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Response of adherent cells to mechanical perturbations of the surrounding matrix

Dan Ben-Yaakov,[a] Roman Golkov,[b] Yair Shokef,[b] and Samuel A. Safran *[a]

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We present a generic and unified theory to explain how cells respond to perturbations of their mechanical environment such as the presence of neighboring cells, slowly applied stretch, or gradients of matrix rigidity. Motivated by experiments, we calculate the local balance of forces that give rise to a tendency for the cell to locally move or reorient, with a focus on the contribution of feedback and homeostasis to cell contractility (manifested by a fixed displacement, strain or stress) that acts on the adhesions at the cell boundary. These forces can be either reinforced or diminished by elastic stresses due to mechanical perturbations of the matrix. Our model predicts these changes and how their balance with local protrusive forces that act on the cell's leading edge either increase or decrease the tendency of the cell to locally move (toward neighboring cells or rigidity gradients) or reorient (in the direction of slowly applied stretch or rigidity gradients).

1 Introduction

Recent experiments on cell motility on substrates explore the connection between cell motility, substrate adhesion and active contractility, resulting in a generic picture of how these effects balance to determine cell velocity [1–7].

In this paper, we focus on contractile forces in cells such as fibroblast, muscle or epithelial cells that would be strongly adherent and non-motile in an unstressed and elastically homogeneous environment devoid of mechanical perturbations external to the cell [8]. We show theoretically how these forces are *modified* by mechanical perturbations of the cellular environment, such as other cells, rigidity gradients, and externally applied stresses. We predict the conditions under which each of these perturbations will increase or decrease the "backwards" displacement of the cell and the force exerted on the adhesions by cellular contractility (see Fig. 1), and thus tend to respectively locally either incrementally impede or promote local cell motility or reorientation *. These results are intimately connected to cell activity and homeostasis and we contrast this situation with the case of passive inclusions in an elastic medium; those do not interact for an infinite, isotropic matrix. Our theory provides insight and generic guidelines for the tendency of mechanical perturbations of the matrix to result in local cellular motion (*e.g.*, durotaxis – the tendency of cells to move or align on more rigid substrates) or reorientation (in response to nearby cells or externally applied stress). The adhesions are treated as rigid bodies that displace but do not stretch in the presence of mechanical perturbations. The passive elastic response of the cell and its adhesions to mechanical perturbations of the matrix can also depend on biochemical feedback and our coarse grained model assumes that these effects are reflected in the homeostatic boundary conditions that allow the cell to adjust its contractility which affects the displacement of the adhesions as the cell responds to its mechanical environment.

Experimental evidence shows that feedback effects in cells lead to homeostasis in which certain mechanical properties of the cell are preserved even if the substrate rigidity is changed [9,10] or as the substrate is slowly stretched [11]. The applicability of various interpretations of cellular homeostasis to the observed response of cells to generic mechanical perturbations of the matrix is examined in the Conclusions section. Homeostasis is possible in cells that can alter their energy expenditure (*e.g*, by modifying acto-myosin or ATP production or activity) to maintain their local, homeostatic, boundary conditions even in the presence of changes in their mechanical

[a] *Dept. Materials and Interfaces, Weizmann Institute of Science, Rehovot, Israel. Fax: 972 8 934 4138; Tel: 972 8 934 3362; E-mail: sam.safran@weizmann.ac.il*
[b] *School of Mechanical Engineering, Tel Aviv University, Tel Aviv, Israel.*

* We discuss the case where the contractile and adhesion forces are uniformly distributed on the cell perimeter. We thus focus only on local force balances in the combined membrane-protrusion-adhesion-contraction system and not on the global motion of the cell which can depend on the non-uniform distribution of the adhesions and contractility. Our use of the terms "cell motion or reorientation" refer to local force imbalances that would destabilize strongly adherent and non-motile cells; our model predicts the tendency to move and not the subsequent dynamics. Motion in highly motile, non-adherent cells (in the absence of mechanical perturbations of the matrix) is often determined by a globally inhomogeneous distribution of contractile and protrusive forces [3].

For example, in keratocytes, the larger contractility at the back of the cell tends to promote motion at its front.

environment. Symmetric, rigid inclusions (that do not regulate the forces that the produce in response to their mechanical environment) in isotropic, infinite and linear elastic matrices, do not elastically interact via their mutual deformations of the matrix [12]. We show below that even in cases where rigid inclusions would not interact, biologically active cells can, in some cases, elastically interact due to feedback and homeostasis. Cell elastic interactions have been discussed in previous studies [8] that assumed a constant "force dipole" moment attributed to the contractile cell and asked how the energy invested by the cell in deforming the elastic matrix could be minimized. However, those studies did not account for the force balance that leads to the adhesion displacement considered here nor for feedback due to homeostasis by which the force dipole itself may change in the presence of mechanical perturbations of the matrix.

2 Biophysical background and proposed model

Substrate adhesion forces and active contractility are two important factors that govern cell motility. Actin polymerization that takes place adjacent to the plasma membrane of the cell (*i.e.*, at its leading edge) generates protrusion forces that tend to move the membrane leading edge in the "local forward" direction, see Fig. 1, as well as forces due to actin retrograde motion that move the adhesions near the leading edge in the local backward direction. These protrusions are attached at their non-polymerizing end to the adhesions that couple the cell to the substrate. Motion of the adhesion along the substrate gives rise to a frictional force [13] (due, in part, to dissociation of bonds between the adhesion complexes and the surface) as well as elastic stresses in the substrate that may oppose such motion. The adhesions also experience forces from cell contractility due, for example, to the actin cortex or to larger-scale stress fibers. In general, contractility can also affect actin treadmilling or retrograde motion and thus influence the magnitude of the protrusion forces. A model for cell spreading that considers the dynamical forces balance of all these effects was presented in Ref. [14,15]. There are additional forces that arise from the intrinsic tension of the bilayer membrane that resists the protrusion forces. However, experiments show [1] that these do not play an important role in determining the cell velocity. In addition, the detailed coupling of the protrusive forces to the membrane also regulate cell motion. However, these membrane-localized effects are not expected to change in the presence of the mechanical perturbations of the matrix considered here. For that reason we focus on the forces that act on the adhesions which are coupled to the matrix and are thus sensitive to mechanical perturbations of the elastic surroundings of the cell. In principle, a wide variety of biochemical, molecular-level changes can all lead to the same velocity which is determined by the "lumped" effects of the protrusion, adhesion and contractile forces. Here, we denote the net protrusive forces as those which move cells in the locally forward direction and the net contractile forces as those that oppose this motion. Furthermore, we highlight the role of mechanical perturbations of the substrate (such as other cells, rigidity gradients or external static stretch) in destabilizing well-adhered cells. This means that we consider the cell, after it has reached its dynamic, mechanical equilibrium and is now fully spread [14]. Perturbations (*e.g.*, stochastic protrusions of the cell boundary) of the system away from this state give rise to a mechanical force that opposes such perturbations and tends to restore the equilibrium state. In addition, we limit ourselves to the case of well-adhered cells for which the stochastic protrusions due to sporadic attempts of the cell to probe its substrate (e.g., via filopodia) are too weak to overcome the total restoring force that maintains the adhesion of the cell with the result that the cell remains non-motile, even while its boundary may still locally and temporarily fluctuate. It is in this context that we ask how mechanical perturbations of the matrix surrounding the cell change the force balance that gave rise to the equilibrium in the absence of the mechanical perturbations. The latter destabilize the quiescent situation (in which the cell boundary may fluctuate but in which the cell shows no average motion) and cause the cell to move in a deterministic manner either towards or away from the source of the perturbation. The speed at which this will occur depends on the cell-substrate friction but here we only focus upon the destabilizing effect of the mechanical perturbations of the substrate, and limit our predictions to the magnitude and sign (forward or backward with respect to the leading edge of the cell) of adhesion displacement compared with its position for the well-adhered and non-motile cell in an unperturbed elastic environment. This is done by calculating the forces on the adhesion and requires a treatment of the matrix deformations in the presence of both mechanical perturbations and cellular forces. Cellular activity implies that these forces can vary in a complex manner and we model the situation for the case where the cell maintains a homeostatic stress, strain or displacement.

Experimental evidence for cellular, mechanical homeostasis in the absence of neighboring cells, rigidity gradients or external stretch has been presented in several studies [9–11] where it was inferred that fibroblast cells maintain a fixed local deformation of the pillars at small to moderate values of the rigidity of the substrate on which they are placed. These experiments, done on pillared substrates, found that the average force per pillar in this regime increased linearly with the pillar elastic modulus. However, since the stress exerted by the cell (determined by acto-myosin contractility and the adhesions) cannot increase indefinitely, the force exerted by the cells saturates to a constant value above a characteristic rigidity. This suggests that cells can switch their behavior from homeostatic fixed lo-

cal pillar displacement to homeostatic fixed force as the rigidity of their elastic surroundings increases [16–18]. We note that these experiments, performed on pillars, only provide information about the conditions at the cell boundary where the adhesions are located and on homeostasis with respect to local rigidity changes of the substrate but not necessarily on cellular homeostasis in the presence of long-range effects such as other cells or rigidity gradients. In addition, the translation of the results of the pillar experiments to cells in an elastic continuum may indicate [19] that in the continuum, the analogue of fixed force is fixed stress at the cell boundary while the analogue of fixed pillar displacement may be fixed strain at the cell boundary. Indeed, since the experimental determination of cellular homeostasis in the presence of mechanical perturbations is not yet clear, we consider below the three possibilies of stress, strain and displacement homeostasis.

The homeostasis scenarios for *active* cells contrast with the mechanical response of non-responsive (rigid) inclusions in elastic media. Such inclusions may locally expand or contract their environment (*e.g.*, due to differences in thermal expansion of the inclusions and the matrix or due to local, elastic matrix deformations induced by the inclusions). However, they are not be able to regulate the stresses, displacements or forces (via changes in adhesion area) they impose in response to the mechanical state of their environment. In addition, we focus here on effects related to active cell contractility and not to the passive, elastic stresses that the cell exerts on its adhesions; for cells that are much more rigid than their environment, the passive cell elastic response can be ignored since the cells are negligibly stretched by mechanical perturbations of the matrix. It is indeed an interesting challenge to combine both the passive elastic stress induced in matrix by stretching soft cells and their active, contractile response [4,20] but a comprehensive theory of both involves physical and chemical degrees of freedom that are outside the scope of this paper †

To predict how the adhesions are displaced in the presence of external mechanical perturbations of the matrix, we introduce a simple model that predicts in a unified manner how cells respond to distant elastic perturbations of their environment even though they are limited to probing (via local cytoskeletal forces and adhesions) only the *local* displacement or stress in their immediate neighborhood. The forces that act on the leading edge of the cell include those due to stochastic protrusions that push the leading edge in the forward direction, retrograde flow of actin in the backward direction as well as those arising from the adhesions that are coupled to the actin (via transmembrane integrins) as well as to the cytoskeleton and the substrate. While the details of these forces depend on the interactions of many molecular components involved here we denote protrusive forces as the *net* forces that tend to move an adhesion in the locally forward direction and contractile forces as the *net* forces that tend to move the adhesion in the opposite manner. In turn, the adhesions are acted upon by cellular contractility (that pulls the upper surface of the adhesion in the direction of the nucleus) as well as forces that arise from the mechanical perturbations of the elastic medium; these act on the lower surface of the adhesion that is in contact with the substrate. The net force on the adhesions in the presence of other cells, rigidity gradients or external stress determines whether the protrusion forces can move or reorient the cell toward or away from these perturbations. While the calculations shown here are for a simple, symmetric geometry, we use them to motivate predictions of the trend for the cell to *locally* increase or decrease the contractile force it exerts on its adhesions. This then modifies the balance of protrusive, frictional and contractile forces on the combined membrane-protrusion-adhesion-contraction system (see Fig. 1) to determine cellular motion or reorientation in response to the mechanical perturbations of the cellular environment.

The discussion presented above suggests that the local dynamics of the combined membrane-protrusion-adhesion-contraction system can be written phenomenologically as a Langevin equation for the velocity of a given adhesion, modeled as a point and displaced from its position in the absence of mechanical perturbations of the matrix; the cells we consider are non-motile when such perturbations are absent. The displacement is denoted by $\vec{u}_c$ whose time derivative (denoted by a prime) is:

$$\gamma \vec{u}_c{}' = \vec{f}^c + \vec{f}^e + \vec{f}^p \qquad (1)$$

γ is proportional to the dynamic friction between the adhesion and substrate. The contractile force on the adhesion is $\vec{f}^c$ which in general, can be a function of the adhesion position on the cell boundary as is the protrusive force $\vec{f}^p$. The forces due to the perturbations of the mechanical environment, $\vec{f}^e$ that are transmitted to the adhesion via the elastic medium or substrate can also modify the effective value of $\vec{f}^c$ depending on the homeostasis conditions. This equation applies only if the sum of the forces on the right hand side of the equation is greater than the static frictional force; otherwise, there is no local motion or reorientation of the cell contour. This is because rebinding of the bonds coupling the adhesions to the substrate stabilizes the system even in the presence of external force [25]. We can therefore also consider the case where, in the absence of mechanical perturbations to the matrix, the static frictional force dominates the right hand terms and the cell is strongly adherent and stationary. When the sum of the contractile and mechanical environmental forces are larger than

† Cellular adhesions in fibroblasts exert stresses [21] of the order of $5\text{nN}/\mu\text{m}^2$ (equivalent to 5kPa) which is indeed the magnitude of the shear modulus of such cells on stiff substrates [22]. However, the measured modulus includes both the effect of the passive elasticity of the cross linked cytoskeletal components as well as the effective stiffening [23,24] due to the molecular motors that give rise to cellular contractility.

the static frictional forces, the cell can move or reorient in the direction of the local protrusion force. Realistic systems are not spherically symmetric so that the change in the force balance induced by the presence of other cells, rigidity gradients, or external stress only occur in specific directions. In that case, the protrusion forces will tend to move or reorient the cell in those directions where the local contractility forces have been sufficiently decreased by the mechanical perturbations of the matrix. Whether the friction is dynamic or static is important to the details of the motion. But our more modest goal here focuses on the first step: predictions of the direction and magnitude of the displacement of an adhesion in a non-motile, well-adhered cell in the presence of mechanical perturbations of its elastic environment. To do this in a robust manner that does not depend on any particular microscopic model, we treat the biological activity of the cell via a homeostatic, mechanical boundary condition at the cell-matrix interface; this presents a well defined scenario which is then analyzed using the theory of elasticity. Our continuum approach cannot resolve the spacing between the adhesions and the membrane. However, the advantage of our treatment is its independence of the details of how the cytoskeletal structure, myosin activity and adhesion size and density are regulated by the cell in achieving the local deformation or stress dictated by the cell's genetic program.

3 Cell response to mechanical perturbations of the matrix

3.1 Theoretical model

We analyze the simplest possible geometry of a spherical, contractile cell so that the contour, of a cell centered at the origin, is specified by a constant radius R_c. The previous discussion of the force balance and homeostasis is applicable to a spherical average over the entire cell; our model does not resolve effects on the scale of a single adhesion and coarse grains over the entire ensemble. Recent experiments also show that homeostasis involves global effects[26]. Homeostasis implies that the contractile apparatus of the cell is biologically programmed to respectively exert either (i) a fixed, local stress (force in the radial direction on the cell boundary, per unit area) σ_c, (ii) the trace of the strain ε_c, equivalent to the local volume change or (iii) local radial displacement $u(R = R_c) = u_c$ of the adhesion, that we assume is identical to the displacement from mechanical equilibrium of the matrix at the cell boundary due to a non-slip bond between them (see Fig. 2). Note that σ_c is the stress the cell exerts on the matrix and is opposite in sign to the matrix response to this stress (defined below as σ_{rr}). An important case that we consider below is where the periodic unit cell boundary at $R = R_b$ is held fixed. For cells that apply radial contractile forces, $\sigma_c < 0$, the elastic counter stress in the matrix is then positive which tends to compress the matrix and pulls the adhesion in the forward direction. If the cell is itself compressed by its own contractile, radial forces, the displacement $u_c < 0$ and the resulting strain in the matrix is stretched, $\varepsilon_c > 0$ which will be attained by the adhesion being displaced in the backward direction. If the cell is not itself compressed, (applicable to a very rigid cell relative to the matrix), but establishes strain homeostasis that locally compresses the (possibly much softer) matrix, the strain in the matrix, ε_c will be negative; this will be accomplished by the adhesion being displaced in the forward direction. Since, as shown below, the adhesion displacement is linearly proportional to the homeostatic stress, strain or displacement, different signs for these quantities will result for different displacements of the adhesion by mechanical perturbations of the matrix, in some cases representing an attraction (or motion towards other cells, rigidity gradients or alignment in the direction of applied stress) and in others a repulsion.

We consider the matrix as an isotropic and homogeneous, linearly elastic medium. A calculation of the interaction between two such cells including the homeostasis condition of fixed displacement or stress at the boundary of each cell, even in the presence of the other, is complex and requires one to consider an infinite series of "induced force dipoles"[27]. However, the symmetry of the problem dictates that at the mid plane between the two cells, the perpendicular displacement of the matrix should vanish. We use that to motivate a particularly simple and tractable geometry by considering the interactions of a periodic array of contractile cells in an elastic matrix as in Fig. 2a. At the mid plane between cells – each of which pulls in opposite directions – the matrix displacement in the direction perpendicular to the mid plane is zero. The smallest volume contained by the intersection of all the mid plane boundaries (termed in condensed-matter physics, the Wigner-Seitz or periodic cell bounding volume) is the region in which the elastic problem must be solved. We use the term cell to denote the biological cell and denote the Wigner-Seitz bounding volume as the periodic unit cell. For simplicity of calculation and exposition, we replace this volume by a sphere of diameter $2R_b$, which is proportional to the center-to-center distance between nearest-neighbor, but somewhat distant cells.

Within this geometry, we calculate the stresses exerted at the adhesion site for the different homeostasis conditions. Stress (strain) homeostasis means that the cell regulates its contractility so that even in the presence of mechanical perturbations to its environment, the stress (strain) exerted by the cell at the adhesion located at R_c is fixed at σ_c ‡ (strain fixed at a value of ε_c). Displacement homeostasis means that the cellular contractility adjusts itself so that the displacement of the adhesion at the cell boundary, $R = R_c$, is fixed at a value

‡ The stress in the matrix at R_c is thus $-\sigma_c$.

of u_c, independent of the cell's mechanical environment. This predicts that if the cell was non-motile in the absence of other cells, rigidity gradients or slowly varying external stretch, that it would remain so even after these perturbations are applied since the adhesions will not move. The force balance on the adhesion that determines whether and how the cell moves or reorients in response to changes in its mechanical environment is obtained by solving for the matrix stress in the region $R_c < R < R_b$ using the equations of linear elasticity with the boundary condition $u(R_c) = u_c$ and $u(R_b) = 0$ for the case of a periodic array. Since the speed of sound in the matrix is typically very fast (1000m/s for the longitudinal mode and 1m/s for the shear mode [28] which imply times of microseconds for micrometer distances), the forces due to the other cells, rigidity gradients or external stretch and the contractile forces rapidly reach mechanical equilibrium and determine the displacement of the adhesion, $\vec{u}_c^e$, due to these effects relative to the adhesion position, $\vec{u}_c^0$, in the absence of the mechanical perturbations of the cellular environment.

Thus, when $\vec{u} = \vec{u}_c^e$ the sum of the contractile and environment forces, $\vec{f}^c + \vec{f}^e$, vanishes; this is equivalent to minimizing the elastic and contractile energies with respect to u_c . We next consider the quasi-stochastic protrusive forces that act on time scales of seconds or longer [4], much slower than the equilibration times of the contractile and mechanical perturbations of the matrix (such as other cells, applied stretch or rigidity gradients). We also predict whether the elastic, matrix force that opposes the protrusive forces is increased or decreased, relative to its value in the absence of mechanical perturbations of the matrix. The force that opposes the protrusions, $\delta\vec{f}_m$, is equal to the change in the contractile and the matrix perturbation forces when the adhesion (or matrix) displacement is different from u_c^e due to the protrusive forces. In our spherical geometry, $\delta f_m = (\partial(f_c + f_e)/\partial u_c)|_{u_c=u_c^e}\ (u_c - u_c^e)$. Assuming that the protrusion forces are ineffective (due to friction and contractility) in causing cell motility in the absence of the mechanical perturbations of the matrix, f_c and f_e predict the tendency of the cell to respectively move/reorient or remain adhered in the presence of other cells, rigidity gradients or external stretch.

We next consider the mechanical equilibrium of the contractile and matrix perturbation forces that include the effect of an elastically coupled cellular array that implies zero displacement of the matrix at the boundary of the periodic unit cell, $u(R_b) = 0$; this is dictated by symmetry since neighboring cells pull the medium in opposite directions. For cells in the same periodic array but with no elastic coupling between them (*e.g.*, due to disconnection of the matrix at the periodic boundaries), the boundary condition is one of no stress: $\sigma(R_b) = 0$; this is applicable to an isolated cell in a matrix of finite extent, R_b. The difference between the equilibrium displacements of the adhesion or matrix at $R = R_c$ for the isolated cell (or elastically disconnected array) and the elastically coupled array indicate how cellular interactions influence the tendency for the adhesion to displace toward or away from the other cells. This can be interpreted as an attraction or repulsion of the cells from each other that takes into account contractility, elasticity, interactions and homeostasis. The protrusion forces function as a type of noise that allows the cell to explore its surroundings so that the friction can be overcome to allow motion of the adhesions.

The radial stress $\sigma_{rr}(R_c)$, exerted by the elastic medium on the cell, is – in mechanical equilibrium – the negative of the stress exerted by the cell on the matrix, σ_c. The local stress is written [29,30] in terms of the local matrix displacement, $u(R)$ as :

$$\sigma_{rr}(R) = \frac{4\mu}{3}\left(\frac{\partial u(R)}{\partial R} - \frac{u}{R}\right) + K\left(\frac{\partial u(R)}{\partial R} + 2\frac{u}{R}\right) \qquad (2)$$

where μ is the shear modulus and K is the bulk modulus of the matrix. At the boundary of the biological cell, the matrix stress is thus $\sigma_{rr}(R_c)$. The term proportional to K is the compression while the term proportional to μ is the shear stress. The compressive strain is

$$\varepsilon(R) = \left(\frac{\partial u(R)}{\partial R} + 2\frac{u}{R}\right) \qquad (3)$$

The term which multiplies $4\mu/3$ in Eq. 2 is the shear strain and it is reasonable to assume that contractile cells with homeostatic strain impose a fixed compressive strain at the cell-matrix boundary and not a fixed shear strain.

The stress is calculated from the equations of mechanical equilibrium of the elastic medium. For our spherically symmetric situation of radial displacements of the matrix due to cell contractility [29,30], the displacement is determined from the equilibrium equation:

$$\frac{d^2u(R)}{dR^2} + \frac{2}{R}\frac{du(R)}{dR} - 2\frac{u(R)}{R^2} = 0 \qquad (4)$$

The equilibrium elastic equation for radial displacements in spherical geometry, Eq. 4 predicts $u(R) = \alpha R + \beta/R^2$: where α and β are determined from the boundary conditions at $R = R_c$ and $R = R_b$. The term proportional to α represents a volume change, while the term proportional to β is a shear (shape) change. This can be seen from Eq. 2: the local compression in spherical geometry is equal to $\partial u(R)/\partial R + 2u(R)/R$ which vanishes for $u(R) = \beta/R^2$. The shape change or shear in spherical geometry is equal to $\partial u(R)/\partial R - u(R)/R$ which vanishes when $u(R) = \alpha R$. The radial stress given by Eq. 2 is then:

$$\sigma_{rr}(R) = -\frac{4\mu\beta}{R^3} + 3\alpha K \qquad (5)$$

We use the subscript uu to denote solutions of the elastic equations appropriate for boundary conditions in which the displacement is fixed at boundaries of both the biological cell (where $u(R_c) = w_c$) and the periodic unit cell (where $u(R_b) = w_b$) and find:

$$u_{uu}(R) = w_c R_c^2 \frac{(R_b^3 - R^3)}{R^2(R_b^3 - R_c^3)} + w_b R_b^2 \frac{(R^3 - R_c^3)}{R^2(R_b^3 - R_c^3)} \quad (6)$$

which yields $u_{uu}(R) = \alpha_{uu} R + \beta_{uu} \frac{1}{R^2}$ with $\alpha_{uu} \approx \left(w_b - w_c \left(R_c/R_b\right)^2\right)/R_b$ and $\beta_{uu} \approx R_c^2 \left(w_c - w_b \left(R_c/R_b\right)\right)$. This is a simplified formula, appropriate to the case in which the cell density is dilute, $R_c \ll R_b$. The general case is easily calculated using the method described here.

For the case where the cell imposes a fixed stress at its boundary, $\pi_c = -\sigma_{rr}(R_c)$, we use Eq. 2 to write the boundary conditions and denote the displacement by the subscripts σu to indicate that the biological cell imposes fixed stress but the periodic unit cell boundary imposes a fixed displacement. We find:

$$u_{\sigma u}(R) = \pi_c R_c^3 \frac{(R_b^3 - R^3)}{R^2(3KR_c^3 + 4\mu R_b^3)} + w_b R_b^2 \frac{(3KR_c^3 + 4\mu R^3)}{R^2(3KR_c^3 + 4\mu R_b^3)} \quad (7)$$

When the cell imposes a fixed compressive strain at its boundary, we use the subscript ε to indicate this and find:

$$u_{\varepsilon u}(R) = \frac{\left(R^3 - R_b^3\right)\varepsilon_c}{3R^2} + \frac{R_b^2 w_b}{R^2} \quad (8)$$

Note that for a contractile cell whose homeostasis is achieved by compressing (densifying) the matrix in its vicinity, $\varepsilon_c < 0$ so that when the boundary at $R = R_b$ is held fixed ($w_b = 0$ in Eq. 8) the cell adhesion is displaced in the forward direction ($u_{\varepsilon u}(R_c) > 0$). The cell indeed imposes a negative compressive strain at the cell-matrix interface due to the fixed displacement boundary condition at $R = R_b$. If the cell is contractile and relatively soft, one can consider a case where the cell itself contracts and the matrix is stretched near the cell, corresponding to $\varepsilon_c > 0$.

If instead of displacement, an external stress π_b is fixed at $R = R_b$, we indicate this with a second subscript of σ. The solution of the elastic equations predicts:

$$u_{u\sigma}(R) = w_c R_c^2 \frac{\left(3KR_b^3 + 4\mu R^3\right)}{R^2\left(3KR_b^3 + 4\mu R_c^3\right)} + \pi_b R_b^3 \frac{\left(R^3 - R_c^3\right)}{R^2\left(3KR_b^3 + 4\mu R_c^3\right)} \quad (9)$$

While,

$$u_{\varepsilon\sigma}(R) = \frac{\varepsilon_c\left(3KR_b^3 + 4\mu R^3\right)}{12\mu R^2} - \frac{R_b^3 \pi_b}{4\mu R^2} \quad (10)$$

and

$$u_{\sigma\sigma} = \pi_c R_c^3 \frac{\left(4\mu R^3 + 3KR_b^3\right)}{12K\mu R^2\left(R_b^3 - R_c^3\right)} + \pi_b R_b^3 \frac{\left(3KR_c^3 + 4\mu R^3\right)}{12K\mu R^2\left(R_b^3 - R_c^3\right)} \quad (11)$$

Once the matrix strain is determined for the different boundary conditions, Eq. 2 is used to determine the corresponding expression for the stress in the medium.

3.2 Cell-cell interactions

We begin with the most dramatic example of how activity and homeostasis sometimes result in elastically mediated cell-cell interactions in situations where non-active inclusions in elastic matrices do not interact and show below how a similar illustration can also explain durotaxis. The solutions for the radial matrix displacement as given above are now analyzed for various boundary conditions. For the case of the periodic array of cells the symmetry boundary condition at $R = R_b$ is $u(R_b) = 0$. At the cell boundary, $R = R_c$, we consider the cases of fixed homeostatic cell stress, σ_c, strain ε_c and fixed homeostatic cell displacement, u_c. The local displacement of the matrix at each point is given by Eqs. 6-11 with $w_b = 0$

In an infinite system, the results of the previous section show that a single cell that exerts a fixed stress σ_c imposes a displacement of the medium at its boundary of $u_c^\infty = R_c\sigma_c/(4\mu)$. For a cellular array with a homeostatic cellular stress, $\sigma(R_c) = -\sigma_c$, Eq. 7 gives the displacement, $u_{\sigma u}(R_c)$, of the adhesion or the medium at the cell boundary in mechanical equilibrium. We define $u_c^e = u_{\sigma u}(R_c)$ evaluated for $\pi_c = \sigma_c$ and $w_b = 0$ (zero displacement of the boundary at $R = R_b$): $u_c^e = u_c^\infty - R_c\left(\sigma_c(3K + 4\mu)/(16\mu^2)\left(R_c/R_b\right)^3\right)$ in the limit $R_c \ll R_b$. Since $\sigma_c < 0$ for contractile cells, the adhesion is displaced in the forward direction compared to an isolated cell; this represents an attractive interaction between cells in a periodic array. This will tend to move the cells closer to each other or to orient towards each other in the case of anisotropically shaped cells. If the cells in the array were disconnected elastically from each other, an elastically incoherent system, equivalent to a finite medium of size R_b with a stress-free boundary, the equilibrium displacement at the cell boundary would be from Eq. 11 with $\pi_c = \sigma_c$ and $\pi_b = 0$ to yield: $u_c^e = u_c^\infty + R_c\left(\sigma_c(3K + 4\mu)/(12K\mu)\left(R_c/R_b\right)^3\right)$ (in the limit $R_c \ll R_b$) which displaces the adhesion in the backwards direction (closer to the nucleus) compared to an isolated cell in an infinite medium. Thus, the interactions of cells in an elastically connected array leads to effective attractions compared with both a single cell in either an infinite medium or a finite, stress-free matrix. § Strain homeostasis for the case

§ As shown in Appendix A, for the case of homeostatic stress, the attraction of the central cell to its neighbors is also reflected in the expression for the total elastic energy which decreases as the distance between cells in the array decreases and scales as $\left(R_c/R_b\right)^3$.

of $\varepsilon_c < 0$ results in the same qualitative trend of attraction but now the displacement of the adhesion (compared to its displacement in an infinite matrix) is independent of the elastic constant of the surrounding medium and for the periodic cellular array $u_c^e = \varepsilon_c (R_b^3 - R_c^3)/R_c^2$ which implies displacement of the adhesion in the forward direction since $\varepsilon_c < 0$ is expected if the cell is fairly rigid relative to the matrix and regulates its activity to compress its elastic surroundings.

For the case of stress homeostasis, the change in the sum of the contractile and elastic forces when the adhesion is displaced from its mechanical equilibrium position due to protrusion forces, δf_m, is proportional to σ_c if there is sufficient time during a protrusion for σ_c to maintain its homeostatic value. In that situation, the stress that opposes the protrusion does not depend on the boundary conditions at $R = R_b$ (*i.e.*, free or clamped, $u_b = 0$ conditions due to the cell array). If homeostasis cannot be achieved during the protrusion, δf_m is proportional to inverse of the derivative of the displacement as a function of σ_c. This is equivalent to the derivative of the stress with respect to the displacement. In this case, a single cell in an infinite elastic medium, $\delta f_m \propto -4\mu/R_c \, (u_c - u_c^\infty)/R_c$ where u_c is the instantaneous displacement in Eq. 1. For an elastically coherent, periodic array $\delta f_m \propto -\left(4\mu + (3K+4\mu)(R_c/R_b)^3\right)(u_c - u_c^e)/R_c$, in the limit $R_c \ll R_b$. The negative sign indicates that the residual stress opposes the protrusion forces that tend to move or orient the cell in the forward direction. We see, however, that for the periodic array, the cellular stress is *more negative* compared with the corresponding stress for the single cell in an infinite medium or for a disconnected, periodic array, where we find that the term in $1/R_b^3$ is positive, thus reducing the absolute magnitude of the matrix stress that opposes the protrusions.

We now consider the case of homeostatic displacement of the adhesions at the cell boundary. The solution of the elastic equations for the periodic array using Eq. 6 with boundary conditions $u(R_c) = u_c$ and $u(R_b) = 0$ predicts that the stress in the medium due to cell contractility at $R = R_c$ is $\sigma(R_c) = -4\mu \, u_c/R_c - R_c^2 \, u_c \, (3K+4\mu)/R_b^3$ in the limit that $R_c \ll R_b$. For the case of free (zero stress) boundary conditions $\sigma(R_b) = 0$, applicable for a cell in a periodic array of cells that are elastically incoherent or for a cell in a finite matrix of size R_b with stress-free boundary conditions, the stress in the medium due to cell contractility is $\sigma(R_c) = -4\mu \, u_c/R_c + 4R_c^2 \, u_c \, \mu \, (1 + 4\mu/(3K))/R_b^3$. The net stress that opposes the protrusion forces that tend to displace the adhesions from $u_c^e = u_c$ is obtained from the derivative of the stress at the cell boundary with respect to u_c times $u_c - u_c^e$. The opposing force defined in the text is $\delta f_m \propto \left(-4\mu - (3K+4\mu)(R_c/R_b)^3\right)/R_c$, for $R_c \ll R_b$. The medium stress that opposes the forward motion of the protrusion force is thus larger (more negative) than its value for an isolated cell in an infinite medium or for a cellular array that is elastically incoherent where the term proportional to $1/R_b^3$ is positive and reduces the stress that opposes the protrusions.

In summary, stress or strain homeostasis that act to compress the medium near the cell, ($\sigma_c < 0$ or $\varepsilon_c < 0$ respectively) tend to displace the adhesion in a periodic, elastically coherent cellular array in the forward direction compared to its displacement in the case of a free (or elastically disconnected) boundary at $R = R_b$ by an amount proportional to $R_c \, (\sigma_c/\mu) \, (R_c/R_b)^3$. This represents an effective attraction between cells and is consistent with the total elastic energy (see Appendix A) which decreases as the cells are brought closer together. However, for stress homeostasis, the stress that opposes the protrusive forces for a cell array is either the same or larger (by an amount proportional to $\mu \, (R_c/R_b)^3$) than the free case, depending on whether the cellular stress can achieve homeostasis or not on the time scale of the protrusion. In the latter case, the larger stress reduces the effectiveness of the protrusions in moving the cells closer together. However, for small displacements of the adhesion from their equilibrium position, this stress is small and the protrusions can be effective in providing the destabilization that moves the cell in the forward direction. For the situation in which the displacement is fixed due to homeostasis, the adhesion displacement is fixed at a constant value of $u_c^e = u_c$ independent of the boundary conditions far from the cell. Thus, the adhesion position remains the same whether the cell is isolated in an infinite medium or is in a periodic array of other cells, independent of whether they are elastically connected. The stress that opposes the protrusions is larger for the case of an elastically connected, periodic array. This represents an effective repulsion between cells and is consistent (see Appendix A) with the total elastic energy of this system which increases as the cells are brought closer together.

The elastic interactions of active cells that can regulate their contractility to maintain stress, strain or displacement homeostasis at their boundaries are dramatically different from the lack of interaction of spherically symmetric, rigid (that cannot change their shape or forces they exert) inclusions in an isotropic and infinite elastic matrix. In the latter case, each inclusion exerts a fixed local displacement or local stress which is independent of the presence of the other inclusions (see Fig. 3a). There is no regulation of the stress, strain or displacement by the rigid inclusion as there is for biological cells, expressed in our model by keeping the local conditions fixed at either u_c, ε_c or σ_c even in the presence of other cells. For an infinite system, the solution of the equilibrium equations predict that the matrix displacement varies as $1/R^2$ with no term linear in R. Thus,[12] the matrix deformation induced by each rigid inclusion in an infinite system is a pure shear which does not couple the purely compressive stress or displacement of its spherically symmetric neighboring inclusion. Therefore, the

ability for an inclusion to respond to changes in its mechanical environment by varying the stress, strain or displacement at its boundaries is crucial to mediate elastically driven, cell-cell interactions, for the symmetric geometry considered here. In the context of biological systems, this ability is related to cell activity while in the context of non-biological inclusions this might be related to the ability of the inclusion to modify its shape or elasticity in response to the matrix stresses generated by mechanical perturbations. An explicit calculation that shows that the interactions between rigid inclusions in a periodic array vanishes, is given in Appendix B. This demonstrates that for the periodic geometry considered here, it is the regulation of the boundary condition by the inclusions that results in a non-zero interaction between them. A calculation of the interactions between two inclusions that can adjust (or actively regulate, in the case of cells) the stress that they exert on the medium in order to maintain fixed stress, strain or displacement boundary conditions (see Fig. 3b) will be presented elsewhere [27]. This case is analogous to a force dipole moment on one inclusion that is induced by the stress field due to its neighbor, which yields the elastic analogy of the van der Waals interaction. We find that the interaction energy between two such inclusions, each of which maintains a fixed displacement at its boundary even in the presence of the other, is repulsive and decreases with cell separation D as $1/D^6$. For a three-dimensional arrangement of inclusions with typical distance D between neighbors, we can integrate this interaction energy over all the interacting pairs in the system and find that the incremental force scales as $1/D^3$, which is consistent with the elastic energy of our spherical-unit-cell approximation of the many-cell, periodic system, Fig. 2. The $1/D^6$ behavior of the incremental force or interaction energy is reminiscent of the van der Waals interaction; both effects are due to induced polarizations of the dipoles in the two bodies [31].

3.3 Durotaxis

Similar elastic calculations in the spherical geometry can also be used to understand the conditions under which cells may exhibit durotaxis – the tendency to migrate from soft to rigid environments – as well as the propensity of cells on a rigid substrate to align parallel to the boundary with softer substrate [32–34]. We consider a cell in a finite elastic matrix of radius R_b with bulk modulus K and shear modulus μ. If the medium outside this matrix (*i.e.* in the region $R > R_b$) is infinitely rigid, the boundary condition of zero displacement, $u(R_b) = 0$, at $R = R_b$ applies. The presence of a very soft medium in the outer region, $R > R_b$, can be approximated by a zero stress boundary condition, $\sigma(R_b) = 0$, for $R = R_b$. The problem then maps to the case of cells in a periodic array where a very soft medium for $R > R_b$ corresponds to elastically incoherent cells (or a single cell in a finite matrix of size R_b with stress-free boundary conditions) and a very rigid medium for $R > R_b$ corresponds to the elastically coherent situation in which the cells interact via their mutual deformations of the matrix. Using the results of the previous section allows us to predict that cells that fix their boundary stress or boundary strain are attracted to the rigid medium (at the boundary $R = R_b$) while cells with homeostatic displacement would be repelled from a neighboring medium with very large rigidity The attractive interaction results in cells that tend to migrate towards the rigid medium and the repulsive interaction would predict the opposite. In the Conclusions section, we relate these predictions to experimental observations.

3.4 External matrix stretch

We now consider incremental forces applied by biological cells to their adhesions in the presence of external mechanical stresses that are applied slowly enough so that the cell can adjust its contractility and the homeostatic stress, strain or displacement are reached adiabatically (*i.e.*, at each step of the process). We assume that the external stress is applied after the cell has achieved its homeostatic stress, strain or displacement after being plated on the substrate. In the spherical geometry, the external stress or displacement is isotropic and we take it to have a constant value of σ_b or u_b at the boundary of the periodic unit cell that delineates the matrix surrounding each cell. Eqs.6-8 are used with these boundary conditions for the cases of homeostatic cellular displacement, stress and strain. If the distance between cells in the periodic array is large enough, the intercellular forces may be negligible compared with the applied stresses.

As above we see that cells with a homeostatic displacement at their boundary adiabatically maintain this value and their adhesions are not displaced by the external stretch. Thus, if the cell was adherent and non-motile before application of the external stretch, it remains in this state and does not "react" to very slowly applied, externally applied displacements or stresses. On the other hand, cells with a homeostatic stress or strain at their boundary do modify their displacements in the presence of slowly applied stretch. The elastic equations are solved with the boundary condition $u(R_b) = u_b$ that corresponds to the displacement due to the external stretch; the strain in the spherical geometry is u_b/R_b and the equilibrium position of the adhesion, u_c^e is shifted forward by an amount $R_c\,(1+3K/(4\mu))\,(u_b/R_b)$. If the quasi-stochastic protrusive forces that drive cell motion in the forward direction are instantaneously larger on one part of the cell boundary, the cell will then tend to move in the direction of the stretch. The residual stress that resists the protrusive force is the same as for the case of the cellular array with no external stretch where $u(R_b) = 0$.

4 Conclusions and comparison with experiment

Our results predict that stress or strain homeostasis results in cells whose adhesions are displaced closer to neighboring cells, to a medium which is more rigid, and in the direction of slowly applied stretch for cases where cell homeostasis results in a local compression of the medium near the cell; in the opposite case, cells are repelled by their neighbors or rigidity gradients. Since cells may translate very slowly in viscoelastic matrices, a more practical comparison of theory and experiment for asymmetrically shaped cells can be obtained by observations of how the relative orientation of *non-spherical* cells such as fibroblasts or muscle cells changes in the presence of other non-spherical cells[34,35]; the attractions we predict imply orientations in the direction of nearby cells or the direction of slowly applied stretch. The residual stress that opposes protrusion-induced cell motility is opposed by an elastic stress that is independent of the location of the other cells, more rigid medium or orientation of the applied stretch when the homeostasis can be established dynamically during the protrusion process. Otherwise, the stress that opposes the protrusions is increased by a factor proportional to $(R_c/R_b)^3$. Displacement homeostasis implies that the adhesions are not displaced by mechanical perturbations of the elastic medium but the residual stress that opposes the protrusions is even more negative by a term proportional to $(R_c/R_b)^3$. This makes the protrusive forces less effective in the direction of the other cells, rigidity gradients or applied stretch, consistent with an effective repulsion. We note that our results indicate that displacement homeostasis implies that the cell expends more energy in maintaining fixed displacement when it is closer to other cells, increasing rigidity regions or slowly applied stretch; stress homeostasis implies the opposite (see the energy calculations in Appendix A). However, the present discussion focuses on forces and displacements of the adhesions since these are indeed mechanical responses even in living cells. Whether the expenditure of more (or less) energy to maintain homeostasis results in cell motion away from (or towards) the mechanical perturbation of the environment is an interesting question that deserves further experimental attention.

We now address the experiments[10] that measured whether the force or displacement of pillars onto which cells adhered was fixed as the elasticity of pillar was varied in order to elucidate whether we should consider fixed stress, strain or displacement boundary conditions in our predictions of cellular response of relatively distant, mechanical perturbations of the matrix. The pillar experiment demonstrated either fixed force or displacement only with respect to rigidity changes of the pillars. ¶. As mentioned in the introduction, the translation of the results of the pillar experiments to cells in an elastic continuum suggest that in the continuum, the analogue of fixed force is fixed stress at the cell boundary while the analogue of fixed pillar displacement may be fixed strain at the cell boundary. Finally, we note that recent experiments suggested that the homeostatic quantity that is independent of substrate rigidity is the work done by a cell in deforming its environment (in the absence of mechanical perturbations) [37]. This is not a local quantity and implies global coordination of the cytoskeletal forces and adhesions in response to rigidity changes. Of course, both this experiment as well as those that measured the local cellular forces or displacement of pillars examined homeostasis only as far as substrate rigidity changes are concerned. Whether this homeostasis persists in the presence of mechanical perturbations such as other cells, rigidity gradients (at a distance from the cell) or static stretch, remains to be measured. For this reason, we have presented our predictions for the three most likely types of homeostasis of stress, strain and displacement. Experiments that directly measure which homeostasis is applicable in response to mechanical perturbations have yet to be done; however, we can still discuss how comparison of our predictions with existing experiments on cell response to other cells, durotaxis or other cells can shed light on whether the cells keep fixes stress, strain or displacement as their mechanical environment if modified.

Experiments[38] have reported that cells on soft substrates attract each other while those on rigid substrates do not. While in some experimental studies cells aligned parallel to the direction of a static or quasi-static stress field[11,39–41], other experiments find that some types of cells remain randomly oriented[42]; the rigidity dependence of these observations has not yet been fully quantified. Our predictions can be summarized as follows: (i) For homeostatic stress or strain: Adhesions are displaced towards those of neighboring cells (cells are attracted to nearby cells) when the cell activity homeostasis results in a counter force in the matrix that locally compresses the medium near the cell. This occurs either for a cellular stress $\sigma_c < 0$ or an imposed strain $\varepsilon_c < 0$ that tends to locally densify the matrix (in the case that the cell is more rigid than the matrix); the same physics explains that cells are also attracted to regions of higher rigidity. Our expressions

¶ We note that it is also possible that in the experiments in which the pillar rigidity was changed the cells modulate maintained fixed force on the pillars for both rigid and deformable pillars, but modified their mechanical coupling to the pillars as a function of the pillar bending rigidity An example of this has been discussed[16,36] where the experiments on soft pillars are interpreted in terms of homeostatic cell stress that is applied to adhesions whose size increases with rigidity so that the force applied by an adhesion to the pillar (product of the stress and adhesion area) increases with rigidity. But this is not the only possible type of feedback that can allow for explanation of the experiments that differ from the "naive" interpretation of fixed displacement homeostasis.

for the displacement and stress predict that for fixed homeostatic stress the incremental strain at the cell boundary in these cases scales as $(R_c/R_b)^3$ which can be tested experimentally by varying the intercell spacing, R_b in the periodic array. For homeostatic stress of the order of the matrix rigidity, this predicts an incremental strain of a few percent for intercellular spacings that are three times larger than the cell size. Similar predictions can be made for homeostatic strain. In addition, the theory suggests that cells will align with the direction of static stretch. If the homeostatic strain or stress imposed by the cell tends to result in a local expansion of the matrix near the cell due to a cellular stress, $\sigma_c > 0$, or a locally imposed strain $\varepsilon_c > 0$ (perhaps due to the cell itself contracting in the case that it is softer than the matrix), the adhesions are displaced in the backward direction and the cell is repelled from nearby cells or from a region of higher rigidity. This also suggests that cells will not tend to align with the direction of static stretch. (ii) For homeostatic displacement, cells do not respond to the mechanical perturbations induced by other cells, rigidity gradients or static stretch since the adhesions are always displaced in the same manner no matter what the mechanical state of the surroundings. Determining in some first-principles manner whether cellular homeostasis in a given system fixes the stress, strain or displacement at the cell boundary is outside the scope of the elastic theory presented here since by definition, homeostasis depends on cellular activity. Experiments [10] on discrete, pillared substrates where it is relevant to discuss the force or displacement of a pillar, indicate homeostatic displacement for soft substrates and homeostatic stress for rigid substrates. The translation of this to a continuum elastic matrix may allow for control of the local stress, strain or displacement at the cell boundary. However, homeostatic displacement is more difficult to justify in continuum elastic matrices where the relative displacements of the matrix or cell (i.e., the strain) should govern the physics. Models that include the cell, substrate and adhesion elasticity [19] are consistent with homeostatic strain on substrates that are softer than the cell and homeostatic stress on more rigid substrates. These models include cell activity as an effective pre-stress of the cell due to its contractility, but do not account for biological feedback effects. For this reason, we have chosen a minimal elastic theory in which the homeostasis enters as a boundary condition which is obtained from observations.

Our prediction of cell-cell attractions for either homeostatic stress or strain can be compatible with observations [38] which showed attractions on soft substrates but short-ranged repulsions upon contact on rigid substrates. The displacement of the adhesions under homeostatic strain $\varepsilon_c < 0$ that results in a local compression of the matrix (applicable to substrates that are softer than the cell), are independent of substrate rigidity and predict attractive interactions – at a distance – of nearby cells. The induced negative strain in the matrix is reasonable even for contractile cells, so long as the cell is more rigid than the matrix. In that case, it is elastically more favorable for the matrix to be compressed compared with the cell; when the matrix is compressed, it pulls the adhesions towards the neighboring cells as shown quantitatively in our theory. When the homeostasis switches to constant stress $\sigma_c < 0$, (applicable to substrates that are more rigid than the cell) in which the relatively softer cell itself may contract under its own contractility, the reaction stress in the matrix is positive and tends to compress the medium; the displacements of the adhesions are again in the forward (attractive) direction, but are predicted to decrease inversely with the substrate rigidity. For very rigid substrates, the elastic interactions are negligible compared with the "noise" that dictates random cellular motion and the cells are therefore observed to move randomly, repelling each other by their excluded volume only upon contact. Typical experiments on durotaxis [32] indicate that cells are attracted to the more rigid regions of their surroundings; this is consistent with our predictions for homeostatic contractile stress ($\sigma_c < 0$) or strain (with $\varepsilon_c < 0$) but not displacement.

For slowly applied stretch, the experimental situation is not completely clear. In some experimental studies cells aligned parallel to the direction of a static or quasi-static stress field[11,39–41], other experiments find that some types of cells remain randomly oriented[42]; the rigidity dependence of these observations is only now beginning to be quantified[43]. On soft substrates (relative to the cell) we expect the passive elastic response of the cell to be negligible and for the cell to respond to external stretch via its coupling to cellular contractility. For this case, our model suggests that the cell will align parallel to the direction of static stretch for either strain or stress homeostasis with $\varepsilon_c < 0$ or σ_c respectively. On rigid substrates (relative to the cell), the cell itself is stretched and the combined response of its own passive elasticity and active contractility may be more complex.

Finally, we remark that for rapidly varying cyclic stress, neither stress nor displacement homeostasis may be able to be maintained; the cell contractility cannot "catch up" to the instantaneous value of the matrix displacement. To understand cell alignment under applied cyclic stretch with frequencies in the range of 1Hz or larger, it may be necessary to more deeply examine the biological mechanisms that regulate homeostasis[44–46] and how these can or cannot respond to rapidly varying mechanical perturbations of the cellular environment. Such a "biological penalty" may be the reason that cells subjected to cyclic stretch tend to align away from the stretch direction[42,47–52]. A more specific model of the molecular effects and time scales that govern cell reorientation in response to cyclic stretch has also been considered [53]. More recent quantitative measurements of such effects[54] have been interpreted in terms of the passive elastic response of the cell that then reorients the cytoskeleton. In any case, this dynam-

ical problem is outside the scope of our work that focuses on the role of homeostasis and how it controls cellular response to mechanical perturbations of its elastic environment. Further experiments on all three types of environmental perturbations under controlled conditions of cell density and matrix rigidity may distinguish between the different homeostatic cases. Such measurements will provide information how an external mechanical probe can be used to infer information about cell activity and function.

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A Elastic energy of the medium due to cell contractility

In the body of this paper, we presented explicit calculations of the displacement of the cell boundary due to the presence of mechanical perturbations of the medium such as other cells, rigidity gradients and external, slowly applied stretch. These forces act to move the adhesions at the cell-matrix interface. Here we also present calculations of the elastic energy of the matrix due to cell contractility. Minimization of this energy by the elastic medium gives rise to the driving stresses that result in the displacement of the adhesions.

The energy of the medium, W is given by one-half of the volume integral of the strain and the stress which (for the cases of interest where either the displacement or the stress at the unit cell boundary R_b is zero) can be transformed by integration by parts into the product of the displacement and force (the product of the stress and area) at the cell boundary:

$$W_{\alpha\beta} = -2\pi R^2 u_{\alpha\beta}(R_c)\ \sigma_{\alpha\beta}(R_c) \tag{12}$$

where $\alpha = u, \sigma$ and $\beta = u, \sigma$ denote the four possible boundary conditions at R_c and R_b respectively. Since the corrections to the displacement due to the boundary conditions at R_b (due to other cells, a rigid external matrix or applied stress) scales as $1/R^2$ and the stress scales as the displacement divided by R, the corrections to the energy due to the boundary condition at R_b are expected to scale as $1/R_b^3$ as we now show from explicit calculations.

In the limit that $R_b \gg R_c$, we find that for the case where there is no applied stretch so that either the displacement (W_{uu}, $W_{\sigma u}$) or stress ($W_{u\sigma}$, $W_{\sigma\sigma}$) at $R = R_b$ is zero:

$$W_{uu} = 8\pi\mu R_c u_c^2 + \frac{\pi R_c^4 u_c^2 (6K+8\mu)}{R_b^3} + \ldots \tag{13}$$

$$W_{\sigma u} = \frac{\pi R_c^3 \sigma_c^2}{2\mu} - \frac{\pi R_c^6 \sigma_c^2 (3K+4\mu)}{8\mu^2 R_b^3} + \ldots \tag{14}$$

$$W_{u\sigma} = 8\pi\mu R_c u_c^2 - \frac{8\pi\mu R_c^4 u_c^2 (4\mu+3K)}{3K R_b^3} + \ldots \tag{15}$$

$$W_{\sigma\sigma} = \frac{\pi R_c^3 \sigma_c^2}{2\mu} + \frac{\pi R_c^6 \sigma_c^2 (3K+4\mu)}{6K\mu R_b^3} + \ldots \tag{16}$$

We see from these equations that compared with the energy ($W_{u\sigma}$, $W_{\sigma\sigma}$) in the free boundary case where the stress at $R = R_b$ is zero, the elastic energy (W_{uu}, $W_{\sigma u}$) of a cell in a periodic cell array or for a cell with an infinitely rigid medium in the region $R > R_b$ where the displacement is zero at $R = R_b$, is lower for the case of stress homeostasis ($W_{\sigma u}$) and higher for the case of displacement homeostasis (W_{uu}). This respectively represents an effective attraction or repulsion of the cell to the other cells in the array or to the infinitely rigid medium (durotaxis). However, since cell behavior is not necessarily governed by energy minimization but rather by the response to force, this paper focuses on the forces on the adhesions and whether they displace the adhesions toward or away from the boundaries.

B Elastic interaction of responsive vs. rigid inclusions

The interaction energy of two rigid inclusions each of which induces a fixed, symmetric force that deforms the surrounding matrix,[12] vanishes. For a realistic geometrical arrangement of such spherical inclusions in space, the displacement field generated by each inclusion, although isotropic around it, is non-isotropic with respect to the center of a neighboring cell some distance away (see Fig. 3) For multiple rigid inclusions, the displacements generated by all neighboring spheres will not cause the total displacement to be isotropic for each cell, as in our spherical-unit-cell approximation. Nonetheless, we can consider active biological cells or responsive non-biological inclusions that sense the anisotropy in the total displacement field due to the presence of other cells around them, and regulate their active displacement so that the sum of the displacements generated by other such cells or inclusions and by themselves will be isotropic. To achieve this, it is necessary that the active or responsive system generate an anisotropic displacement (to cancel the displacements at the cell boundary induced by the other contractile cells). Therefore, the incremental elastic energy has a non-zero term due to the interactions between cells, similarly to the interaction between a single cell and the

spherical wall which we used above in our one-dimensional model for the boundary condition that simulates the effects of the neighboring cells. Details of such a calculation for two responsive inclusions will be presented elsewhere[27].

To explicitly demonstrate that the interaction of rigid inclusions vanishes even in the many-body case of a periodic array of cells, we have calculated cell-cell interactions in a, non-spherical (*e.g.*, cubic) Wigner-Seitz periodic unit cell appropriate to cuboidal inclusions on a cubic lattice. The inclusions are described by an eigenstrain tensor ε^*_{ij}, for which the Fourier components are given by[55]:

$$\varepsilon^*_{ij}(p,q,r) = \frac{8\phi}{\pi^3}\frac{\sin(\frac{p\pi a}{L})(\sin(\frac{q\pi a}{L})\sin(\frac{r\pi a}{L})\cos(\frac{p\pi x}{L})\cos(\frac{q\pi y}{L})\cos(\frac{r\pi z}{L})}{pqr}\delta_{ij}, \tag{17}$$

leading to the Fourier components of the displacement field:

$$u_x(p,q,r) = \lambda\,\frac{\sin(\frac{r\pi a}{L})\sin(\frac{p\pi x}{L})\cos(\frac{q\pi y}{L})\cos(\frac{r\pi z}{L})}{(p^2+q^2+r^2)qr} \tag{18}$$

$$u_y(p,q,r) = \lambda\,\frac{\sin(\frac{q\pi a}{L})\sin(\frac{r\pi a}{L})\sin(\frac{p\pi a}{L})\sin(\frac{q\pi y}{L})\cos(\frac{r\pi z}{L})\cos(\frac{p\pi x}{L})}{(p^2+q^2+r^2)rp} \tag{19}$$

and

$$u_z(p,q,r) = \lambda\,\frac{\sin(\frac{r\pi a}{L})\sin(\frac{p\pi a}{L})\sin(\frac{q\pi a}{L})\sin(\frac{r\pi z}{L})\cos(\frac{p\pi x}{L})\cos(\frac{q\pi y}{L})}{(p^2+q^2+r^2)pq} \tag{20}$$

Here

$$\lambda = \frac{8}{\pi^4}\frac{3\lambda+2\mu}{\lambda+2\mu}L\phi \quad , \tag{21}$$

where p,q,r are the Fourier components indices, ϕ is the relative length change, $2L$ is the distance between two inclusions (and the size of a Wigner-Seitz cell), and $2a$ is the cuboidal inclusion linear size.

The total elastic energy is given by a volume integral:

$$U = \int \mathrm{dV}\sigma_{\mathrm{ij}}\left(\varepsilon_{\mathrm{ij}} - \varepsilon^*_{\mathrm{ij}}\right) \tag{22}$$

where $\varepsilon_{ij} = \frac{1}{2}\left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i}\right)$ is the strain tensor, and $\sigma_{ij} = \lambda \mathrm{Tr}(\varepsilon - \varepsilon^*)\delta_{\mathrm{ij}} + 2\mu(\varepsilon_{\mathrm{ij}} - \varepsilon^*_{\mathrm{ij}})$ is the stress tensor. Substituting Eqs. 17-20 into the stress and strain tensors, and calculating the integral shows that the elastic interaction energy vanishes. This particular example shows that for rigid inclusions each of which introduces a pure volumetric deformation, the elastic interactions vanish[27].

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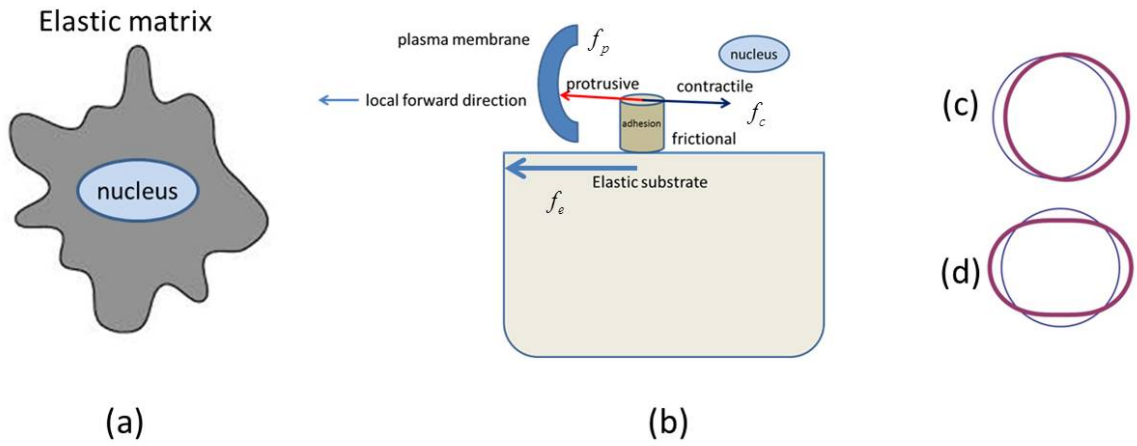


Fig. 1 (a) Top view of an initially spherical, contractile cell which develops protrusions. (b) Side view of the cell on a line from the protrusion to the nucleus. The local balance of forces on the combined membrane-adhesion system that determine whether the cell locally would tend to move or reorient or whether it remains adherent and stationary. The protrusions generally exert local forces in the "forward" direction on the cell membrane and are connected, via the adhesions, to the contractile forces that generally (see text) act toward the cell nucleus. The arrow shown in the elastic substrate represents the forces due to elastic perturbations of the cellular environment (that can displace the adhesion in either direction) such as other cells, rigidity gradients or external stretch that are coupled to the adhesions via the elastic medium or substrate. (c) Schematic of an initially spherical cell (thin blue line) with a protrusion that tends to move the cell to the right (thick red line). (d) Schematic of protrusions that tend to orient the cell along the horizontal direction (thick red line). If the cell is initially well adhered and stationary, the motion (c) or orientation (d) will only occur if the forces due to the presence of other cells, external stress or rigidity gradients tend to move the adhesion in the forward direction sufficiently reduce the contractile forces to allow the protrusion forces to move or orient the cell.

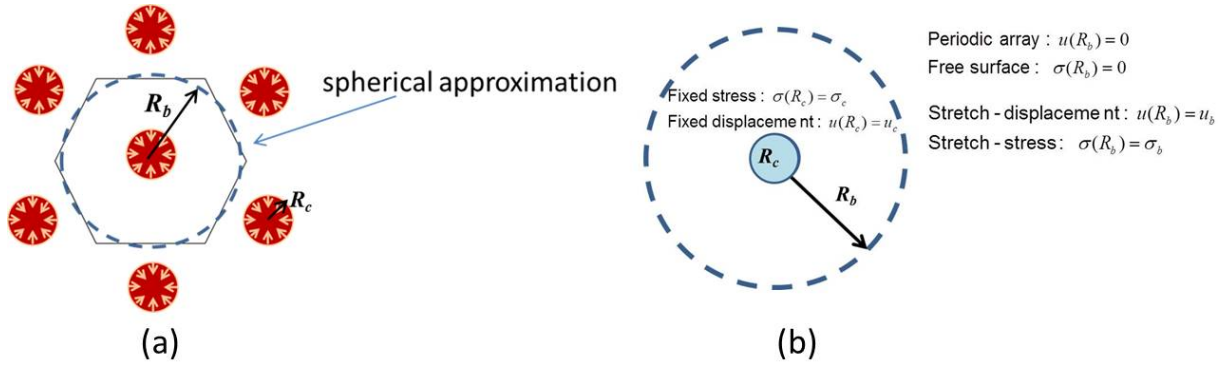


Fig. 2 (a) Periodic array of contractile cells. The Wigner-Seitz, periodic unit cell is approximated by a sphere at $R = R_b$. (b) Contractile cell of radius R_c in medium bounded by R_b. Homeostasis implies that the stress or strain that the cell applies at its boundary $R = R_c$ are fixed.

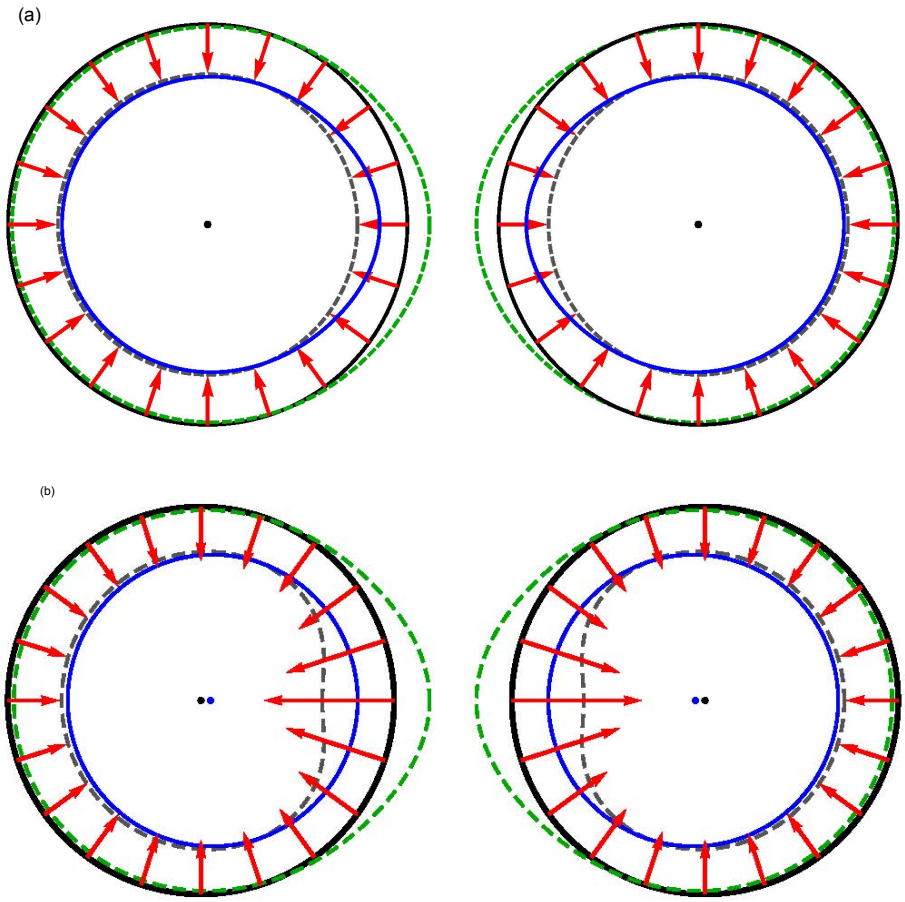
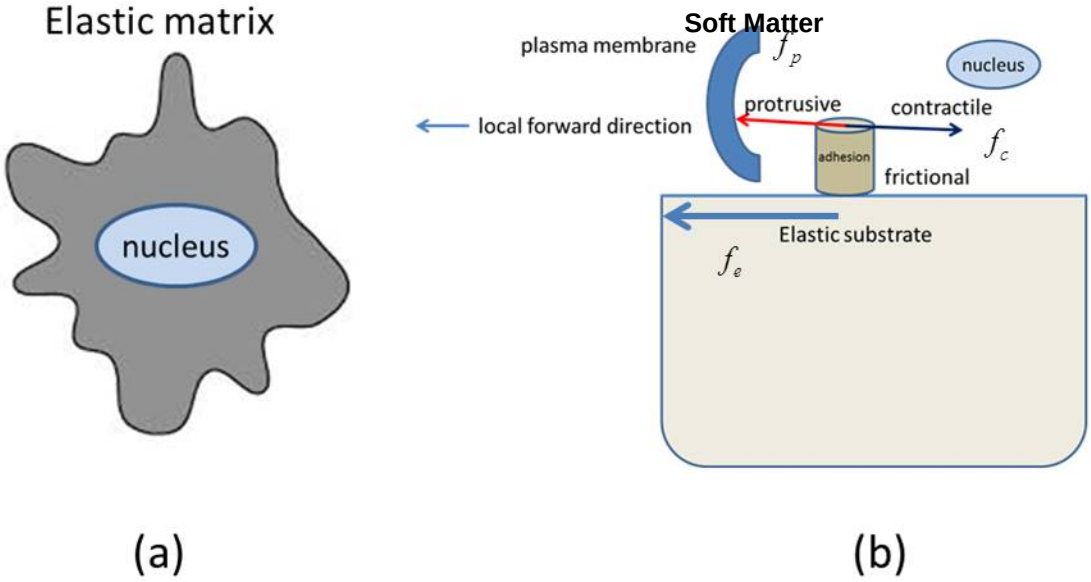
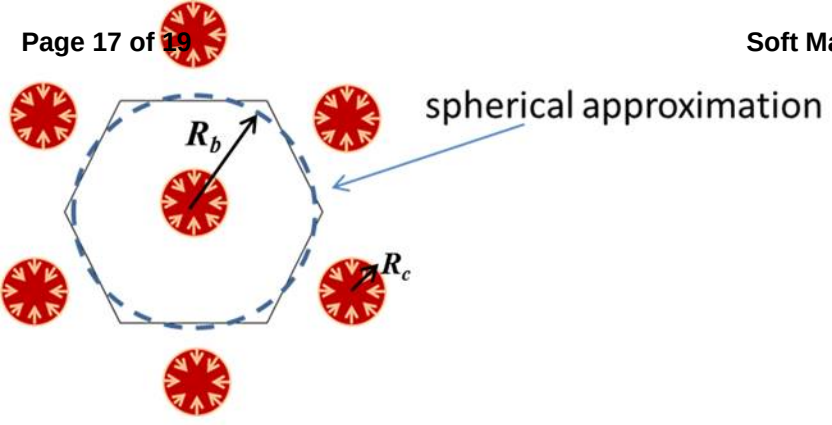
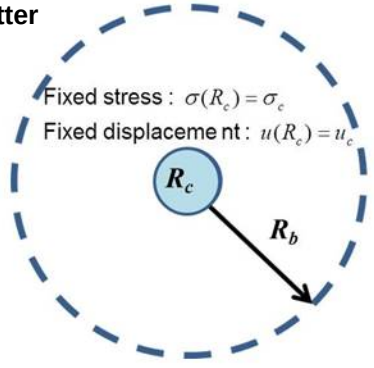


Fig. 3 Two contractile spheres in an infinite elastic medium: The solid black lines show the displacement of each sphere before it becomes contractile and the solid blue lines show the displacements upon interaction and regulation. The gray dashed line is the self displacement of each sphere (caused by its own, local contractility), and the green dashed line is the displacement caused by the neighboring sphere. The red arrows depict the contractile forces applied by each sphere. For illustration purposes, the initial distance between the spheres was taken set to $2.5R_c$, and the self displacement to $0.25R_c$. (a) Passive spheres that apply a fixed isotropic elastic stress at the sphere boundary $R = R_c$. (b) Active spheres that regulate the force they apply in order to maintain a fixed value of the local displacement at $R = R_c$ even in the presence of other cells.





(a)



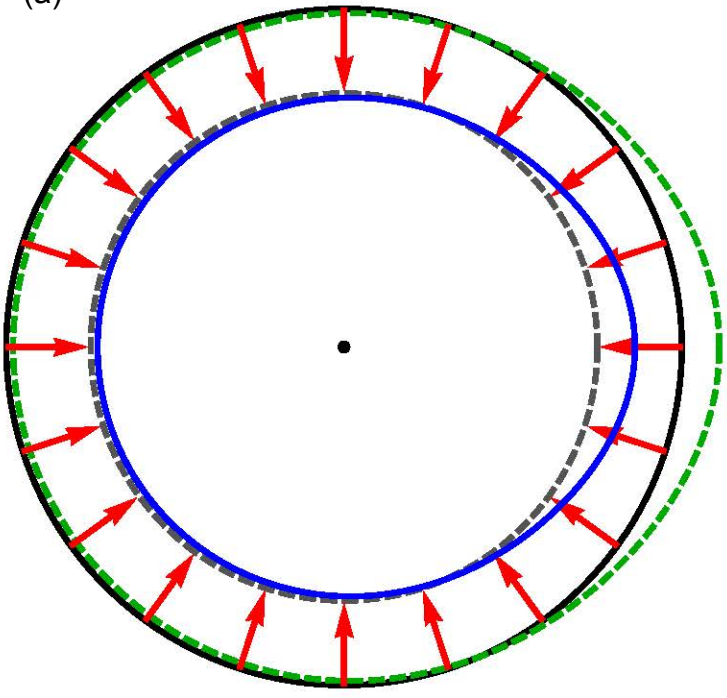
(b)

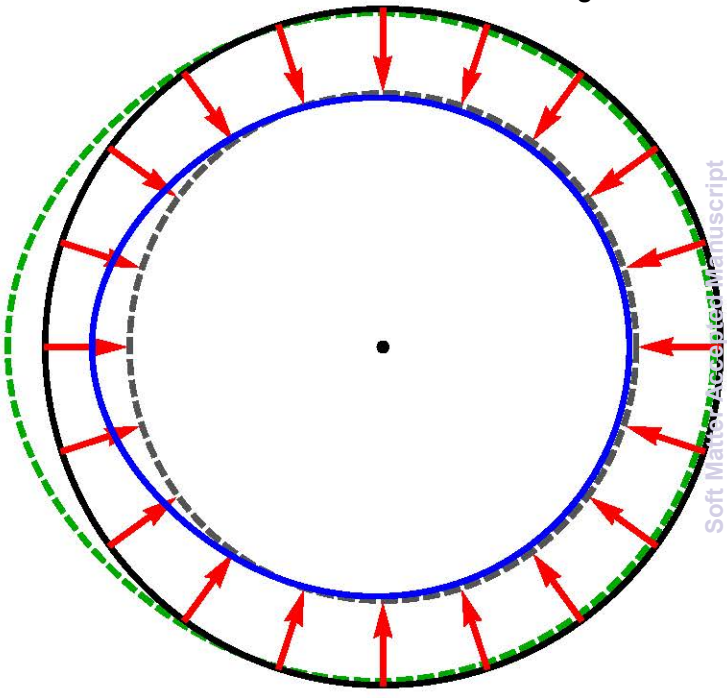
Periodic array : $u(R_b) = 0$

Free surface : $\sigma(R_b) = 0$

Stretch - displaceme nt : $u(R_b) = u_b$

Stretch - stress : $\sigma(R_b) = \sigma_b$





(b)