Collective Motility, Mechanical Waves, and Durotaxis in Cell Clusters

Youyuan Deng\textsuperscript{1,2}, Herbert Levine\textsuperscript{1,3}, Xiaoming Mao\textsuperscript{1}, and Leonard M. Sander\textsuperscript{1,5,*}

\textsuperscript{1}Center for Theoretical Biological Physics, Rice University, Houston, Texas 77030-1402, USA; \textsuperscript{2}Applied Physics Graduate Program, Rice University, Houston, Texas 77005-1827, USA; \textsuperscript{3}Department of Physics, Northeastern University, Boston, Massachusetts 02115, USA; \textsuperscript{4}Department of Physics, University of Michigan, Ann Arbor, Michigan 48109-1040, USA; \textsuperscript{5}Center for the Study of Complex Systems, University of Michigan, Ann Arbor, Michigan 48109-1107, USA; \textsuperscript{*}E-mail: lsander@umich.edu

When epithelial cell clusters move in a collective manner on a substrate, mechanical signals play a major role in organizing the coherent behavior. There are a number of unexplained experimental results from traction force microscopy for a system of this type (MDCK cell clusters). These include: the internal strains are tensile even for clusters that expand by proliferation; the tractions on the substrate are conﬁned to the edges of the cluster; in many cases there are density waves within the cluster; there is collective durotaxis of the cluster even though individual cells show no effect; and for cells in an annulus there is a transition between expanding clusters with proliferation and non-proliferating cases where cells rotate around the annulus. We formulate a simplified mechanical model which explains all of these effects in a straightforward manner. The central feature of the model is to use a molecular clutch picture which allows “stalling” – inhibition of cell contraction and motility by external forces. Stalled cells are passive elements from a physical point of view and the un-stalled cells are active. When we attach cells to the substrate and to each other, and take into account contact inhibition of locomotion, we get a simple picture that gives all the mechanical results noted above.

1. Introduction

Eukaryotic cells can often move by a judicious use of forces generated by their cytoskeleton and applied to their surroundings (1). The observed motion can range from individual cells moving through extracellular space to the coordinated collective motion seen during developmental morphogenetic processes such as gastrulation. In fact, many processes that are important in biology and medicine involve the collective motility of epithelial cell sheets and clusters. In addition to morphogenesis, this type of motion is important during tissue repair, and cancer invasion (2); for a recent review see (3). A particularly striking example occurs as part of the progression of inflammatory breast cancer, where the rapid progress of the disease has been connected to collective cell motion (4, 5).

Aside from its direct biological relevance, the phenomenon of collective cell motion is of great interest from the perspective of non-equilibrium physics. Individual cells are active particles (6), able to use their stores of ATP to remain far from equilibrium, do work on their surroundings and on their neighbors, and more generally evade many of the features we associate with non-active materials. During collective motion, these cells coordinate their activity by mechanical coupling, for example by connections such as adherens junctions (7). This coordination can further be modulated by signaling processes, helping to determine cellular front-back polarity (8) which affects the directionality of applied forces. How the interplay of all these effects give rise to the observed phenomenology is a challenging conceptual problem. In this paper we address the problem with a simplified mechanical model that helps explain many of the features which have been observed. For simplicity we focus on one dimensional geometries (lines of cells moving on a substrate), although extension to two dimensions is straightforward. Collective motion in 3d is more challenging as the cells do not have a convenient flat surface upon which to exert traction and will require physics beyond what is discussed here.

Collective motility has been studied in a wide variety of experiments, for a wide variety of cell types (9). From the physics perspective, major progress has been made by utilizing a convenient choice of cell, Madin-Darby Canine Kidney (MDCK) cells, moving on well-defined substrates that can be patterned by standard lithography techniques. Our primary concern relates to the physical forces between the cells, and these have been measured in several studies using traction force microscopy (10–12). There is significant evidence from this body of work that the interaction between cells that produces collective behavior is primarily mechanical.

The mechanics of the clusters has some odd features: for example in (10) it was shown that the mechanical stress in the center of a cluster is primarily tensile even though there is cell division and the cluster continually expands in size. In these experiments tension and cell density varied on the scale of millimeters. Conversely, in (11, 12) it is shown that the intercellular tension increased up to a plateau within a few cells of the boundary. In these newer experiments it was shown that most of the traction on the substrate comes from the outer parts of the cluster – in terms of net force applied, it is as if the center is not attached to the substrate. Our model gives a plausible explanation for both behaviors, and shows that a key parameter is the rate of cell division.

There have been additional findings regarding the mechanics of these collectively moving cells. Sometimes, mechanical...
waves are observed within the cluster, waves that move towards the edge faster than the overall expansion speed (12). It is not yet clear exactly what is the mechanism behind these waves. Most recently, an experiment which confines one-dimensional clusters of cells in annular rings (13) has shown a fascinating transition between growth with expansion and collective unidirectional motility without cell division. Again, a convincing explanation for this transition is lacking.

Individual cells can be sensitive to the stiffness of their environment, leading to the phenomenon of durotaxis (14–17) where cells move up stiffness gradients. It is therefore interesting to consider whether cells moving collectively can exhibit increased stiffness sensitivity. Several papers have (11, 18) investigated this type of collective durotaxis. In these cases individual cells exhibited negligible durotaxis, but the cluster did systematically expand towards the stiffer side. The salient feature seems to be that the cell mass acts as a giant single cell, as if the cells deep within the cluster were not connected to the substrate, as above. Our model, as we will see, gives rise to collective durotaxis without special assumptions.

There have been a number of attempts to formulate theoretical models which can explain the forces in the cell clusters and concomitant motility, e.g. (11, 19–21). These models take a variety of forms. Some authors have modeled the cell cluster as a continuous active medium. In (19) the cluster is treated as a viscous fluid with an effective viscosity and friction coefficient which interacts with a nematic-like polarization field. Continuum models are also used to investigate questions regarding the stability of the advancing tissue boundary (22–24). In (20) a continuum model is proposed for wave propagation using an assumed feedback between strain and an internal variable of the cell cluster; here there are phenomenological parameters whose exact significance is not clear. At the other end of the spectrum are models which attempt to fully resolve the shape degrees of freedom of the individual cells. These include cellular Potts models (25), vertex models (26) and phase-field approaches (27, 28). There has been only limited successes in using these models to study the detailed mechanical state of the cluster and the existence of the aforementioned waves. Finally, there are simplified cell approaches, ranging from the extreme of treating the cell as a single point (29) on upward to more complex collections of subcellular point-like elements (30). In (21), cells are treated as composed of two force centers coupled by a contractile spring and which interact with other cells via adhesion forces. The theory includes cell proliferation and an important mechanism for cells in close contact, namely contact inhibition of locomotion (CIL); see (3) and references therein. This effect describes the tendency for cells that collide to move away from each other. In the model described below we include these two effects. In (11) the cells are treated individually using a molecular clutch scheme (31) much like the one we propose in the work below. However, the observed feature that the tractions are localized at the edges of the cluster was put in by hand in (11).

In the following we present a one-dimensional model for cells connected to a substrate by bonds that represent focal adhesions, and the internal dynamics is given by a version of the molecular clutch scheme given in (31). Cells are joined by bonds and their motion is modulated by CIL. In our version of CIL, when two cells have a head-to-head collision, one or both of them (chosen at random) reverse polarization; in (21) a more general version is used. As will be explained in detail below, this polarization affects the distribution of adhesion sites, as is commonly seen in experiment (32); adhesions are formed in the front and are disassembled in the rear. The dynamics of each cell undergoes a cycle of contraction and protrusion, as was originally proposed for single cells (33, 34). What is new here is that the contraction is directly coupled to inter- and intercellular forces through the clutch mechanism. Namely, if the cell tensile stress is too high, the cells will not be able to contract and will instead “stall”. As we will see below, this notion of stalled cells is key to explaining many of the observed features of cluster mechanics. These features, to be discussed in detail below, include the aforementioned waves, the existence of collective durotaxis, and transitions from expanding clusters to collectively translating ones.

2. One dimensional model

In this section we describe a simplified, one-dimensional model for collective motility based on the molecular clutch concept (31). This enables us to give a unified account of many mechanical features of epithelial cell clusters.

A. Cell Motility and the Molecular Clutch Model. The starting point for our model is the assumption of a motility cycle where cells contract and partially detach from the substrate by breaking adhesive bonds, which can then re-attach after cell protrusion. We need explicit algorithms governing what happens to the cell position and to the forces during all the stages of this process. In (21, 35), each cell is considered to be composed of two subcellular elements that interact with a pre-defined active contractile spring force law. Other work (33, 34) assumes that the cells have a fixed contraction speed during that part of the cycle. Both of these assumptions are rather simplified views of the complex process of myosin motor mini-filaments walking along actin fibers.

Fig. 1. One-dimensional model for a single cell. The red lines are springs with spring constant $k$, representing adhesions to substrate. The adhesions detach with rate $k_{off}$ and attach with rate $k_{on}$. The cell length contracts according to Eq. 1. At the start of a contraction cycle, more adhesions form in the front half than in back. In the figure, the long bonds represent adhesions that attached to the substrate and the shorter ones represent ones that have detached. The heights of cellular components are for illustration only — the model is one-dimensional.

Here we use a variant of the molecular clutch model (31). In this more realistic account, the molecular motors that drive contraction have a nontrivial force-velocity curve and thereby allow the cell to stall (i.e. pause contraction) when the tension applied to the cell is too large. As shown in Figure 1, we model the cell body as a contracting one-dimensional “bar"
which is uniformly compressed around the mid-point by the contracting actin cytoskeleton. We take the forces generated by myosin motors to be concentrated at the midpoint, thus dividing the cell into front/back halves of equal lengths $L(t)$. The retarding force acting against contraction is generated by the adhesions to the substrate and the connections to the other cells; see below. This force is the same as the tension, $T$, at the cell midpoint. The condition for stalling is that $T$ is greater than $T_s$, the stall tension.

In a time step of length $dt$, the half-length contracts from $L$ to $L - dL$, where

$$dL = \begin{cases} \frac{dt}{2} v_j (1 - T/T_s) & \text{if } T < T_s, \\ 0 & \text{if } T \geq T_s. \end{cases} [1]$$

The cell starts each contraction cycle with half-length $L_0$. It then contracts for multiple steps according to Eq. 1, before reaching the minimum half-length of $(1 - r_{contr})L_0$. Afterwards, it reverts to $L_0$ by a step defined as protrusion, thereafter entering the next contraction cycle. This picture of contraction/protrusion is completed by modeling the dynamics of cell's attachment to the substrate.

We assume a rigid substrate approximation. The cell is attached to substrate with elastic adhesion bonds, which describe trans-membrane proteins such as integrin. We represent these as a number of springs with rest length zero. Consider a cell whose midpoint has coordinate $x_c$. At the beginning of a contraction cycle, a series of springs is formed with one end on cell body at $x_i^{(c)}$ and the other on substrate at $x_j^{(s)} = x_i^{(c)}$,

$i = -N_{adh,back}, -N_{adh,back} + 1, \ldots, -1, 1, 2, \ldots, N_{adh,front}$, and $N_{adh,back} < N_{adh,front}$, where negative $i$ indicates an adhesion in the back half, while positive the front. In this process, two adhesions of indices $i = -N_{adh,back}, N_{adh,front}$ form at both ends of the cell, and others are drawn by choosing the ratios from two groups of equally spaced probability distributions:

$$r_i^{(c)} = \frac{x_i^{(c)} - x_c}{x_{N_{adh,front}}^{(c)} - x_c}$$

$$\begin{cases} \mathcal{N} \left( \mu = \frac{i}{N_{adh,back}}, \sigma = \frac{1}{N_{adh,back}} \right) & \text{if } i = -N_{adh,back}, -N_{adh,back} + 1, \ldots, -1, \text{ and } N_{adh,back} < N_{adh,front}, \\ \mathcal{N} \left( \mu = \frac{1}{N_{adh,front}}, \sigma = \frac{1}{N_{adh,front}} \right) & \text{if } i = 1, \ldots, N_{adh,front} - 1. \end{cases} [2]$$

$\mathcal{N}$ denotes the normal distribution truncated to within $[-1, 1]$, so as to always lie within the cell body.

As the cell body contracts, the $x_j^{(s)}$ change, but the absolute coordinates $x_i^{(c)}$ are unchanged. Therefore, the $i$-th adhesion is stretched because $x_i^{(c)} \neq x_i^{(s)}$, and exerts a force $f_i = k (x_i^{(s)} - x_i^{(c)})$ on the cell. The adhesions can detach when $f_i$ becomes large. We take the rate of detachment to be:

$$k_{off} = K \exp(f_i/F_0), \quad \text{where } F_0 \text{ is the critical detachment force.}$$

Mechanical relaxation is a much faster process than a typical biochemical reaction. In our model cells undergo immediate mechanical equilibration by shifting midpoint position $x_c$ after each biochemical change, i.e. each contraction and any detachment/attachment of adhesions. If each half of the cell body is in equilibrium, the tension $T$ in Eq. 1 must be equal to the total force exerted on the half-body by the adhesions with the substrate and with adjacent cells (to be discussed later):

$$T = \sum_{\text{half cell}} (f_i + f_{inter}) [4]$$

Returning to the discussion of protrusion, the cell is allowed to contract by a maximum ratio $r_{contr}$. Then it “protrudes” by inverting $L(t)$ to $L_0$ and placing the back end at the $x_i^{(c)}$ of the current rear-most adhesion, as in the models of (33, 34). In a cluster of multiple cells, each cell is only allowed to protrude to occupy the inter-cellular space, and is prevented from overlapping with the neighboring cell, so they may protrude to a length smaller than $L_0$.

B. Contact Inhibition of Locomotion in Cell Clusters. To make a cluster in our model, the nearest ends of adjacent cells are joined by a spring with elastic constant $k$, and a fixed, non-zero rest length $l_0$. This elastic bond represents not only the separation between cells, but also the elasticity of the cell body. All adjacent cells are joined with springs of the same $k$ and $l_0$.

A subtlety is necessary in expressing the force and potential energy of these inter-cellular springs. For an isotropic harmonic spring with rest length $l_0 \neq 0$, the potential energy is usually taken to be $V(x_1, x_2) \propto \|x_1 - x_2 - l_0\|^2$. That is, the spring is equally inclined to restore the natural length in either direction. To account for volume exclusion, the inter-cellular adhesion should not allow an equilibrium where two connected cells intrude into each other. A more sensible form for our model is $V(x_1, x_2) \propto |x_1 - x_2 - l_0|^2$, with $l_0$ being a vector.

Contact inhibition of locomotion (CIL) is a process during which cells alter their direction of movement to avoid collision. In our one-dimensional model, there are two possible polarities, left or right. The polarity is defined by the distribution of cell-substrate adhesions — the half with more adhesions is the front half; cells always protrude from the front half. When a spring connects front ends of two cells and is being compressed, a head-to-head collision is taking place. Contact inhibition would result in disassembly and assembly of adhesion complexes in front and back, respectively. Our model approximates this by randomly relocating colliding cells’ detached cell-substrate adhesions in the front half to the back with rate:

$$k_r = K' \exp(f_{inter}/F_{CIL}), [5]$$

excluding the $N_{adh,front}$-th one, i.e. one located at the front end. Suppose adhesion $j$ is chosen to move, we then randomly choose an interval in the back half delimited by two adjacent adhesions $j'$ and $j' + 1$, where probability $P(\text{choosing } j' \text{ and } j' + 1) \propto l_{j'} - r_j^{(c)} - r_{j' + 1}^{(c)}$. The $j$-th adhesion then relocates to near the midpoint of $x_j^{(c)}$ and $x_{j' + 1}^{(c)}$:

$$r_j^{(c)} \text{ is drawn from } \mathcal{N} \left( \mu = \frac{r_j^{(c)} + r_{j' + 1}^{(c)}}{2}, \sigma = l_{j'}/8 \right).$$

See Eq. 2 for the meaning of $r_j^{(c)}$ and $\mathcal{N}$. Once the current rear half has more cell-substrate adhesions, the cell flips polarity, i.e. it protrudes from the end which now has more adhesions.

C. Cell Division and the Complete Algorithm. As in (21, 35), we adopt the idea that cells are likely to divide if the inter-cellular tension is large enough. At each step, if a cell’s tension

et al.  
PNAS | July 27, 2020 | vol. XXX | no. XX | 3
T is greater than $T_{\text{div}}$, the critical tension, it divides with constant probability $dt r_{\text{div}}$. Upon division, a newborn cell C’ of the same polarity is inserted next to the current cell C, randomly on the left or right. C’ virtually protrudes in place to avoid overlapping (see the discussion in Section A). The corresponding inter-cellular adhesion is cut to accommodate the new cell, and the nearest ends from adjacent cells are then reconnected. Note that the processes of applying CIL and of cell division are both significant configurational changes, and require mechanical equilibration immediately after each step.

The following is our complete algorithm for simulating collective motility:

- Start each cell with length $2L_0$. Initialize all the adhesions to be at their rest length.

- For each time step $dt$,

  1. For each cell, compute $T$ according to Eq. 4; contract according to Eq. 1; if the cell has reached $r_{\text{contr}}$, protrude; in rare cases when no adhesions remain attached to substrate, wait for next step. Adhesions are stretched. Equilibrate cell cluster by shifting $\{x_c\}$.

  2. For each cell, test for detachment of cell-substrate adhesions using Eq. 3, i.e. detach with probability $k_{\text{off}} dt$. Move $\{x_c\}$ to maintain mechanical equilibrium.

  3. For each cell, attach the free adhesions with probability $K dt$. Equilibrate.

  4. For each cell, apply contact inhibition of locomotion (CIL). Equilibrate.

  5. For each cell, test for cell division, i.e. divide with probability $dt r_{\text{div}}$ if $T \geq T_{\text{div}}$. Equilibrate.

Model parameters are listed in Table 1.

| Symbol | Meaning | Value |
|--------|---------|-------|
| $L_0$  | cell’s (maximum) half-length at the beginning of each contraction cycle | $5 \mu m$ |
| $v_f$  | cell’s free(maximum) contraction speed, w.r.t half-length | $5 \mu m/min$ |
| $r_{\text{contr}}$ | cell’s maximum allowed contraction ratio | 20% |
| $T_0$  | cell’s stall tension | 10 nN |
| $l_0$  | rest length of inter-cellular adhesions, also the initial inter-cellular separation | $5 \mu m$ |
| $k$    | spring constant of cell-cell and cell-substrate adhesions | 1 nN/\mu m |
| $K$    | reattachment rate and coefficient in detachment rate expression of cell-substrate adhesion | 10/min |
| $F_d$  | critical force for detachment of cell-substrate adhesions | 0.75 nN |
| $T_{\text{div}}$ | threshold tension of cell division | $0.99 T_s$ |
| $k_{\text{div}}$ | rate of cell division once $T \geq T_{\text{div}}$ | 1/min |
| $N_{\text{adh.}_{\text{back}}}$ | number of adhesions to substrate in back half | 8 |
| $N_{\text{adh.}_{\text{front}}}$ | number of adhesions to substrate in front half | 10 |

Table 1. Parameters in one-dimensional model

3. Simulation Results

A. Cluster Dynamics in the Absence of Cell Division. Several previous models (33, 34) describe a crawling cell’s motility cycle, but do not use the molecular clutch picture. In a reduced “cluster” consisting of a single isolated cell, the previous models are the limit of $T_s \to \infty$ of our current approach. (For an animation of a single free-moving cell, see Supplemental Information (SI) Movie S1.) To start to look at collective effects, we simulated a cluster consisting of two cells, aligned head-to-head. As the simulation starts, the two cells begin to collide. Because of the CIL mechanism, at least one of the two cells will eventually change its polarity. When one cell flips, the two cells will move together as a translating cluster. In rare cases, both cells will flip at the same time. (In a larger cluster this would eventually give rise to a static cluster, once the cells have moved far enough apart for the intercellular spring to exert enough force to reach the stall condition.) See SI Section 1 and SI Movies S2, S3 for more details. We will see that these two basic choices, a static cluster with an equilibrated tug-of-war versus a translating state, also characterize multicellular clusters. The tug-of-war case is more common when there are a large number of cells in the cluster.

In order to represent a large cell cluster, we repeated the procedure above for 50 cells connected by springs with random initial polarization, and used the dynamics described in Section 2. In Figure 2, we show results from one simulation for the polarization, cell contraction speed, force between cell and substrate, and inter-cellular tension. Note that after initial transients the colony settles down with large domains of like polarization. We always find that, eventually, the majority of the cells on the left are moving left and those on the right, to the right. In this case, we have “perfect” domains of similar size.

There are interesting features shown in Figure 2 that correspond to the experimental observations. The traction force is confined to the edges of the colony, even though all of the cells are attached to the substrate. The interior cells are mostly stalled, and the forces on either end of each domain approximately balance. Only at the edges are the cells pulling outwards; cf. (11, 12). Also, note that, as a function of time, the width of the cell colony saturates. In our model, there is a possible equilibrium between the traction forces at the edges and the internal force on the individual cells. Of course, in experiments, when cells are stretched too much they tend to divide. We will treat this effect in the next section.

We can give a qualitative explanation of some of the behavior seen in experiments by noting that in the case shown in Figure 2 most of the cells are stalled. Thus the only cells that are active elements (i.e. are contracting) are at the edges. The stalled cells are passive elements, and we can think of them as being dragged by the cells at the edges. The pattern is then rather easy to understand: as the dynamics proceeds, the interior cells will adjust as if they were a line of masses connected by springs, subject to viscous friction (from the attachment/detachment to the substrate), and pulled by forces at the ends. Clearly, the steady state will have the masses equally spaced, the inter-cellular tensions equal, and the traction on the substrate confined to the edges. This qualitative picture reproduces much of the experimental behavior in (11, 12). In SI Section 3 we give a semi-quantitative estimate of the number of active cells at the opposing edges.
The above argument suggests the domains of left- and right- polarized cells need not be equal in size for this state to be achieved. Having more stalled cells in one of the domains should not affect the overall balance of forces. Similarly, having a few “rebelling” cells, that is cells of the wrong polarity embedded inside large domains should not matter if these are not part of the edge layer. These predictions are consistent with simulations shown in SI Section 2. If however, there are not enough cells in one of the opposing domains to form a complete boundary layer and therefore not have enough cells to counteract the pulling by the active cells on the other side, the cluster will move. This is shown in Figure 3; even though not all the cells have the same polarity, the cluster moves to the left. The interior cells are not quite stalled; many of the cells contract but the interior tension still is nearly uniform. The cells exert a drag on the substrate throughout the cluster due to the “friction” of the adhesions, and the traction force with substrate still concentrates near boundaries but is non-zero in the interior. We can also have cases where nearly all the cells eventually have the same polarity, see SI Section 2.

Fig. 2. Space-time plot of a cell cluster of the one-dimensional model. (a) Polarity and half-length $L(x)$ of the cells. +− signs correspond to right/left polarities. (b) Cell contraction speed. Note that many of the cells barely contract, i.e. they are (nearly) stalled. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs. (e) Tension at each cell’s midpoint at $t = 1000, 3000, 5000$. Note how the interior cells gradually become stalled. (f), (g) Same quantities as in (c),(d), respectively, at $t = 1000, 3000, 5000$. Dashed lines in (e)-(g) correspond to the equivalent of the stall tension. Notation: $x_c$ is the coordinate of the cell midpoint; $x_{mid}$ is the coordinate of midpoint of the intercellular adhesions.

Fig. 3. Space-time plot of a cell cluster of the one-dimensional model. Not all cells eventually have the same polarity but there is overall translation. (a) Polarity and half-length of the cells. (b) Cell contraction speed. Note that many of the cells contract at least to some extent, (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs.

B. Cell Division and Mechanical Waves. We now turn to the effect of cell proliferation using the scheme outlined in Section C. In Figure 4 we show the effects of proliferation on cell clusters. We chose a value of $F_{div} = 0.99 T_s$ giving rise to occasional proliferation events. Since the randomness in initial polarity mainly leads to different domain sizes (which is not our focus in this section) we started 50 cells in two equal domains, with 25 cells on left and right with left/right polarity, respectively. Thus there is no CIL. Note that the cluster now
expands indefinitely, as expected by the fact that stalled cells in the interior will eventually divide.

Very striking effects can be seen in Figure 4. Whenever a new cell is added, there is a strong local density perturbation. This appears to launch a density wave in the cluster which propagates to the boundary. We associate this with the experimental observation of (12). Note that this effect arises naturally in our model: we do not need an extra feedback mechanism as in (20). We understand the waves as arising simply because there is a time delay for a cell to start to move from its stalled state to accommodate the presence of the new cell.

Also, as intermittent waves launch and spread towards the boundary they change the inter-cellular tension pattern. With more frequent proliferation, the average interior tension decreases and the tension pattern shifts away from having a flat plateau to increasing gradually towards the center. As there is a peak at the center, cell division events are more likely near the center, where intra-cellular tension reaches $T_{div}$ more easily. Although such division would cause instant stress relief, the rapid propagation away from the initiation point of the waves quickly restores the center to be the most tensile. This behavior could account for the observation of (10) where the tension gradient is not confined to the surface layers; we note that the experimental data comes from a 2d system and represents an average over some distance in the transverse direction, and this may smooth out the structure as compared to our 1d simulation results.

To get a qualitative picture of the waves, we see that each wave arm consists of two edges: the serial release from stalling (upper edge) and the serial restoration of stalling (lower edge). As in Fig. 4, the edges are approximately parallel between different wave arms. For the upper edge, inserting a new cell moves adjacent cells with a finite velocity, because of the aforementioned drag by the substrate. Also, it is only when the neighboring cells protrude that the perturbation is transmitted further away in the most effective way. For the lower edge, the cluster features a characteristic speed of sequential restoration of stalling, as is approximately the speed of initial onset of stalling. An estimate for this wave speed is presented in the SI Section 4.

Other local perturbations could launch waves, of course, such as the collisions of clusters and the removal of barriers to growth. This is observed in some experiments; see (12). The point is that the cell cluster is a medium that supports propagating waves which can be initiated by various perturbations.

C. Periodic Boundary Conditions. In the experiment of (13), cells move along a 1d annulus. Initially, clusters expand but once the ends contact each other around the annulus there is a transition between growth with expansion and collective motility (rotation) without cell division.

To treat this case, we simulated a cluster growing in a 1d periodic domain, ignoring any possible effect due to the ring curvature. An extra intercellular spring between the two outermost cells in our colony is added when the cluster has grown enough to “fill the annulus”. Specifically, this size occurs at the 5000-th step where the left- and right-most cells are joined by an adhesion; see Figure 5. Note that in these simulations we have enhanced the rate of cell division by taking $F_{div} = 0.95 T_s$ (instead of 0.99 $T_s$) to speed up the cluster growth. As can be seen, our simulation directly captures the observed transition.

The mechanism underlying the transition is that when the two outer ends of the cluster collide as the cells fill the annulus (i.e., when the new spring is attached), the CIL process occurs. In our simulations the cluster always chooses one or the other polarity, and starts to move, i.e. rotate around the annulus. In some cases a few cells do not change to agree with the majority polarity, and are dragged along; an example of this is shown in SI Section 6. The reversal of polarity takes place in a wave (the sloping border between red and blue in Figure 5a.) The nature of this wave is, we believe, similar to that of the density waves mentioned above. Both waves involve finite delay in response to mechanical perturbations. There is a characteristic time for reversal of polarity, the inverse of the rate in Eq. 5. The speed of the reversal wave is of the order of the cell length divided by this time.

D. Collective Durotaxis. The phenomenon of collective durotaxis has been observed for cell clusters of the type we have been considering (11, 18). In fact, for these cases single cell durotaxis is not observed (presumably because the gradient in
stiffness is too small to be significant on the length scale of a single cell), but a large cluster does move up the stiffness gradient. The interpretation for the effect given in these references is that the cells in the interior of the cluster are not connected to the substrate so that the cluster acts as a giant cell with one end in the stiff part and the other on the compliant part of the substrate. In this section we give a qualitative treatment of collective durotaxis using our model, without making this assumption.

Durotaxis, motion directed by stiffness gradients (14–17), is commonly observed for single cells as well as clusters. It is always observed that when cells are sensitive to stiffness they move towards stiffer regions, never the reverse. The general understanding of this phenomenon in the literature relies on one of two separate mechanisms. One approach assumes that cells move up gradients because they move faster on stiff substrates, so that if they wander in to such a region they will wander away (15, 16). This is sometimes called population durotaxis because it is not evident at the level of a single cell. Another mechanism relies on the fact that cells can sense stiffness (36) and tend to grow stronger and longer lived adhesions on stiff substrates. This leads to durotaxis at the single cell level (34) because the end of the cell in the stiff region (usually the front) will not detach as quickly as the end in the compliant part, leading the back to peel off and leading to motion up the gradient. Doering et al. (17) have argued that the latter mechanism is far more likely for single cells. For clusters it is unclear what mechanism to choose.

In this work we consider the substrate to be perfectly stiff. A proper account of elastic effects is beyond our scope (and will be the subject of future work). However, within our framework we can try to introduce proxies for substrate elasticity to get a qualitative answer. Within our model, there are two plausible mechanisms that we can invoke. We note that a spring connected to a compliant substrate shares load and becomes, in effect, a softer spring. This suggests making the spring constant, \( k \), space dependent, with larger \( k \) on stiffer substrates. Note that larger \( k \) should lead to faster cell speed since it makes detachment faster (recall Eq. 1 and see SI Section 7). This mechanism would give population durotaxis if all the cells were mechanically uncoupled. Alternatively, we can try to mimic the effects of forming more and stronger focal adhesions on stiffer surfaces by letting \( F_d \) depend on space. A larger \( F_d \) makes it harder to detach as would be the case if there are more adhesions. Thus \( F_d \) should be larger in stiffer regions. Single-cell level durotaxis is not being considered in either mechanism; \( k \) or \( F_d \) is taken to depend on cell midpoint coordinate, \( x_c \), and is uniform within individual cells.

In Figure 6 we show results for both mechanisms. As in Section B, we started with 50 cells with two equal-sized domains, with cell division enabled. An average shift in the center of mass is calculated from 50 independent samples for each mechanism/parameter. It is clear that there is collective durotaxis in both cases. The net translation seen here should not be confused with that illustrated in Figure 3: the overall translation direction there was random, due to the prominently imbalanced domains set by random initial polarities without proliferation. As expected, as the gradient of stiffness increases, the translation of the center of mass of the cluster increases, though a tendency of saturation seems to appear. We made no...
Adhesion is treated as a set of springs connecting points along width. The limiting case here is when all the cells are polarized would not care about their substrate interaction. Thus, as is shown directly in SI Section 8, without proliferation the cluster cannot sustain durotaxis. In that case, interior regions merely supply a passive friction from attachment/detachment of bonds, but the left/right active pulling edges have different thickness to compensate the different pulling capabilities of individual cells. Once the cluster reaches the saturation width, no overall translation is possible. In our view, proliferation breaks such a deadlock.

4. Summary and Conclusions

Here we have introduced a simple mechanical model for cells that are attached to each other by molecular springs and hence move collectively. This model is based on the notion that cells undergo a contraction-protrusion cycle and that these processes are modulated by adhesion to the substrate and by intercellular forces. To account for the fact that contraction is based on myosin mini-filaments walking along actin fibers, we have used the molecular clutch formulation of the connection between the stress state of the cell and the contractile velocity. Adhesion is treated as a set of springs connecting points along the cell to a rigid substrate. These adhesive springs come and go and cell polarity determines which half of the cell has a higher number of such adhesions. We have also incorporated a simplified form of the well-established biological mechanism CIL, contact inhibition of locomotion. This form of CIL consists of reversing the polarity and hence moving adhesive sites of two cells engaged in a head-on collision. This model is formulated in one dimension, i.e. for a moving line of cells.

Given its relative simplicity, it is remarkable how many interesting aspects of collective cell motility this model is able to encompass. As shown in detail, the eventual mechanical state of a cell cluster can be of one of several types. In the absence of any cell division, the cluster size eventually must saturate. If the cluster is relatively symmetric, that is there are a significant number of cells in the right-polarized domain engaging in a tug-of-war of with a significant number of left-polarized cells, the cluster will stop moving altogether and the intercellular tension will exhibit a broad plateau. This type of pattern is perhaps similar to what was observed in (10), where the tension gradient exists over several millimeters worth of tissue. The wave effect offers an alternate explanation for this behavior than that provided in (21) which assumed that cells never reach stall forces anywhere inside the tissue. Interestingly, as far as we know waves were not seen in the original experiments of Trepata et al (10), which might therefore be consistent with the lack of any stalling behavior under those conditions.

A last set of results concerns simulating a recent experiment where cells were constrained to move along an annulus. As the cluster expands, the two ends eventually collide and the cluster transitions to the coherent motion state with almost all the cells having the same polarity and no division taking place. We observe that this transition takes place by a polarity reversing wave that eventually leads to a large preponderance of cells moving the same way. Again, wave-like phenomena have been seen in colliding tissues (38). There can be individual “rebellious” cells that maintain the “wrong” polarity, but these have little effect on the overall cluster behavior. Rebellious cells might be detected by measuring the relative position of intracellular structures such as centrosomes (39).

There are a number of directions in which our model should be extended. It should be straightforward in principle to create a two-dimensional version of our system. This extension has already been accomplished for a contraction-protrusion model of single cell motility, with the major changes being that now both force and torque need to be balanced at each step of the simulation and the fact that polarity now becomes a vector which determines the direction of the protrusion (34). A different generalization involves incorporating the effects of substrate elasticity. Given that the entire measurement strategy involved in traction force microscopy relies on having a flexible substrate, it is clearly important to understand when this feature makes a difference. In addition, as we have seen there is clear evidence in favor of collective durotaxis, namely that the cluster can respond to relatively small substrate stiffness gradients, sufficiently small as to preclude single-cell durotaxis. We have shown phenomenologically how making the spring constant or the detachment force depend
on position can lead to weak durotaxis, but it would definitely be useful to replace this approach with one that correctly accounts for substrate elasticity, as well as include possible parameter changes at the single-cell level due to some form of mechanosensing.

Cells are extremely complex mechanical objects and of course one cannot expect to describe all their phenomenology with simple models. However, at least for collective behavior we may expect (or at least hope) that many of the biological details are not critical when it comes to grasping the essence of what can occur. The results reported here should give us added confidence in this physics-based approach.

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Supporting Information Text

1. Contact Inhibition Of Locomotion, Two-Cell Demonstration

Figures S1, S2 respectively are two cases of one and both cells flipping after a head-to-head collision.

2. Different Outcome Set By Randomness

Running the simulations without division but with random initial polarity gives a variety of different final states. The two examples shown in main text are one with nearly symmetrical polarity domains (two domains of roughly the same cell numbers), and one with asymmetrical domains and bulk translation. Figure S3 is a critical case between the two. It is also possible to end up with asymmetrical domains that does not translate in bulk (Figure S4, also see discussion in the next section), and with all cells having the same polarity (Figure S5). More rarely, “rebellious” cells can live inside a domain of opposite polarity, where the overall tension makes polarity flip difficult (recall the rules of contact inhibition of locomotion). Examples are Figures S6, S7.

3. Active Edge Cells

As shown in the main text, a cluster of sufficient number of cells eventually reaches a saturation width. In cases without bulk translation, the interior cells are (nearly) stalled, while outer cells on both sides are actively contracting and exerting traction on substrate. There exists a typical width of this active edge, i.e. number of active edge cells. For simplicity, we shall ignore the possibility of having rebellious cells inside domains for this section. We have seen the cluster sometimes maintaining the state without overall translation, even though the number of cells on both domains are unequal. The following qualitative picture explains this: Active edge cells generate traction such that inter- and intra-cellular tension builds up gradually from the boundary, which eventually stalls the interior cells, and stays flat further toward the center. Even if the two domains have unequal numbers of cells, as long as both form a complete active edge, the net traction cancels. Only if a polarity domain totals too few cells to have a complete active edge, there is overall translation toward the other side.

It is possible to have a semi-quantitative estimate of the number of active boundary cells. The following approach gives a lower bound of number of edge cells necessary to stay active. For simplicity, define the notation for states \( a, d \) = attached, detached, respectively. Consider a spring in a force field, where it either has zero tension (detached) or a fixed tension \( F \) (attached). State transition probabilities according to the detachment probability in the main text are

\[
P(d,t|a,t-1) = dt K \exp(F/F_d) \tag{[1]}
\]

\[
P(a,t|a,t-1) = 1 - dt K \exp(F/F_d) \tag{[2]}
\]

\[
P(a,t|d,t-1) = dt K \tag{[3]}
\]

\[
P(d,t|d,t-1) = 1 - dt K \tag{[4]}
\]

Further, set unconditional probabilities \( x(t) = P(d,t), \quad 1 - x = P(a,t) \), then

\[
x(t+dt) = x(t)(1 - dt K) + (1 - x(t)) dt K \exp(F/F_d) \tag{[5]}
\]

It yields

\[
\frac{dx}{dt} = \frac{x(t+dt) - x(t)}{dt} = -x(t)K + (1 - x(t))K \exp(F/F_d) \tag{[6]}
\]

Say given enough time, the system reaches steady state, i.e. \( \frac{dx}{dt} \bigg|_{t \to \infty} = 0 \). Then, we get

\[
x(\infty) = \frac{\exp(F/F_d)}{1 + \exp(F/F_d)} \tag{[7]}
\]

This gives the expected steady-state spring tension:

\[
E[\text{spring tension} \big|_{t \to \infty}] = E[\text{spring tension} \big|_{t \to \infty}] = (1 - x(\infty))F = \frac{F}{1 + \exp(F/F_d)} \tag{[8]}
\]

In the numerical simulations except those of durotaxis, \( F_d = 0.75 \). This gives the maximum of \( E[\text{spring tension} \big|_{t \to \infty}] \) to be 0.209, approximately. That is, if each spring is allowed to optimize its pulling strategy, it can at most exert an expected tension of 0.209. To accumulate toward stall tension of 10, at least 50 springs are needed. That is around 3 cells, considering each cell having 18 adhesions to the substrate. In reality, cells cannot pull in the most efficient way. The traction gradually decreases from the outermost inward. As can be read from the space-time plot of traction, the traction decreases significantly since around 6-th cell from the outermost.
4. Wave Speed Of Sequential Stalling

As set out in the main text, the wave incurred by insertion of new cells (by cell division) has two edges, an upper edge of serial release from stalling, and an lower edge of serial restoration of stalling. To separate different wave fronts for clearer demonstration, we ran a simulation where division rate $k_{\text{div}}$ is reduced to 0.01, from 1 in the main text (Figure S8).

The slope of the lower edge can be estimated by noting that those edges are parallel to the initial onset of stalling (the purple slopes). Note the following: Initial expansion speed is close to free single cell speed $v_1$ (segment AD); stalling of the center cells is very soon after expansion starts (point C); the time of point D,E is when the cell cluster reaches the maximum width before division, and is the same as the time point that all interior cells are stalled; this width $\Delta x_{DE} = x_E - x_D$ can be estimated by setting all interior intercellular springs to have been stretched to stall tension $T_S$; by noting that midline coordinate does not change, $x_D = x_C - \frac{\Delta x_{DE}}{2}$

$$\text{wave speed of lower edge} = \text{slope of purple segments}$$

$$= \frac{x_E - x_C}{t_E - t_C}$$

$$= \frac{\Delta x_{DE}/2}{t_D - t_A}$$

$$= \frac{\Delta x_{DE}/2}{(x_A - x_D)/v_1}$$

[9]

Point A is set by initial condition, so all quantities in the last line are known at this point. This is surely, a very rough, semi-quantitative estimation.

5. More Frequent Cell Division

Figure S9 is the sample with a lower division threshold $T_{\text{div}} = 0.9 T_s$ mentioned in main text.

6. Periodic Boundary Condition, A Case With Rebellious Cells

Figure S10 is a sample where a rebellious cell persists and temporarily induces its neighbor to be rebellious, but has little effect of the overall sustained rotation.

7. Larger Spring Constant Of Cell-Substrate Adhesions Leads To Faster Cell Speed

As mentioned in the main text, larger $k$ should lead to faster cell speed since it makes detachment faster. In Figure S11, for each value of $k$, we simulated 50 samples of free single cell and calculated the mean / standard deviation of each sample’s average speed. A positive relationship is clear. Although we have not studied the precise mechanism of durotaxis in our model, the proxy of larger $k$ in stiffer region is hereby shown to lead to faster speed. It is therefore consistent with the proposed mechanism that faster cell speed on stiffer substrates leads to population durotaxis (1, 2).

8. No Proliferation, No Durotaxis

To trying to answer the qualitative question of whether collective durotaxis can happen without single-cell durotaxis, we did two numerical experiment by varying elastic constant $k$ and detachment threshold $F_d$ respectively. The answer is yes only when cell division enabled. If proliferation is disabled, center of mass shift is significant only in the initial expansion (Figure S12). In these cases, the cluster manages to compensate for different individual traction capability by differing numbers of active edge cells on both sides (Figures S13, S14). We do not claim to have solved the quantitative problem of modelling durotaxis. As in Figures S15, S16, the bulk shift is obviously smaller than experimental results (3).

Methods

The simulation is performed by a custom-built program. We utilized the optimization function (for mechanical equilibration) and others from DLIB (4).
Fig. S1. Space-time plot of two cells initially aligned head-to-head. Left cell flips polarity. (a) Polarity and half-length of the cells. (b) Cell contraction speed. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs. Notation: $x_c$ is the coordinate of the cell midpoint; $x_{m,cd}$ is the coordinate of the midpoint of the intercellular adhesions.
Fig. S2. Space-time plot of two cells initially aligned head-to-head. Both cells flip polarity. (a) Polarity and half-length of the cells. (b) Cell contraction speed. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs. Notation: $x_c$ is the coordinate of the cell midpoint; $x_{mid}$ is the coordinate of the midpoint of the intercellular adhesions.
Fig. S3. Space-time plot of a cell cluster of the one-dimensional model. Very slow bulk translation. (a) Polarity and half-length of the cells. (b) Cell contraction speed. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs. Notation: $x_c$ is the coordinate of the cell midpoint; $x_{mid}$ is the coordinate of the midpoint of the intercellular adhesions.
Fig. S4. Space-time plot of a cell cluster in the one-dimensional model. More cells have polarity to the right, but there is still no overall translation. (a) Polarity and half-length of the cells. (b) Cell contraction speed. Note that many of the cells barely contract, i.e., they are (nearly) stalled. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e., the stretch of the inter-cellular springs. Notation: $x_c$ is the coordinate of the cell midpoint; $x_{mid}$ is the coordinate of the midpoint of the intercellular adhesions.
Fig. S5. Space-time plot of a cell cluster of the one-dimensional model. All cells eventually have the same polarity. (a) Polarity and half-length of the cells. (b) Cell contraction speed. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs.
Fig. S6. Space-time plot of a cell cluster in the one-dimensional model. Rebellious cells persist but has little effect over collective pattern. (a) Polarity and half-length of the cells. (b) Cell contraction speed. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs. Notation: $x_c$ is the coordinate of the cell midpoint; $x_{mid}$ is the coordinate of the midpoint of the intercellular adhesions.
Fig. S7. Space-time plot of a cell cluster in the one-dimensional model. Rebellious cells persist but have little effect on collective pattern. (a) Polarity and half-length of the cells. (b) Cell contraction speed. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e., the stretch of the inter-cellular springs. Notation: $x_c$ is the coordinate of the cell midpoint; $x_{mid}$ is the coordinate of the midpoint of the intercellular adhesions.
Fig. S8. Space-time plot of a cell cluster in the one-dimensional model. (a) Polarity and half-length of the cells. (b) Cell contraction speed. The red segment is a trajectory of a free single cell; the parallel purple segments are slope of the lower edges. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs. Notation: $x_c$ is the coordinate of the cell midpoint; $x_{mid}$ is the coordinate of the midpoint of the intercellular adhesions.
Fig. S9. Space-time plot of a cell cluster in the one-dimensional model. Division threshold tension is tuned down for more frequent division: $T_{div} = 0.9 T_s = 9$. (a) Polarity and half-length of the cells. (b) Cell contraction speed. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs. Notation: $x_c$ is the coordinate of the cell midpoint; $x_{mid}$ is the coordinate of the midpoint of the intercellular adhesions.
Fig. S10. Space-time plot of a cell cluster that keeps expanding until two outermost cells are joined by a spring at $t = 5000$. (a) Polarity and half-length of the cells. Note the persistent “rebellious” cell. (b) Cell contraction speed. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs. Note that the rightmost new spring since $t = 5000$ is the one connecting two outermost cells. Notation: $x_c$ is the coordinate of cell midpoint; $x_{mid}$ is the coordinate of midpoint of intercellular adhesions.
Fig. S11. Average cell speed vs $k$, the spring constant of cell-substrate adhesions. Error bar is standard deviation.
Fig. S12. Durotaxis: Center of mass shifting with time, under two durotactic mechanisms and different changing rate along spatial axis, division disabled. Each line is the average over 50 samples, where the filled area signifies standard deviation among samples. 

(a) Changing per-cell cell-substrate adhesion elastic constant $k$ spatially. cell-cell adhesion is unchanged from previous simulations. $k = \max(0.2, 0.4 + \text{slope} \times x_c)$. 

(b) Changing the per-cell cell-substrate adhesion $F_d$ spatially. $F_d = \max(0.2, 0.4 + \text{slope} \times x_c)$. 

Youyuan Deng, Herbert Levine, Xiaoming Mao, and Leonard M. Sander
Fig. S13. Space-time plot of a cell cluster in the one-dimensional model without proliferation. No sustained durotaxis observed. Elastic constant $k$ of cell-substrate adhesions is varied spatially according to cell midpoint position $x_c$ and is same within each cell. $k = \max(0.2, 0.4 + 1e - 3 * x_c)$. (a) Polarity and half-length of the cells. (b) Cell contraction speed. Note that many of the cells barely contract, i.e. they are (nearly) stalled. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs. Notation: $x_c$ is the coordinate of the cell midpoint; $x_{mid}$ is the coordinate of the midpoint of the intercellular adhesions.
Fig. S14. Space-time plot of a cell cluster in the one-dimensional model without proliferation. No sustained durotaxis observed. Detachment threshold $F_d$ of cell-substrate adhesions is varied spatially according to cell midpoint position $x_c$ and is same within each cell. $F_d = \max(0.2, 0.4 + 1e - 2 * x_c)$. (a) Polarity and half-length of the cells. (b) Cell contraction speed. Note that many of the cells barely contract, i.e. they are (nearly) stalled. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs. Notation: $x_c$ is the coordinate of the cell midpoint; $x_{mid}$ is the coordinate of the midpoint of the intercellular adhesions.
Fig. S15. Space-time plot of a cell cluster in the one-dimensional model with proliferation. Elastic constant $k$ of cell-substrate adhesions is varied spatially according to cell midpoint position $x_c$ and is same within each cell. $k = \max(0.2, 0.4 + 1e^{-3} \cdot x_c)$. (a) Polarity and half-length of the cells. (b) Cell contraction speed. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs. Notation: $x_c$ is the coordinate of the cell midpoint; $x_{mid}$ is the coordinate of the midpoint of the intercellular adhesions.
Fig. S16. Space-time plot of a cell cluster in the one-dimensional model with proliferation. Detachment threshold \( F_d \) of cell-substrate adhesions is varied spatially according to cell midpoint position \( x_c \), and is same within each cell. \( F_d = \max(0.2, 0.4 + 1e^{-3} \cdot x_c) \). (a) Polarity and half-length of the cells. (b) Cell contraction speed. (c) Traction force on each cell by substrate due to the adhesions. (d) Inter-cellular tension, i.e. the stretch of the inter-cellular springs. Notation: \( x_e \) is the coordinate of the cell midpoint; \( x_{\text{mid}} \) is the coordinate of the midpoint of the intercellular adhesions.
Movie S1. Animation of a free moving cell

Movie S2. Animation of two cells colliding. One of them flipped polarity.

Movie S3. Animation of two cells colliding. Both of them flipped polarity.

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