r_{\alpha \beta} \) which has components \( r_{\alpha \beta, x} \) and \( r_{\alpha \beta, y} \).

Similarly, for the torque on cell \( \alpha \) by cell \( \beta \), we take a derivative of the elastic potential with respect to \( \theta_\alpha \)

\[
- \frac{\partial W_{\alpha \beta}}{\partial \theta_\alpha} = -\frac{P^2(1 + \nu)}{8\pi Er_{\alpha \beta}^3} \left( -6(\nu - 1) \sin 2\theta_\alpha - (\nu - 2) \sin 2(\theta_\alpha - \theta_\beta) + 15\nu \sin 2(\theta_\alpha + \theta_\beta) \right). \tag{10}
\]

B. Nondimensionalization of Langevin equations

We begin with the Langevin equation for cell position stated on the first line of the Model section of the main text.

\[
\frac{dr_\alpha}{dt} = -\mu_T \sum_\beta \frac{\partial W_{\alpha \beta}}{\partial r_\alpha} + \sqrt{2D_T} \eta_{\alpha, T}(t), \tag{1}
\]

where \( D_T \) is the effective translational diffusivity quantifying the random motion of an isolated moving cell, with \( \eta \) as a random white noise term whose components satisfy \( \langle \eta_i(t)\eta_j(t') \rangle = \delta(t - t')\delta_{ij} \). Note that \( \eta \) - the noise term describing active cell motility - has units of \( t^{-1/2} \).

\( W_{\alpha \beta} \) is a long-range elastic potential (full form shown in kdwrit equation number) when \( d \leq r_{\alpha \beta} \leq 3d \) and a steric spring given by

\[
W_{\alpha \beta} = \frac{1}{2}k(d - r_{\alpha \beta})^2 \quad \text{when} \quad 0 < r_{\alpha \beta} \leq d.
\]

We now choose characteristic time, length, and energy scales. Let \( r^* = \frac{r}{d} \) be a dimensionless distance vector scaled by cell size, let \( W^*_{\alpha \beta} = \left( \frac{P^2}{16Ed^5} \right)^{-1} W_{\alpha \beta} \) be a dimensionless energy scaled by elastic energy at cell length separation, and let \( t^* = \frac{\mu^2T}{16Ed^5k_B T_{\text{eff}}} \) be a dimensionless time scaled by an elastic interaction.

Non-dimensionalizing our translational Langevin equation using the above characteristic scales gives us the following equation

\[
\frac{dr^*_\alpha}{dt^*} = -\sum_\beta \frac{\partial W^*_{\alpha \beta}}{\partial r^*_\alpha} + \sqrt{\frac{2}{A}} \eta^*_{\alpha, T}(t^*), \tag{2}
\]

where

\[
A \equiv \frac{P^2\mu_T}{16Ed^5D_T} = \frac{P^2}{16Ed^5k_B T_{\text{eff}}} \tag{3}
\]

is a dimensionless parameter that is the ratio of characteristic elastic energy to an effective temperature called the effective elastic interaction.

The Langevin equation for cell orientation is given by

\[
\frac{d\hat{n}_\alpha}{dt} = -\mu_R \sum_\beta \left( \hat{n}_\alpha \times \frac{\partial W_{\alpha \beta}}{\partial \hat{n}_\alpha} \right) + \sqrt{2D_R} \eta_{\alpha, R}(t) \tag{4}
\]

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where $\hat{n}$ is the cell orientation and $D_R$ is the effective rotational diffusivity quantifying the random reorientations of an isolated moving cell.

Nondimensionalizing eqn. 4 with the same scales as in the translational Langevin equation and assuming $\mu_R d^2 = \mu_T$ and $D_R d^2 = D_T$ gives us

$$\frac{d\hat{n}_\alpha}{dt^*} = -\sum_\beta (\hat{n}_\alpha \times \frac{\partial W_{\alpha\beta}^*}{\partial \hat{n}_\alpha}) + \sqrt{\frac{2}{A}} \eta_{\alpha,R}(t^*) .$$ \[5\]

C. Numerical methods

We can rewrite the nondimensionalized Langevin equations obtained in Appendix B in a discrete form as follows:

$$r_{\alpha}^*(t + \Delta t) = r_{\alpha}^*(t) - \sum_\beta \frac{\partial W_{\alpha\beta}^*}{\partial r_{\alpha}^*} \Delta t + \sqrt{\frac{2}{A}} \eta_{\alpha,T} \sqrt{\Delta t} , \tag{1}$$

and

$$\theta_{\alpha}^*(t + \Delta t) = \theta_{\alpha}^*(t) - \sum_\beta \frac{\partial W_{\alpha\beta}^*}{\partial \theta_{\alpha}^*} \Delta t + \sqrt{\frac{2}{A}} \eta_{\alpha,R} \sqrt{\Delta t} , \tag{2}$$

where $\theta_{\alpha}^* \equiv \tan^{-1} \frac{y_{\alpha}}{x_{\alpha}} + \theta_{\alpha}$ is the angle between $\hat{n}_\alpha$ and the lab frame x-axis. A schematic of all the variables used is shown in Supp. Fig. S3. The time step used in the simulations is $\Delta t = 0.000625$. Each cell is initialized at a random orientation and position inside the simulation box - a square with length $L$. The position and angle of each cell is updated at every interval $\Delta t$ according to equations C1 and C2 with periodic boundary conditions. The simulations are run for $4 \times 10^4$ time steps with annealing of the effective temperature to avoid metastable states and to promote cell activity before many contacts form. The cells are kept at the final effective temperature for $1/4$ the total simulation time which equates to roughly ten hours of experimental time, an appropriate time after which to report cell configurations.

D. Phase portrait of simulation final snapshots on compressible and incompressible substrates

Fig. S4b is shown in the main text. We now show the phase portrait for the low $\nu$ case in Supp. Fig. S4a. While the trends and general dependence on $A$ and $N$ is the same for both values of Poisson’s ratio, we can see from the $A = 10$, $N = 300$ cases that $\nu = 0.1$ forms longer chained, larger ringed structures than the $\nu = 0.5$ system which forms compact structures of tight rings and many junctions.

Supp. Fig. S4. Simulation snapshots of final configurations in the parameter space of number of cells and $A \equiv \frac{E_c}{k_B T_{eff}}$, the ratio of the characteristic elastic interaction strength and noise, for $\nu = 0.1$ (left) and $\nu = 0.5$ (right). At lower packing fractions, cells form segments of branches and stems. At lower $A$ values, cells remain isolated. At higher values of $A$ with sufficient packing fraction, cells form space spanning network configurations characterised by rings, branches, and junctions. At higher packing fractions, parallel chains occur frequently in these networks.
E. Computational Analysis of Networks

Identifying clusters. Each cell is assigned to a cluster by assigning an initial cell to zeroth cluster. Then the cells in its neighbor list - a list identifying all other cells that are within $1.2d$ of the central cell - are assigned to this cluster. The neighbor list of each of these neighbors are assigned this cluster label in an identical way. Once all neighbor lists have been exhausted, we search for unassigned cells and repeat the process with an incremented cluster number until every cell belongs to one or the other cluster. Once each cell belongs to a cluster, cell-cell distances are checked. If the distance between any two cells within the same cluster is greater than or equal to the size of the simulation box, we consider that realization of the network to be percolating. This calculation is done at the final time step of forty simulations per data point shown in Fig. 3 for dipoles and ten simulations per data point for diffusive sticky disks. The average value and corresponding error are then plotted as a function of packing fraction $\phi$ in Fig. 3a and of the effective elastic interaction parameter $A$ in Fig. 3b.

Identifying junctions/branches. Final configurations of cells, like those shown in Figs. 1 and 2, are re-plotted with elongated black markers on cell positions along the direction of the dipole axis. This gives us networks like those shown in Supp. Fig. 11. These images are imported into imageJ (3), Gaussian blurred, intensity thresholded, binarized, and skeletonized. By then using the "Analyze Skeleton" plugin in ImageJ, we obtain skeleton information including the full branch length distribution and junction counts (4).

Identifying rings. Instead of using the "Analyze Skeleton" plugin in ImageJ, we invert the binarized image described above and utilize the "Analyze Particles" function of ImageJ to obtain a distribution of rings and ring areas in the networks.

F. Critical packing fraction dependent on box size

In the main text, the connectivity percolation we report is for a specific box size $L = 26.66d$. This curve is subject to shift and/or dilate/contract under varying the box size (Supp. Fig. S5). The box size we chose to use in the main text is appropriate (given our characteristic length scale of $\approx 50\mu m$ to compare to in vitro experiments of cells on compliant substrates.

\[ v = 0.1, A = 10 \]

Supp. Fig. S5. Critical packing fraction of elastic dipoles decreases with increasing box size. Due to the highly anisotropic nature of elastic dipolar interactions, dipoles will percolate at lower critical area fraction as (near the transition) area scales as $L^2$ whereas cluster size scales as $L^{d_f}$ where $d_f$ is the fractal dimension. Thus, the critical packing fraction will go as $L^{2-d_f}$ where $d_f - 2 < 0$.

G. Mapping the effective interaction parameter to substrate stiffness

We have considered the effective elastic interaction, $A$, to be the model parameter which encodes stochasticity, cell forces, and substrate stiffness. We wish now to relate this parameter to an easily accessible and measurable experimental value - substrate stiffness. By casting $A$ in
Mapping $A$ to $E$. Four curves characterized by various choices of optimal substrate stiffness $E^*$ for $A_0$ are shown. Range of $A$ values mapped to $E$ decreases as the choice of optimal stiffness increases.

**Table S1. Contractility and optimal stiffness of various cell types.**

| $P_0$ (J) | $E^*$ (kPa) | Cell Type          |
|-----------|-------------|--------------------|
| $10^{-12}$ (6) | 1-10 (8, 9) | Endothelial        |
| $10^{-13}$ (10) | 0.1 (11, 12) | Neuron             |
| $10^{-9}$ (13) | 20 (11)     | Smooth Muscle      |
| $10^{-11}$ (14) | 10 (14)     | Astrocyte          |

Adaptivity of the cell traction is modeled by considering a force dipole magnitude that scales with substrate stiffness as (15),

$$P(E) = P_0 E / (E + E^*).$$

110 terms of substrate stiffness, we aim to predict trends with varying substrate stiffness, which can be directly tested in experiment. It is known from traction force experiments (such as Ref. (5)) that cells on elastic substrates adapt their forces to substrate stiffness. Adherent cells on softer substrates build fewer and smaller focal adhesions. With increasing substrate stiffness, cells spread more and exert stronger traction forces which saturate to a constant value beyond a typical substrate stiffness $E^*$ which depends on cell type (table S1). This mechanical adaptivity of the cell traction is modeled by considering a force dipole magnitude that scales with substrate stiffness as (15),

$$P(E) = P_0 E / (E + E^*).$$
Supp. Fig. S8. Percolation peak width as a function of $A_0$. (a) Percolation probability as a function of substrate stiffness for various values of $A_0$. (b) Blue curve shows the analytic expression for percolation peak width quadratic in $A_0$ gives good agreement with mappings from simulation data, shown in red dots obtained from (a), except at lower values of $A_0$ where the analytical expression breaks down due to an assumption of transition ($p_{\text{max}} \approx 1$). (c) Percolation probability as a function of elastic substrate stiffness where the optimal stiffness is assumed to be 1 kPa. Percolation peak is centered on critical stiffness and has a width dependent on both packing fraction and effective temperature.

Plugging in 1 to our definition of $A$ gives us $A = \frac{P_0^2}{16k_B T_{\text{eff}} d^3}$, which has a non-monotonic dependence on substrate stiffness, as seen in Supp. Fig. S6, reaching a maximum of $A_0$ where $A_0 \equiv \frac{P_0^2}{16k_B T_{\text{eff}} d^3}$ at $E = E^*$. We now have a mapping from effective interaction parameter $A$ to substrate stiffness $E$ which we can directly relate to experiments. We know from experiments of endothelial cells on elastic substrates, that cells can form networks or remain isolated from one another depending on the substrate rigidity (8). This is to say that there is a range of viable substrate stiffnesses over which cells will self-assemble into vascular networks. We now ask if we can predict the viable range of stiffnesses that accommodate network formation. We use the metric of percolation probability to quantify the tendency for network formation. We know that we can make these predictions numerically as we now have a mapping from $A$ to $E$ for our simulations. Percolation vs. substrate stiffness curves are shown in Supp. Fig. S8b for various values of $A_0 \equiv \frac{P_0^2}{16k_B T_{\text{eff}} d^3}$, the effective elastic interaction without stiffness dependence.

We now seek to develop an analytic treatment of the substrate dependent percolation metric and obtain a closed form expression which predicts the range of substrate stiffnesses conducive to network formation. Supp. Fig. S7 shows that percolation vs. $A$ is well fit by a hyperbolic tangent function with two fit parameters $A^*$ and $k$ such that

$$p = .5 \tanh \left( \frac{A - A^*}{k} \right) + .5,$$

[2]

where $p$ is the percolation probability. We will use the full width at half maximum (FWHM) to represent the range of values over which networks are formed. In order to find the FWHM, which we will call $\Sigma$, of the above function, we set $p$ equal to half of its maximum value, which we assume is 1. This gives us the condition $A = A^*$, which we can rewrite in the following way

$$E^2 + \left( 2E^* - \frac{A_0}{A^*} \right) E + E^{*2} = 0.$$

[3]

Thus, we obtain an expression for the FWHM of our percolation curves given by

$$\Sigma = \sqrt{\left( \frac{A_0}{A^*} - 2E^* \right)^2 - 4E^{*2}}.$$

[4]

Supp. Fig. S8a shows a comparison of peak width for various values of $A_0$ computed with 4 and computed numerically from Supp. Fig. S8b. The plot shows great agreement between the analytical prediction and numerical results except at values of $A_0$ that are close to the analytical
solution condition $\frac{kT}{\ln(\kappa) A^*} \geq 4E^*$. This disparity, shown in the inset of Supp. Fig. S8a is due to the assumption that the maximum percolation value is 1, which is not the case for the red curve in Supp. Fig. S8b.

Thus, $4$ provides us a closed form expression, valid over a large interval of parameter space, for the range of substrate stiffnesses over which cells will form percolating networks. This value is dependent on cell forces, effective temperature, cell size, and a fit parameter $A^*$ which represents the position of the percolation transition. In particular, it predicts that higher force dipole magnitude ($P_0$), lower noise ($T_{\text{eff}}$), and higher cell density corresponding to lower required elastic interaction for percolation ($A^*$), all lead to wider peaks in percolation vs substrate stiffness.

H. Junction count shows similar behavior to neighbor counts

Supp. Fig. S9. Junction density shows a trend similar to neighbor counts. (a) Junction density vs. $A$ shows at low $A$, few cells are part of a junction. As $A$ increases, irreversible networks structures are formed where the $\nu = 0.5$ systems exhibit a greater capacity to form junctions - structures which produce greater neighbor counts. (b) Junction density vs. $\phi$ (or $N$) increases as a function for all $N$ when $\nu = 0.5$. Junction density begins to decrease at highest $\phi$ as the system begins to form more parallel strings in the low $\nu$ case.

Another metric that can be used to probe the morphologies of our branched networks is junction density - the number of cells connected to a node after skeletonizing normalized by the total number of cells. Junction density is shown in Supp. Fig. S9 as a function of $A$ (left) and $\phi$ (or $N$) (right). Unsurprisingly, junction density vs. $A$ follows the same trend as neighbors vs. $A$ shown in Supp. Fig. S18 since junctions are structures which promote higher neighbor counts. In contrast with neighbors vs. $\phi$, our highest value of packing for junction density exhibits a different trend. While neighbor counts continue to increase, junction density slightly decreases for $\nu = 0.1$. This is due to the ground states explored in the previous section. At our highest packing fraction, we begin to transition out of the dilute regime where we know $\nu = 0.1$ dipoles will form parallel strings. Tightly packed parallel strings will have a high neighbor count but no junctions.

I. Dependence of percolation on packing fraction and elastic interactions

While we report several percolation curves in Fig.3 of the main text, Supp. Fig. S10 shows a percolation contour map in $(A,N)$ space. At low $A$, $\nu = 0.5$ percolates more reliably than the low $\nu$ counterpart as the system is more resilient to noise. At $A \geq 5$, however, $\nu = 0.1$ percolates more reliably with fewer cells as we have seen the lower $\nu$ system forms more extended structures in general. The full percolation map then shows that networks at low Poisson’s ratio are more efficient with respect to number of cells, whereas the high Poisson’s ratio networks more efficient with respect to effective elastic interaction.

J. Irrigation area reveals a marginal efficiency for networks at low Poisson’s ratio

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. We can then determine the density of interconnections within our networks. Supp. Fig. S11 shows the filled area fraction of our networks as a function of homogeneous dilation factors. Realizations of these dilated networks at different dilation factors are shown as shaded regions in part c and d. This plot shows that the lower $\nu$ case increases area coverage as a function of dilation faster than the higher $\nu$ case. This reinforces the result in Fig. 6e as area fraction growth rate is maximized when cell overlap and proximity is minimized. Since higher values of Poisson’s ratio produce networks with more compact structures of higher neighbor counts, its area coverage does not scale with dilation as strongly as the lower $\nu$ case.
Supp. Fig. S10. Percolation contour plots show \( \nu = 0.1 \) is more efficient with respect to \( N \) while \( \nu = 0.5 \) is more efficient with respect to \( A \). (a) Color represents percolation probability in \((A,N)\) space for \( \nu = 0.1 \) (left) and \( \nu = 0.5 \) (right). For \( A < 5 \), \( \nu = 0.5 \) is more percolating while for \( A \geq 5 \), \( \nu = 0.1 \) is more percolating.

Supp. Fig. S11. Substrate compressibility alters area coverage of networks. (a) Fraction of available area covered by simulated networks at \( A = 10 \) and \( \phi = .33 (N = 300) \), as the cell area is uniformly inflated by a dilation factor. \( \nu = 0.1 \) exhibits greater area for given dilation than the \( \nu = 0.5 \) case. This is due to the fact that higher values of \( \nu \) produce networks with more compact structures like junctions and 4-rings. These structures overlap one another when inflated unlike sparse networks with long branches. (b) Ratio of area coverage of \( \nu = 0.1 \) to \( \nu = 0.5 \) for \( A = 10 \) and \( \phi = .33 (N = 300) \). The plot increases sharply past unity then saturates to one at area limited dilation. (c) Visualizations of homogeneous dilation of a representative \( \nu = 0.1 \) network. (d) Visualizations of homogeneous dilation of a representative \( \nu = 0.5 \) network.

K. Branch length analysis of simulation box and a larger composite box shows boundary errors are minimal

We utilize skeletonization in ImageJ for branch length, junction count, ring count, and robustness. ImageJ, however, does not account for the periodic boundary conditions by which cells interact. These boundary errors could lead to an incorrect analysis for the aforementioned metrics. To study the effect of these boundary errors, we report the branch length histograms and average branch lengths for both the original simulation box results and a box comprised of a 3x3 replication of the original simulation box in order to reconstitute periodicity and minimize boundary errors. Supp. Fig. S12 shows that the error due to the boundary effects are minimal which lets us use the original box size for a
Supp. Fig. S12. Branch length distributions for both the original simulation box and a composite box reveal minimal boundary effects. (a) Branch length distribution for \( A = 10 \) and \( N = 300 \) with average branch length for our original simulation box. (b) Branch length distribution for \( A = 10 \) and \( N = 300 \) with average branch length when the original simulation box is made into an identical 3x3 grid of the simulation snapshots to reconstitute periodicity and study the effect of this boundary effect. Histograms are qualitatively similar and the error for the average branch lengths for both \( \nu = 0.1 \) and \( \nu = 0.5 \) is less than 5%. Thus, for computational feasibility of robustness studied in Fig. 7d, we use the original simulation box snapshots.

L. Simulation results for different choices of translational and rotational diffusivity

Supp. Fig. S13. Network formation tendency robust to differing diffusion coefficients. (left) Networks formed from assumption stated in main text \((D_T = d^2 D_R)\). (middle) Networks form when \( D_T = 0.5d^2 D_R \). (right) Networks form when \( D_T = 2d^2 D_R \).

While the results we present in the main text are for systems in which we choose the rotational and translational diffusivity to be proportional...
\(d^2 D_R = D_T\), this is not required to be the case for cells. The random movements of cells are caused by their internal physico-chemical activity, and the diffusivities are therefore not constrained by the fluctuation-dissipation theorem. Supp. Fig. S13 shows that both \(\nu = 0.1\)(top) and \(\nu = 0.5\)(bottom) systems give rise to similar network configurations, whether the rotational diffusion is half or double its translational counterpart. This suggests that a different choice of rotational and translational diffusivity does not change the tendency of dipoles to form networks.

**M. Simulation results for different choice of interaction cutoff range**

Supp. Fig. S14. Network formation is qualitatively similar for longer cutoff ranges. (left) Networks formed from cutoff stated in main text (\(r_{\text{cut}} = 3d\)). (middle) Qualitatively similar network structures form when \(r_{\text{cut}} = 8d\). (right) Qualitatively similar network structures form when \(r_{\text{cut}} = 13d\).

In the main text, we cut off the long range elastic interactions at a value of \(3d\), consistent with physiological limitations seen in cell culture experiments. Supp. Fig. S14 shows utilizing much longer cutoff distances (\(8d\) or \(13d\)) does not qualitatively change the assembly behavior.

**N. Global ground states from on-lattice simulations**

We can gain intuition on the common motifs in the network morphology, especially in the higher density situations, by constraining the cell dipoles to a lattice. This corresponds to a situation where the dipoles have only rotational freedom, but the translation diffusion is very low leading to cells staying in their original positions. Previous Monte-Carlo simulations of contractile force dipoles on a hexagonal lattice with rotational freedom showed the difference in ground state configurations between two different values of the Poisson’s ratio of the elastic substrate - \(\nu = 0.1\) and \(\nu = 0.5\) (7). In Supp. Fig. S15, we reproduce these results using our Brownian dynamics simulations. Supp. Fig. S15a shows the ground state configurations of elastic dipoles where \(\nu = 0.5\) in orange and \(\nu = 0.1\) in blue. The dipoles tend to align globally at lower values of Poisson’s ratio while at the higher Poisson’s ratio, the dipoles exhibit a mutual perpendicular alignment into “4-rings”. The mutual alignment of the dipoles is measured by a nematic order parameter magnitude, \(S = 2\langle\cos^2 \theta\rangle - 1\). Supp. Fig. S15b shows this order parameter as a function of effective elastic interaction. At low values of \(A\), noise destroys any order and coherent collective orientation. As \(A\) increases, elastic interactions overcome stochastic effects and cells align with each other (become orthogonal to their neighbors) when \(\nu = 0.1(0.5)\). Knowing the preferred configurations of these force dipoles in such a constrained case as a hexagonal lattice provides us the intuition with which we can understand the behavior of cells.

**O. Experimental validation of primary model assumption - contractile force dipoles**

**Traction Force Microscopy**: GFP-HUVECs were seeded on fibronectin-coated 1.1kPa PAA hydrogels containing embedded 1\(\mu\)m fluorescent beads (Fisher) at a density of \(8 \times 10^3\) cells/cm\(^2\). After 20 hours of culturing, 1X trypsin (Corning) was added to the HUVEC cultures, effectively cleaving, and removing the cells from the PAA hydrogel. Images of target areas were collected before and after the addition of trypsin and were subsequently stacked and corrected for drift with the “Linear Stack Alignment with SIFT” function in FIJI (16). Once aligned the images were uploaded into MATLAB where the bead displacements and traction forces were calculated using a previously published Fourier transform traction cytometry algorithm (17). A primary assumption of the model we have used in this work is that cells tend to exert...
Supp. Fig. S15. Final snapshots from on-lattice simulations of contractile for dipoles with only rotational freedom reveals ground state structures. Contractile force dipoles assume a global state of what previous literature has referred to as “4-rings” \((\nu = 0.5)\) when \(\nu = 0.1\) blue. Contractile force dipoles assume a global state of linear strings \((\nu = 0)\) when \(\nu = 0.5\) system becomes entirely unordered or ant unordered (4-rings). (b) Nematic order parameter, \(S \equiv 2 \langle \cos^2 \theta \rangle - 1\) where \(\theta\) is the difference between cell orientation and the average director, reveals as elastic interactions overcome noise, the \(\nu = 0.1\) system becomes entirely ordered (strings) while the \(\nu = 0.5\) system becomes entirely unordered or ant unordered (4-rings).

P. Experimental branch lengths

One of the metrics we have used to characterize the morphology of our simulated dipole assemblies is branch length. While it is difficult to directly compare simulation to experiment with branch lengths due to there not being a common length scale, we report the branch lengths for two different seeding densities and post seeding times (Fig. S17). The branch lengths show a saturation in substrate stiffness.

Q. Model network morphological features depend on substrate compressibility given by Poisson’s ratio

While percolation is by nature a global quantity describing the whole network, we now employ more local metrics to classify the topology of our networks. Figs. S18a-d show characteristic networks of \(N = 300 (\phi = 0.33)\) cells for \(\nu = 0.1\) (top) and \(\nu = 0.5\) (bottom) when the system is well past the percolation transition \((A = 10, \text{right})\), and at the shoulder of the transition \((A \leq 1, \text{left})\). The particles in these snapshots have been given artificially elongated bodies along their dipole axis to emphasize the backbone of the network and aid the image analysis process, detailed in SI section E. The relative number of the different topological features of these networks, e.g. open ends, junctions, and rings, will determine the average number of neighbors (or coordination number, \(z\)) of each cell dipole.

Fig. S18e shows that the average number of neighbors increases with effective elastic interaction \(A\) (for \(N = 300\) fixed) and cell number density (for \(A = 10\) fixed), for both \(\nu = 0.1\) and \(\nu = 0.5\) that saturates in \(A\). This quantity is calculated for the final simulation configuration of three networks per value of Poisson’s ratio. The saturating neighbor count for each Poisson’s ratio is reached for percolating networks, and corresponds to the disparate topological features characteristic of these two cases. The higher \(\nu = 0.5\) (incompressible) substrate case shows a higher saturating neighbor \((z > 3)\), which indicates the preeminent structures inherent to this network are junctions and tighter rings (with up to 4 neighbors), consistent with the characteristic simulation snapshots in Fig. S18d. The saturating neighbor count for low \(\nu = 0.1\) (more compressible) substrates is lower \((2 < z < 3)\). This suggests that these networks exhibit long chains as well as more interconnected structures like junctions and rings, consistent with the characteristic simulation snapshots shown in Fig. S18b. This trend is seen over a wide range of packing fractions as shown by the inset in Fig. S18e. The qualitative differences between the two types of networks ultimately arise from the different orientational dependencies of the deformation induced by a dipole, as shown in Fig. 1d, with a transition expected at \(\nu = 0.3\) \((\nu = 6)\). We note that these results are for a relatively dilute regime \((\phi = 0.33)\), whereas in the limit of complete packing, neighbor count would saturate to a maximum possible value of 6.
Supp. Fig. S16. Cells exert dipolar forces on substrate. (a) Full field of view - nucleus stained in blue. White box shows the region of analysis for single cell traction force microscopy. (b) Traction force microscopy analysis for single cell shows displacements are radially inward and peaked at two regions on either side of the cell nucleus suggesting anistropic contractile dipolar force production.

Interestingly, the networks on the lower Poisson ratio substrates exhibit a saturating neighbor count ($z \approx 2.6$), that is very close to that for the predicted rigidity percolation threshold for elastic fiber networks ($z_c = 2.67$) (18, 19). This may be attributed to the self-assembled linear chains that mimic semiflexible polymers (20), with bending rigidity of a “polymer” of disks, set by the dipolar interaction strength, $A$. This implies that although we do not measure the rigidity of networks in simulation, the connectivity percolation is closely related to it and predicts the onset of rigidity percolation threshold as well. Such a transition from isolated, fluid-like, motile cells to a mechanically rigid state has been shown to be biologically important for epithelial cells during development and disease (21), and may also be relevant to network-forming endothelial cells.

Similarly to percolation, Fig. S18f shows that neighbor counts exhibit peaks over intervals of substrate stiffness centered around the characteristic substrate stiffness (chosen to be $E^* = 1$ kPa) and can be narrowed and decreased by increasing effective temperature and decreasing packing fraction. This result is consistent with Fig. 1 where cells on substrates that are too soft or too stiff remain isolated, and have fewer neighbors than those in network configurations that form at the optimal stiffness range.

R. Full Order Parameter

In the main text, we show a phase diagram of order parameter OP ≡ $\Theta(p - p_T)p + \Theta(p_T - p)(.25 + .5(s - s_T))$ where $p$ and $s$ are the normalized and scaled values of percolation and shape parameter, respectively, and $p_T$ and $s_T$ are the threshold values of percolation and shape factor set to .7 and .95, respectively. There, we limit substrate stiffness (x-axis) to the maximum value tested in our experiments. We note here, however, that the model predicts a prominent non-monotonicity of this order parameter (Fig. S19).

S. Order Parameter Thresholds informed by largest cluster and spatial extent

In the main text, we classify experimental systems into percolating “networks” if the average percolation is greater than $p_T = 0.7$; into elongated “chains” if the percolation is below $p_T$ and shape factor above $s_T = 0.95$; and isotropic “isolated” clusters if percolation is below $p_T$.
Supp. Fig. S17. Average branch lengths of experimental systems at both low and high seeding density show a saturation in substrate stiffness.

(a) (b) (c) (d) 
$$A \leq 1$$  $$A = 10$$ 
$$\nu = 0.1$$  $$\nu = 0.5$$

Supp. Fig. S18. Neighbor counts reveal relative prevalence of various morphological structures in networks formed by elastic dipolar interactions. (a)-(d) Simulation snapshots of cell assemblies at the shoulder of the percolation transition (left) and well above the percolation transition (right). (e) Number of neighbors as a function of A when $$\phi = 0.33 (N = 300)$$ for $$\nu = 0.1$$ - blue - and $$\nu = 0.5$$ - orange where a neighbor in this context is defined as a cell $$\alpha$$ whose center is within one and a half cell diameters away from cell $$\beta$$ ($$|r_{\alpha\beta}| \leq 1.5d$$). While the number of neighbors is relatively insensitive to A, there is a marked difference between the two values of Poisson's ratio. Across A space, cells on substrates of higher $$\nu$$ values accumulate more neighbors than the lower $$\nu$$ cases. Inset shows number of neighbors as a function of packing fraction for $$A = 10$$. Cells on higher $$\nu$$ value substrates have more neighbors than the low $$\nu$$ case regardless of packing fraction. (f) Number of neighbors as a function of substrate stiffness. Optimal stiffness is assumed to be 1 kPa. $$N = 200 (\phi \approx 0.22)$$ exhibits an average neighbor count of 1-2 indicating the prominence of short chains. $$N = 300 (\phi \approx 0.33)$$ case shows average neighbor counts of 2-3 indicating an abundance of chains, rings, and junctions. The peak in neighbor count over stiffness is taller and wider for lower effective temperature and higher cell density. Each data point and error bar represents the average and standard error of the mean, respectively, of ten simulations.

and shape factor is below $$s_T$$. Supp. Fig. S20 shows that the percolation threshold value corresponds to the largest cluster group (as determined by a density-based spatial clustering algorithm with a max distance set to one percent (22)) account for more than twenty percent of total cell area. The shape factor threshold is chosen as it is greater than two simulation cells in a line - the elongated morphology we want to characterize with this metric.
Supp. Fig. S19. “Phase diagram” of the distinct morphology of cell clusters, based on cell density and substrate stiffness. The color map represents a composite order parameter \( \langle OP \rangle \), detailed definition in the text) designed to capture both the cluster percolation probability \( p \) and the cluster shape parameter \( s \) as a single value. Ranges of the order parameter values \( \langle OP \rangle > 0.7, 0.25 \leq \langle OP \rangle \leq 0.7 \) and \( < 0.25 \) correspond to percolating “networks”, elongated but disconnected “chains”, and isotropic “isolated” clusters, respectively. The background color map is created by interpolating over a set of order parameter values obtained from simulations of varying cell number and elastic interaction strength.

Supplementary Movies

**Movie 1.** \( A = 1 \) - Simulation of contractile force dipoles at the shoulder of percolation when \( \nu = 0.1 \).

**Movie 2.** \( A = 0.625 \) - Simulation of contractile force dipoles at the shoulder of percolation when \( \nu = 0.5 \).

**Movie 3.** \( A = 10 \) - Simulation of contractile force dipoles well past percolation transition when \( \nu = 0.1 \).

**Movie 4.** \( A = 10 \) - Simulation of contractile force dipoles well past percolation transition when \( \nu = 0.5 \).
Supp. Fig. S20. Largest relative cluster group size. Values are the sum of the largest clusters contributions as determined by the DBSCAN algorithm with tolerance .01, corresponding to a one percent difference in relative cluster size. Only those whose largest cluster group contributes at least twenty percent the total cell area are classified as percolating structures.

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