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Modeling mechanosensing and its effect on the migration and proliferation of adherent cells

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Abstract

The behavior of normal adherent cells is influenced by the stiffness of the substrate they are anchored to. Cells are able to detect substrate mechanical properties by actively generating contractile forces and use this information to migrate and proliferate. In particular, the speed and direction of cell crawling, as well as the rate of cell proliferation, vary with the substrate compliance and prestrain. In this work, we present an active mechanosensing model based on an extension of the classical Hill's model for skeletal muscle behavior. We also propose a thermodynamical approach to model cell migration regulated by mechanical stimuli and a proliferation theory also depending on the mechanical environment. These contributions give rise to a conceptually simple mathematical formulation with a straightforward and inexpensive computational implementation, yielding results consistent with numerous experiments. The model can be a useful tool for practical applications in biology and medicine in situations where cell–substrate interaction as well as substrate mechanical behavior play an important role, such as the design of tissue engineering applications.

Keywords: Cell–matrix interaction; Mechanotaxis; Computational model; Mechanobiology

1. Introduction

Research on cell migration and proliferation has drawn the attention of the scientific community during the last decades. It has now become a leading interdisciplinary research field that demands the collaboration of cellular biologists with experts from other disciplines, such as computer modeling and imaging, and biomaterial and mechanical engineering [1]. The relevance that cell motility has gained in biology research is due to its major role in several physiological and pathological processes, e.g. morphogenesis, inflammatory response, wound healing and tumor metastasis [2]. Cell migration and proliferation are also of significant interest in the field of tissue engineering. In fact, the primary function of a scaffold in tissue engineering is to serve as a substrate to which cells can attach, grow and maintain differentiated functions, and all of these processes can be strongly influenced by the scaffold microstructure and mechanical properties, as well as the biological and chemical properties of its surface [3].

Cell movement is guided by input signals from the surrounding environment in order to achieve an appropriate organization of cells and production of extracellular matrix (ECM) within tissues and organs. Migration, in response to gradients of dissolved or surface-attached chemicals, light intensity or electrostatic potential, has been studied for years [4]. More recently, the influence of the stiffness and topography of the ECM or substrate that adherent cells are anchored to has been investigated [4–6]. Among other important results, it has been found that cells crawl better

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on stiffer and more strained substrates, phenomena that have been defined as *durotaxis* and *tensotaxis*, respectively [7]. Moreover, it has been found that the stiffness of the substrate also directs cell proliferation, the proliferation rate being markedly reduced on more compliant substrates [8].

The immediate question that emerges is how are cells able to sense ECM flexibility or prestrain? In the last few years, it has become clear that adherent cells anchor to a substrate and then exert contractile forces in order to explore the properties of their environment, what is a part of the so-called process of *mechanosensing* [9]. These active forces are primarily generated by the actomyosin contractile machinery and transmitted to the ECM by means of transmembrane proteins of the integrin family in so-called focal adhesions [10]. The cytoplasmic domains of integrins are linked to the actin cytoskeleton (CSK) by a network of adapter proteins that form a submembrane plaque [11]. It has been found that cells exert higher contractile forces and show larger stable focal adhesions on stiffer substrates [12–14]. Moreover, the application of external stress/strain on the cell also stimulates focal adhesion formation and increases the tension that the submembrane plaque withstands [15,16]. This tension can trigger molecular reorganization at the adhesion sites or alterations in the conformation of plaque proteins or integrins. That is the reason why the integrin-mediated submembrane plaque tension-dependent mechanism has been hypothesized as a possible mechanosensitive path [10].

In addition to this extensive experimental research, mathematical models and computational simulations can also provide some insight into these matters [17–19]. Efforts have been addressed to the study of mechanosensing, and several models based on mechanics [20], thermodynamics [21] or the dynamics of focal adhesions [22] have been proposed. Cell migration modeling has received considerable attention, with some works aiming at reproducing the influence of chemical and mechanical properties of the substrate on cell locomotion [23,24].

In this paper, we present a model that makes predictions about mechanosensing and cell migration and proliferation. We show how active mechanosensing, albeit resulting from complex molecular processes, can be explained by the interaction of the mechanical behavior of the CSK components, the actomyosin contractile system and the ECM compliance. Applying equilibrium conditions to this simple scheme, equivalent to Hill's model for active muscle behavior [25], we are able to explain many experimental findings obtained for adherent cells on soft substrates. With regard to cell migration, we propose modeling the time and spatial evolutions of cell concentration through the classical transport equation within the framework of continuum mechanics. Our contribution in this direction consists in deriving an expression for the cellular flux from thermodynamic arguments, so that the input of mechanical signals received by individual cells through active mechanosensing is taken into account. Furthermore, the variation of the rate of proliferation depending on the mechanics of the ECM observed experimentally [8] has been also modeled.

The resulting continuum formulation has been implemented in a finite element framework, and computational simulations of cell migration on two-dimensional (2D) gradient-compliant and gradient-strained substrates have been performed, obtaining results that agree with several experimental observations. In order to predict cell–ECM interaction and the influence of the mechanical environment upon cell locomotion and proliferation in a specific biological phenomenon, e.g. fibroblast locomotion in wound healing, mesenchymal cell migration at the interface of a recently placed dental implant or endothelial cell organization in vasculogenesis and angiogenesis, it would be necessary to integrate every particular biological process of interest into this general model. In particular, the model is specifically suited for rational design of tissue engineering applications, since it allows one to understand the interaction between the mechanical state of the environment and cell behavior.

2. Mechanosensing model

The cellular elements that carry out a relevant function in the mechanics of cell mechanosensing and that have been considered in our model are the actin bundles, the actomyosin contractile apparatus and the passive mechanical strength of the rest of the body cell, whose main contribution comes from the CSK microtubules and the membrane (Fig. 1A). The cytoplasmic CSK is linked with the external ECM through focal adhesions and transmembrane integrins that are assumed perfectly rigid for our purposes. This scheme agrees with the tensegrity hypothesis [26], since tensile forces generated in the actin CSK are balanced by the compression of the microtubules and the external substrate. Finally, external forces are also taken into account, being another possible cause of the deformation of the substrate and cell. Note that this model can be applied to adherent cells, irrespective of the nature of their actual environment: plated on elastic substrates, cultured on hydrogels or on the surface of a scaffold or attached to the ECM of a connective tissue. Consequently, the expressions ECM and substrate will be used henceforth without distinction.

Even though the above suggested model, depicted in Fig. 1B, is one-dimensional, if isotropy for the contractile forces exerted by cells is assumed (which, although not the actual case, is sufficiently approximate for the aim of this work and useful in the interests of simplicity), one can interpret forces in each branch of the scheme of Fig. 1B as octahedral or hydrostatic stresses, and the change of length of each element as its corresponding volumetric strain [27]. In such a case, the characteristic value of each spring can be identified with the volumetric stiffness modulus of the representing element. Forces in each branch of the model then have a clear physical interpretation: p_c is a measure of the mean contractile stress generated internally by the myosin II machinery and transmitted through

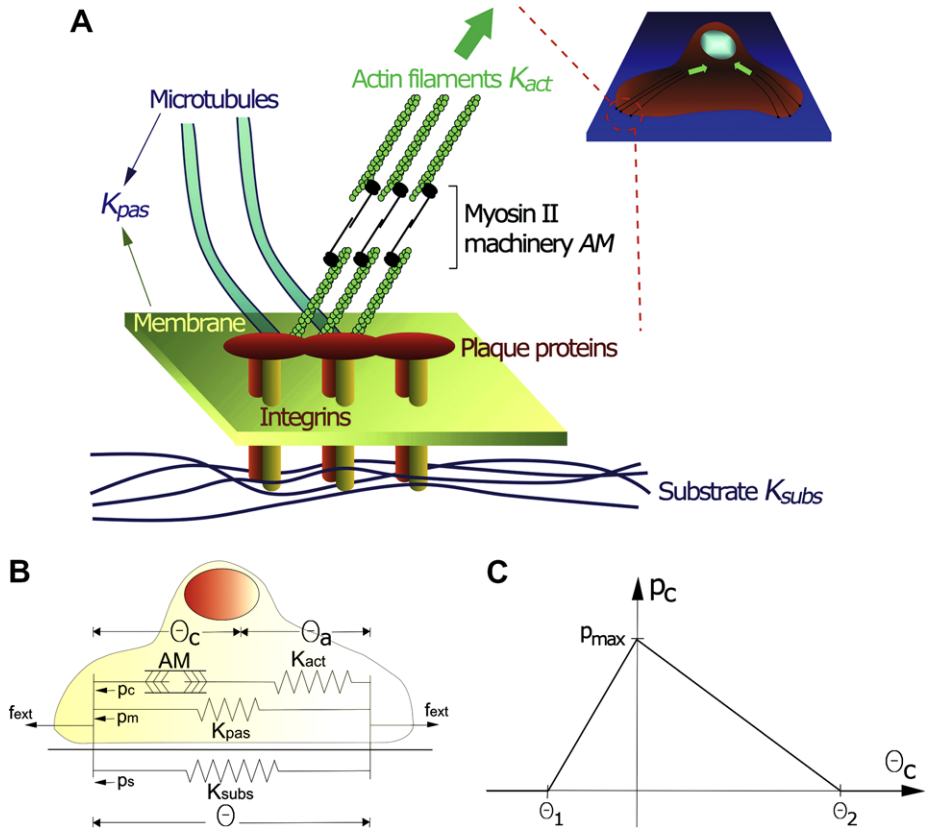

Fig. 1. Mechanosensing model for an adherent cell. (A) Schematic diagram of the relevant mechanical constituents of a cell. (B) Mechanical model corresponding to the previous diagram of the cell. K_{act}, K_{pas} and K_{subs} denote the volumetric stiffness moduli of actin filaments, of the passive components of the cell and of the ECM/substrate, respectively. f_{ext} stands for possible external forces applied to the cell or the substrate. Note that volumetric strain θ can be written in terms of the displacement vector $\mathbf{u}$ of the ECM as $\theta = \nabla \cdot \mathbf{u}$. (C) Dependence of the contractile pressure p_c on the deformation of the contractile element $\theta_c \cdot p_{max}$ stands for the maximum contractile force exerted by the actomyosin machinery, and θ_1 and θ_2 are the corresponding shortening and lengthening strains of the contractile element with respect to the unloaded length at which active stress becomes zero.

the actin bundles; p_m stands for the contractile stress supported by the passive resistance of the cell (CSK microtubules, essentially, but also the membrane); finally, p_s denotes the stress of the ECM. Note that the net stress of the cell, which can be denoted as p_{cell}, is the sum of active and passive stresses, i.e. $p_{cell} = p_c + p_m$, with p_{cell} the stress that the cell effectively transmits to the ECM. Therefore, only a portion of the active force p_c is transmitted to the ECM, since part of it is absorbed by the microtubules. Note also that in Fig. 1A it can be seen that the plaque proteins are subjected to forces from both microtubules (p_m) and actin bundles (p_c) and, therefore, p_{cell} can also be interpreted as a measure of the average cell force that the submembrane plaque is bearing. This is, according to the integrin-mediated mechanosensing hypothesis, the stimulus that triggers the cellular chemical response to external mechanical input signals [10]. Moreover, deformations of the springs of the model in Fig. 1B also possess a direct connection with physical quantities that can be observed in Fig. 1A. θ stands for the local volumetric strains of the substrate and of the whole cell, which, due to the assumption of rigid attachment between cell and substrate, are identical. θ_c represents the change in length of the active contractile element with respect to the resting length to which it will return when unloaded. This deformation represents the actual physical change of the overlap between myosin and actin filaments that occurs when active forces are exerted. Finally, θ_a denotes the deformation of the actin bundles due to the active forces that they transmit.

If all the springs are considered as linear elastic, which is a reasonable initial and simple assumption under moderate cell and substrate strains, the only element behavior that remains to be established is that of the actomyosin contractile system. The active force generated by the cell results from a relative sliding movement between actin and myosin filaments induced by myosin cross-bridges on hydrolysis of ATP [28]. This force has been found to be maximal for an optimal filament overlap, decreasing proportionally when the overlap is reduced [29]. Accordingly, the simple piecewise linear constitutive law shown in Fig. 1C relating contractile stress p_c and deformation of the contractile element θ_c is here proposed.

Applying equilibrium conditions to the mechanical system in Fig. 1B, the following expression for the active pressure p_{cell} transmitted to the ECM by a single cell as a function of the ECM volumetric strain θ is obtained:

$$p_{\text{cell}} = \begin{cases} K_{\text{pas}}\theta & \theta < \theta_1 \\ \frac{K_{\text{act}}p_{\max}}{K_{\text{act}}\theta_1 - p_{\max}}(\theta_1 - \theta) + K_{\text{pas}}\theta & \theta_1 \leqslant \theta \leqslant \theta^* \\ \frac{K_{\text{act}}p_{\max}}{K_{\text{act}}\theta_2 - p_{\max}}(\theta_2 - \theta) + K_{\text{pas}}\theta & \theta^* < \theta \leqslant \theta_2 \\ K_{\text{pas}}\theta & \theta > \theta_2 \end{cases} \quad (1)$$

where $\theta^* = p_{\max}/K_{\text{act}}$.

3. Cell migration model

In a similar way to some mechanobiological models, we consider two main species in our model – cells and ECM – whose respective concentration and density are denoted by n and ρ. As stated in Section 1, we adopt a continuum approach and, consequently, are interested in the spatio-temporal evolution of the volumetric concentration of each specie. Our model is based upon the fundamental conservation law for the concentration of each specie $Q = Q(\mathbf{x}, t)$ at time t and spatial position $\mathbf{x}$:

$$\frac{\partial Q}{\partial t} = -\nabla \cdot \mathbf{J}_Q + f_Q \quad (2)$$

where $\mathbf{J}_Q$ is the flux (rate of outgoing matter per unit area) of specie Q (ECM density ρ or cell concentration n) and f_Q the rate of net production of Q.

In the case of the ECM, the only flux term that appears comes from passive convection, i.e. there is a flow of ECM simply due to its deformation caused by cellular and external loading, so

$$\mathbf{J}_\rho = \rho \frac{\partial \mathbf{u}}{\partial t} \quad (3)$$

where $\mathbf{u}$ denotes the displacement vector at each point of the ECM.

Furthermore, assuming for simplicity that secretion of ECM by cells is not relevant and that ECM degradation is negligible, it is straightforwardly inferred that $f_\rho = 0$.

Cells, however, in addition to passive convection – since they are attached to the ECM, they flow passively as a consequence of its deformation – also exhibit a relative movement with respect to the ECM. In many models, an expression for the flux accounting for this migration is directly postulated [30,31]. In contrast, another route consists in considering all the governing equations of continuum mixture theory (continuity, momentum principles, energy balance and entropy inequality) and finding a constitutive relation for the cellular flux so that the Clausius–Duhem inequality is satisfied [32]. Details of this procedure can be found in a previous work of some of the authors [33]. The final expression that is obtained for the cellular flux, assuming that effects such as haptotaxis or chemotaxis are not significant, reads:

$$\mathbf{J}_n = -D\nabla n + n\frac{\partial \mathbf{u}}{\partial t} + M\frac{\nabla \cdot \boldsymbol{\sigma}_{\text{cell}}}{n} \quad (4)$$

where D is an isotropic diffusion coefficient and constitutes a measure of the degree of motility of a particular type of cell, M is a parameter that for simplicity has been here assumed as a constant scalar and quantifies the influence of durotaxis and tensotaxis on the direction and magnitude of the cellular movement, and $\boldsymbol{\sigma}_{\text{cell}}$ denotes the stress of the cell population. A reasonable assumption consists in considering $\boldsymbol{\sigma}_{\text{cell}}$ as proportional to the net stress of a single cell p_{cell} and to the local cell density n. This holds true under low cell concentrations, but tends to saturate at high cell densities due to contact inhibition and competition for ECM binding sites [30]. That is why, similarly to other authors [31], we propose the following expression for $\boldsymbol{\sigma}_{\text{cell}}$:

$$\boldsymbol{\sigma}_{\text{cell}} = \frac{p_{\text{cell}}}{1 + \lambda n}n\mathbf{1} \quad (5)$$

where λ is a parameter that characterizes the cellular stress saturation and $\mathbf{1}$ denotes the second order identity tensor.

Finally, f_n is any suitable function that accurately defines the kinetics of cell population (mitosis, death, differentiation), eventually depending on the nature of the problem in question (see [34] for a review of population models). A logistic growth law is one of the most general options:

$$f_n = rn\left(1 - \frac{n}{N}\right) \quad (6)$$

where N is the maximum cell carrying capacity of the substrate and r denotes the rate of cell proliferation.

Importantly, it has been shown that the magnitude of cellular traction directly affects the rate of cell proliferation: cells appear to proliferate markedly faster on substrates where they exert higher tractional forces [8]. According to this experimental finding we propose the following expression for r:

$$r = r_{\max}\frac{p_{\text{cell}}}{p_{\text{cell}} + \tau} \quad (7)$$

where $r_{\max}$ stands for the maximum rate of cell proliferation and τ is a parameter that characterizes the dependence of r upon p_{cell}.

The ECM displacement $\mathbf{u}$ is also an unknown that must be quantified in order to determine the contribution of passive convection to ECM and cellular fluxes. For this purpose, the balance of linear momentum of the ECM must be used. This reads as follows:

$$\nabla \cdot (\boldsymbol{\sigma}_{\text{cell}} + \boldsymbol{\sigma}_{\text{ecm}}) + \rho\mathbf{f}_{\text{ext}} = 0 \quad (8)$$

It essentially states that cell stresses $\boldsymbol{\sigma}_{\text{cell}}$ and passive resisting ECM stresses $\boldsymbol{\sigma}_{\text{ecm}}$ must be in equilibrium with the external forces $\mathbf{f}_{\text{ext}}$. Obviously, $\boldsymbol{\sigma}_{\text{ecm}}$ depends on the ECM displacement vector $\mathbf{u}$. If a general linear viscoelastic behavior is assumed for the ECM, what is usually accurate under moderate ECM strains, the following explicit formula is found:

$$\boldsymbol{\sigma}_{\text{ecm}} = \frac{E}{1+v}\boldsymbol{\varepsilon} + \frac{v}{1-2v}\theta\mathbf{I} + \mu_1\frac{\partial \boldsymbol{\varepsilon}}{\partial t} + \mu_2\frac{\partial \theta}{\partial t}\mathbf{I} \quad (9)$$

$\boldsymbol{\varepsilon}$ being the ECM strain tensor ($\boldsymbol{\varepsilon} = \frac{1}{2}(\nabla\mathbf{u} + \nabla\mathbf{u}^\top)$ under the small strain assumption, E and v the Young modulus and Poisson ratio of the ECM and μ_1 and μ_2 its shear and bulk viscosity, respectively.

4. Results

4.1. Mechanosensing: absence of external load

Results are obtained by applying equilibrium conditions to the mechanosensing model of the cell of Fig. 1B. We first consider a cell interacting with an elastic medium with no external force applied either on the cell or on the substrate. In that case, the equilibrium conditions give

$$p_{\mathrm{cell}} = p_{\max}\frac{\alpha}{(\alpha+\beta)(\gamma_1+1)+\gamma_1} \tag{10}$$

where $\alpha = K_{\mathrm{subs}}/K_{\mathrm{act}}$, $\beta = K_{\mathrm{pas}}/K_{\mathrm{act}}$ and $\gamma_1 = -p_{\max}/(K_{\mathrm{act}}\theta_1)$ are nondimensionalized parameters of the model. Under these conditions our model predicts that the cellular stress p_{cell} increases with increasing substrate stiffness (Fig. 2A). This behavior is indeed observed experimentally for fibroblasts on polyacrylamide sheets [13] and for epithelial cells on artificial silicone elastomer micropillar substrates [12]. p_{cell} tends logically to saturate at high stiffnesses, reaching values close to $p_{\max}/(1+\gamma_1)$. Interestingly, as the passive stiffness of the cell (CSK microtubules) increases, the active contractile stress p_{c} increases but the net cell stress p_{cell} decreases (Fig. 2B). This is due to the fact that CSK microtubules absorb a higher portion of the active stress as their stiffness increases.

Under these assumptions it is also possible to compute the energy consumed by the contractile machinery of the cell W_{cell}

$$W_{\mathrm{cell}} = \left(\frac{p_{\max}^2}{2K_{\mathrm{act}}}\right)\frac{(1+\alpha+\beta)(\alpha+\beta)}{[(\alpha+\beta)(\gamma_1+1)+\gamma_1]^2} \tag{11}$$

W_{cell} decreases with increasing stiffness (Fig. 2A), which implies that preferential migration of cells to stiffer regions of their environment is accompanied, according to our model, by a lower investment of resources (e.g. ATP) needed for the build-up of the active force [20].

4.2. Mechanosensing: presence of external load

Another different case is the situation where the strain of the ECM is imposed by means of the application of external loads. When the ECM is subjected to low mechanical strain, both the active stress p_{c} and the passive cell stress p_{m} contribute to the cell stress p_{cell}. However, when the ECM is subjected to high compressive or tensile strains, the active contractile stress p_{c} tends to zero, since the overlapping of actin and myosin filaments is far from optimal. However, the net cell stress $p_{\mathrm{cell}} = p_{\mathrm{c}} + p_{\mathrm{m}}$ increases monotonically with increasing strain θ, due to the contribution of the stress of the passive elements p_{m} (Fig. 2C). Hence, increments of the stiffness as well as prestraining of the substrate have the same increasing effect upon the cell stress p_{cell}. According to the main hypothesis of this mechanosensing model, which states that p_{cell} constitutes the triggering mechanical stimulus for the subsequent chemical response, our model suggests that cells must behave in a similar way on stiff or prestrained substrates. This is precisely what is observed experimentally since cells seem to have the same

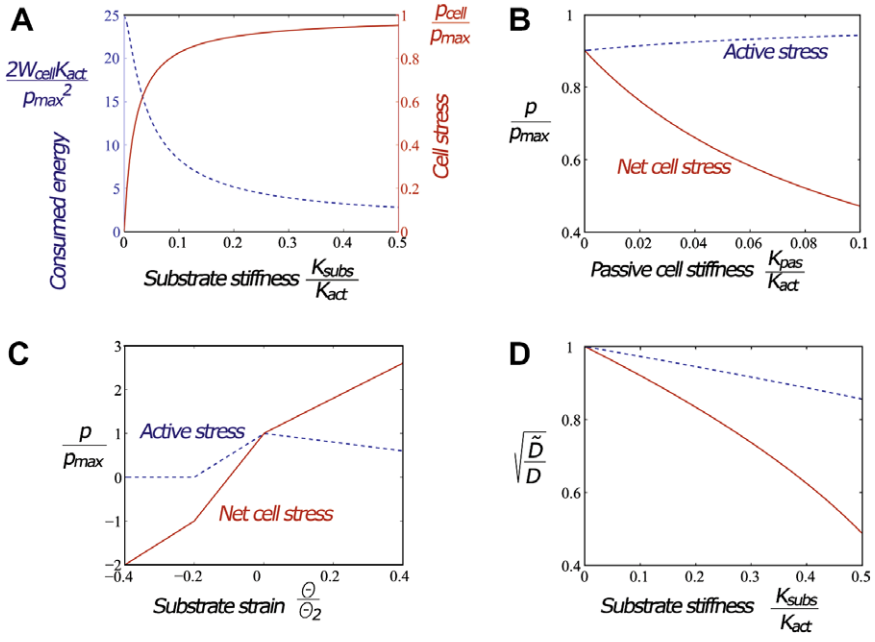

Fig. 2. Predictions of the model. (A) Net cell stress p_{cell} and consumed energy W_{cell} in the absence of an external load, plotted in units of $p_{\mathrm{cell}}/p_{\max}$ and $2W_{\mathrm{cell}}K_{\mathrm{act}}/p_{\max}^2$, respectively, as a function of the normalized stiffness of the substrate, $K_{\mathrm{subs}}/K_{\mathrm{act}}$. (B) Active stress p_{c} and net cell stress p_{cell} in the absence of an external load, plotted in units of $p/p_{\max}$, as a function of the normalized passive cell stiffness, $K_{\mathrm{pas}}/K_{\mathrm{act}}$. (C) Active stress p_{c} and net cell stress p_{cell} in an externally loaded substrate, plotted in units of $p/p_{\max}$, as a function of the normalized substrate strain θ/θ_2. (D) Square root of the normalized effective diffusivity $\tilde{D}/D$, as a function of α, the normalized stiffness of the substrate, $K_{\mathrm{subs}}/K_{\mathrm{act}}$ in the absence of an external load. Solid and dashed lines correspond to normalized cell densities of $0.75\,Mp_{\max}$ and $1.5\,Mp_{\max}$, respectively. The parameter λ was given a value of $(Mp_{\max})^{-1}$. Unless stated otherwise, parameters of the model have been taken as $\alpha = 0.1, \beta = 0.01$ and $\gamma_1 = 0.01$ in all figures.

preference for stiffer environments [4,13] as for prestrained substrates [35,36].

Nevertheless, an important remark should be made concerning the differences between the two possible physical origins of the stimulus p_{cell}: substrate stiffness or ECM/substrate strain. On the one hand, when external loads are not significant, deformation of the substrate is due to active forces exerted by cells and, consequently, p_{cell} is essentially controlled by the stiffness of the substrate K_{subs}. In this case, p_{cell} varies abruptly with K_{subs} on flexible substrates but, once a certain value of K_{subs} is reached, p_{cell} saturates and remains at an almost constant level close to p_{max} (Fig. 2A). This suggests that the cell is able to perceive and respond to the substrate stiffness provided that it is not excessively rigid. On the other hand, when external loads applied on the substrate prevail over cell active forces, p_{cell} is controlled by the strain of the substrate, which now depends on the magnitude of the external load. In this case, the stimulus p_{cell} never saturates, since continuous straining of the cell always causes an increase in p_{cell} (Fig. 2C).

4.3. Migration and proliferation

First, we can extract information from the model by replacing the definition of $\boldsymbol{\sigma}_{cell}$ of Eq. (5) into the third term of the cellular flux of Eq. (4) as follows:

$$J_n = \underbrace{-\left(D - \frac{Mp_{cell}}{n(1+\lambda n)^2}\right)\nabla n}_{\text{Diffusion}} + \underbrace{n\frac{\partial \mathbf{u}}{\partial t}}_{\text{Convection}} + \underbrace{M\frac{\nabla p_{cell}}{1+\lambda n}}_{\text{Duro and Tensotaxis}} \tag{12}$$

From Eq. (12) two features of the model can be drawn. First, according to the third term, cells will tend to migrate towards regions where their net cellular stress p_{cell} is higher, which, according to the results shown in the above paragraph, correspond to stiff or prestrained environments. This is indeed the behavior observed experimentally [4,13,35,36]. Secondly, cells will tend to diffuse to low cell density regions with an effective diffusion coefficient $\tilde{D}$ defined as

$$\tilde{D}(n, p_{cell}) = D - \frac{Mp_{cell}}{n(1+\lambda n)^2} \tag{13}$$

Remarkably, this last formula states that the effective diffusivity of the cell population decreases as the net cell stress increases, e.g. when the substrate stiffness increases. The diffusivity, quantified by means of $\tilde{D}$, is proportional to the square of the mean cell crawling speed. Hence, our model predicts lower cell motility with increasing stiffness of the substrate (Fig. 2D), which has been found for fibroblasts on collagen-coated polyacrylamide substrates [13] and on hyaluronan and fibronectin hydrogels [8]. Moreover, the stiffness-dependent reduction of $\tilde{D}$ is less pronounced with high cell densities. This is logical, since it is reasonable to think that intercellular contact inhibition rather than ECM rigidity directs cell crawling at high cell concentrations. This finding suggests that the two above-mentioned distinctive features of cell behavior, durotaxis and tensotaxis on the one hand and the stiffness-dependent cell locomotion speed on the other hand, can be regulated by the same intracellular mechanism.

4.4. Cell migration and proliferation on a planar substrate

The full model proposed, consisting of the conservation equations of the cellular concentration n and ECM density ρ together with the balance of linear momentum of the ECM, was solved by means of the Finite Element Method using an iterative Newton–Raphson algorithm with an implicit scheme. The simulations were performed using commercial software ABAQUS 6.6. In particular, a 2D square domain was considered, representing the common planar substrates usually used in experiments. Model parameters were obtained from the literature for fibroblasts, one of the most common cellular types used in experimental works of cell migration [37,8]. Only M was adjusted so that dependence of the rate of cell locomotion upon the flexibility of the substrate found experimentally in Ref. [5] was reproduced. A gradient of stiffness along the substrate was considered in the simulation in order to study the ability of the model to reproduce durotaxis. As can be seen in Fig. 3A, cell concentration after 2 days is markedly higher on the stiffer region of the substrate, exactly as was observed experimentally [4]. It is important to note that this is not only due to the biased direction of cell migration towards the stiffer region, but particularly to the increasingly faster rate of cell proliferation as stiffness increases. In fact, the simple stiffness-directed movement of cells is not sufficient to explain the sharp variation in the accumulation of cells depending on the position on the substrate, at least after only 2 days of simulation. It is necessary to simulate a 10–20 times longer period of time in order to obtain such a distribution if proliferation effects are neglected. This could be expected bearing in mind typical values of the diffusion coefficient, which, for fibroblasts, is in the order of 10^{-10} cm^2 s^{-1}, which is equivalent to a cell crawling speed lower than 1 μm min^{-1}. This speed is not sufficient to lead to differences in cell number as pronounced, as found experimentally within 1 day of culture [4] in gradient-compliant substrates whose size is in the order of centimeters. This is why it would be interesting to modify the experimental procedure in such a way that the effects of substrate compliance on cell proliferation and migration could be monitored independently.

Next, a substrate with a gradient of prestrain induced by external loading was also simulated, with the objective of looking into tensotaxis. The results shown in Fig. 3B indicate that after two days of simulation cell concentration is also higher on the more strained region, again in qualitative agreement with experimental findings [35,36]. Similar considerations concerning the partial contributions of proliferation and locomotion also apply to this case.

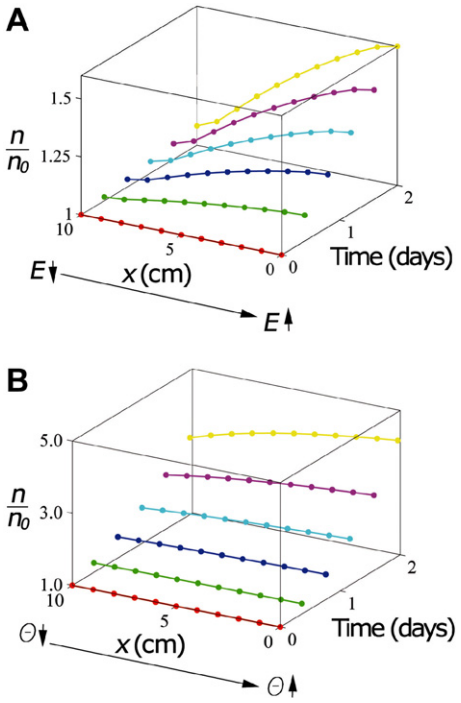

Fig. 3. Result of the computational simulation of the temporal evolution of the spatial distribution of cell concentration on a 2D square substrate. x denotes the position along the symmetry line of the substrate. Cell concentration n has been scaled with respect to the initial cell density n_0, which was taken as 10^3 cells cm^{-2}. (A) Substrate with a gradient of the elastic modulus E. (B) Substrate with a gradient of prestrain θ.

5. Discussion

Here we have presented a mechanosensing model able to explain numerous important experimental observations reported for cell active interaction with deformable substrates. The model is based on two main assumptions: (i) passive behavior of the cell can be studied applying equilibrium conditions to a simple mechanical model where only the mechanically relevant components of the cell are considered; and (ii) active forces are generated by the actomyosin contractile machinery, whose mechanical behavior can be assimilated to that of the skeletal muscle due to the fact that they share the same molecular basis. Moreover, the variables of the model present a clear physical meaning. Remarkably, the net cellular stress p_{cell} can be interpreted as a measure of the mean stress of the protein submembrane plaque. p_{cell} increases for cells placed on stiff or prestrained substrates and thus constitutes for the cell a source of information about the mechanics of the environment, irrespective of the physical origin of the signal, which may be stiffness or strain of the substrate. These results are in agreement with experimental observations and support the idea of the protein plaque mechanical state as one of the possible mechanosensor mechanisms, strongly suggested by experimental studies [10] and also proposed in other modeling works [21].

In our model, cell orientation, and thus active force anisotropy, has not been included, what can be accurate for chondrocytes but not for other cell types, such as fibroblasts, vascular smooth muscle cells or mesenchymal stem cells, which can offer a bipolar morphology, especially on tissue equivalents subjected to anisotropic strain or on micropatterned substrates [38]. However, the prediction of cell alignment is not one of the objectives of this work, nor does it affect the general predictions of the model and conclusions that can be drawn from the results. Cell orientation due to the mechanical environment has been considered in other modeling works [20] and could be taken into account within the original scheme by considering second-order elastic, strain and stress tensors for the spring elements instead of volumetric moduli, volumetric strain and hydrostatic stress, respectively.

Another limitation of the mechanosensing model is that the existence of cell–cell junctions has not been considered. There exist experimental works showing that cells that make cell–cell junctions on a soft substrate can present a morphology and distribution of actin fibers similar to the one observed for isolated cells on stiffer substrates [39]. It could be hypothesized that cells making cell–cell contact feel a higher stiffness of the surrounding medium, since when they exert active forces they deform not only the substrate they are attached to but also the surrounding cells. The effect of cell–cell interactions was considered from a theoretical point of view in the work developed by Ramtani [40].

As a final comment to the mechanosensing model, it should be remarked that, due to its simplicity, it does not constitute an accurate description of the mechanical behavior of the cell. The mechanical response of living cells when subjected to transient and dynamic loads is complex, usually nonlinear and highly dependent on the type of cell, and its simulation requires complex mechanical models (see Ref. [41] for a review). Consequently, the scheme of Fig. 1B should be seen just as a rough approximation to the real mechanical behavior, but from where conclusions regarding cell active forces as well as the mechanosensing mechanism can be drawn.

A cell migration model that considers cell–ECM mechanical interaction and ECM mechanical behavior has also been discussed. Similar models have been proposed since the pioneer work of Murray and collaborators [31]. However, the originality of our work resides in the choice of the approach used for obtaining an expression for the cellular flux. The application of all the typical thermodynamic governing equations in the context of continuum mixture theory to the two species, ECM and cells, requires the dependence of the cellular flux on p_{cell} in order to satisfy the Clausius–Duhem inequality. Importantly, this dependence of cell migration on the net cellular stress is completely in agreement with the hypothesis of considering this stress as a key for cell mechanosensing: according to our model, the net cell stress (or the protein plaque stress, since in our model they are equivalent) appears to be the main mechanical input signal that cells receive from their environment and determines, partially, their behavior, in particular the direction and speed of locomotion. The

transformation of the nature of this mechanical stimulus to a biochemical signal still remains unclear, although alterations of the conformation or organization of plaque proteins have been hypothesized as a possible mechanism [10].

A prediction of the model is that cells tend to move preferentially towards regions where cellular stresses are higher. According to the mechanosensing model, p_{cell} is higher on stiff or prestrained regions and, thus, it is there where cells will tend to move. This result agrees perfectly with observations of durotaxis and tensotaxis, although the compliance-dependent rate of proliferation must be taken additionally into account in the model in order to obtain similar cell accumulations within the short culture periods used in experiments. It is important to note that it is in these regions where the energy consumed by the cell W_{cell} is minimum and, therefore, durotaxis and tensotaxis could be considered the result of a tendency of cells to optimize their resources. However, we are not suggesting that cells actually move according to the principle of minimizing the invested work W_{cell}. This would imply that W_{cell} would constitute the input signal and cells should have at their disposal a suitable mechanism of converting this signal into a biochemical cascade of cellular events that would determine cell behavior. On the contrary, we consider it much more plausible to think of p_{cell} as the mechanical input available to a mechanosensing cell whereas the reduction in the consumption of energy is just an output of the migration process, not its triggering factor.

In summary, we have presented one model that considers some important characteristic aspects of cell behavior: mechanosensing, migration and proliferation. This simple model is able to predict the behavior of adherent cells on elastic substrates under different loading conditions, in agreement with a wide range of experimental findings. In the future, the model should be improved, extending it in order to take into account cell–cell interaction, cell orientation and anisotropy of active forces, which can be important in certain soft media. This model can be easily implemented computationally and, consequently, has potential use for many interesting practical applications in biology and medicine, in situations where cell–ECM interaction as well as the ECM mechanics play an important role, such as the study of wound healing, vasculogenesis or the design of tissue engineering applications. The application of particularized versions of this model is also of interest with regard to the study of certain pathological states where a change in the mechanical properties of a tissue leads to an alteration of the normal behavior of its cells [42].

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References

[1] Chicurel M. Cell migration research is on the move. Science 2002;295:606–9.
[2] Lauffenburger DA, Horwitz AF. Cell migration: a physically integrated molecular process. Cell 1996;84:359–69.
[3] Harley BA, Leung JH, Silva ECCM, Gibson LJ. Mechanical characterization of collagen–glycosaminoglycan scaffolds. Acta Biomater 2007;3:463–74.
[4] Wong JY, Velasco A, Rajagopalan P, Pham Q. Directed movement of vascular smooth muscle cells on gradient-compliant hydrogels. Langmuir 2003;19:1908–13.
[5] Pelham RJ, Wang Y. Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc Natl Acad Sci USA 1997;94:13661–5.
[6] Engler A, Bacakova L, Newman C, Hategan A, Griffin M, Discher D. Substrate compliance versus ligand density in cell on gel responses. Biophys J 2004;86:617–28.
[7] Schwarz US, Bischofs IB. Physical determinants of cell organization in soft media. Med Eng Phys 2005;27:763–72.
[8] Ghosh K, Pan Z, Guan E, Ge S, Liu Y, Nakamura T, et al. Cell adaptation to a physiologically relevant ecm mimic with different viscoelastic properties. Biomaterials 2007;28:671–9.
[9] Discher DE, Janmey P, Wang Y. Tissue cells feel and respond to the stiffness of their substrate. Science 2005;310:1139–43.
[10] Bershadsky AD, Balaban NQ, Geiger B. Adhesion-dependent cell mechanosensitivity. Annu Rev Cell Dev Biol 2003;19:677–95.
[11] Zamir E, Geiger B. Molecular complexity and dynamics of cell–matrix adhesions. J Cell Sci 2001;114:3583–90.
[12] Saez A, Buguin A, Silberzan P, Ladoux B. Is the mechanical activity of epithelial cells controlled by deformations or forces? Biophys J 2005;89:L52–4.
[13] Lo C, Wang H, Dembo M, Wang Y. Cell movement is guided by the rigidity of the substrate. Biophys J 2000;79:144–52.
[14] Cukierman E, Pankov R, Stevens DR, Yamada KM. Taking cell–matrix adhesions to the third dimension. Science 2001;294: 1708–12.
[15] Kaverina I, Krylyshkina O, Benigno K, Anderson K, Wang Y, Small JV. Tensile stress simulates microtubule outgrowth in living cells. J Cell Sci 2002;115:2283–91.
[16] Riveline D, Zamir E, Balaban NQ, Schwarz US, Ishizaki T, Narumiya S, et al. Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and rock-independent mechanism. J Cell Biol 2001;153:1175–86.
[17] Kitano H. Systems biology: a brief overview. Science 2002;295:1662–4.
[18] Maini PK, Baker RE, Chuong CM. The Turing model comes of molecular age. Science 2006;314:1397–8.
[19] Suresh S. Biomechanics and biophysics of cancer cells. Acta Biomater 2007;3:413–38.
[20] Bischofs IB, Schwarz US. Cell organization in soft media due to active mechanosensing. Proc Natl Acad Sci USA 2003;100:9274–9.
[21] Nicolas A, Geiger B, Safran SA. Cell mechanosensitivity controls the anisotropy of focal adhesions. Proc Natl Acad Sci USA 2004;101:12520–5.
[22] Schwarz US, Erdmann T, Bischofs IB. Focal adhesions as mechanosensors: the two-spring model. Biosystems 2006;83:225–32.
[23] DiMilla PA, Barbee K, Lauffenburger DA. Mathematical model for the effects of adhesion and mechanics on cell migration speed. Biophys J 1991;60:15–37.
[24] Zaman MH, Kamm RD, Matsudaira P, Lauffenburger DA. Computational model for cell migration in three-dimensional matrices. Biophys J 2005;89:1389–97.
[25] Hill AV. The heat of shortening and the dynamic constants of muscle. Proc Royal Soc London B 1938;126:136–95.
[26] Ingber DE. Tensegrity. I. Cell structure and hierarchical systems biology. J Cell Sci 2003;116:1157–73.

[27] Timoshenko S, Goodier JN. Theory of elasticity. 3rd ed. New York: Mc Graw-Hill Publishing Co; 1970.
[28] Spudich JA. The myosin swinging cross-bridge model. Nat Rev Mol Cell Biol 2001;2:387–92.
[29] Rassier DE, MacIntosh BR, Herzog W. Length dependence of active force production in skeletal muscle. J Appl Physiol 1999;86:1445–57.
[30] Namy P, Ohayon J, Tracqui P. Critical conditions for pattern formation and in vitro tubulogenesis driven by cellular traction fields. J Theor Biol 2004;202:103–20.
[31] Oster GF, Murray JD, Harris AK. Mechanical aspects of mesenchymal morphogenesis. J Embryol Exp Morph 1983;78:83–125.
[32] Marsden JE, Hughes TJR. Mathematical foundations of elasticity. 1st ed. New York: Dover Publications, Inc; 1994.
[33] Doblaré M, García-Aznar JM. On the numerical modelling of growth, differentiation and damage in structural living tissues. Arch Comput Meth Engng 2005;11:1–45.
[34] Murray JD. Mathematical biology. 3rd ed. New York: Springer; 2005.
[35] Beloussov LV, Louchinskaia NN, Stein AA. Tension-dependent collective cell movements in the early gastrula ectoderm of xenopus laevis embryos. Dev Genes Evol 2000;210:92–104.
[36] Mandeville JTH, Lawson MA, Maxfeld FR. Dynamic imaging of neutrophil migration in three dimensions: mechanical interactions between cells and matrix. J Leukoc Biol 1997;61:188–200.
[37] Shreiber DI, Barocas VH, Tranquillo RT. Temporal variations in cell migration and traction during fibroblast-mediated gel compaction. Biophys J 2003;84:4102–14.
[38] Kurpinski K, Chu J, Hashi C, Li S. Anisotropic mechanosensing by mesenchymal stem cells. Proc Natl Acad Sci USA 2006;103:16095–100.
[39] Yeung T, Georges PC, Flanagan LA, Marg B, Ortiz M, Funaki M, et al. Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. Cell Motil Cytoskelet 2005;60:24–34.
[40] Ramtani S. Mechanical modelling of cell/ecm and cell/cell interactions during the contraction of a fibroblast-populated collagen microsphere: theory and model simulation. J Biomech 2004;37:1709–18.
[41] Lim CT, Zhou EH, Quek ST. Mechanical models for living cells – a review. J Biomech 2006;39:195–216.
[42] Georges PC, Janmey PA. Cell type-specific response to growth on soft materials. J Appl Physiol 2005;98:1547–53.