A mechanical model for guided motion of mammalian cells

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Abstract – We introduce a generic, purely mechanical model for environment-sensitive motion of mammalian cells that is applicable to chemotaxis, haptotaxis, and durotaxis as modes of motility. It is able to theoretically explain all relevant experimental observations, in particular, the high efficiency of motion, the behavior on inhomogeneous substrates, and the fixation of the lagging pole during motion. Furthermore, our model predicts that efficiency of motion in following a gradient depends on cell geometry (with more elongated cells being more efficient).

Motility and directed cell motion play an important role in many biological processes ranging from embryonic development [1–3] to tissue invasion by pathogenic microorganisms [1,2,4,5] and cancer progression [5–8].

Often extracellular cues are used to regulate the decision in which direction the cell will move [9–11]. Depending on these cues one distinguishes between: 1) chemotaxis where directed motion is guided by solvent chemical cues [9]; 2) haptotaxis where substrate-bound cues influence the cell-substrate adhesiveness [12]; and 3) durotaxis where mechanical cues such as substrate rigidity influence the directed motion [6].

Physics-based experimental and theoretical approaches to study these phenomena have attracted considerable interest in the last decade. Many studies have been devoted to bacterial chemotaxis [9,10,13–17] and to Dictostelium discoideum as model system for amoeboid migration [11,18–22]. The main challenge is to analyze and theoretically model the interplay between molecular processes and the emerging macroscopic motion. For amoeboid motion this is further complicated as shape changes have to be taken into account [20–22].

In contrast, the mesenchymal migration of mammalian cells has so far not been studied theoretically. Although many details have been characterized experimentally [7,23,24] theoretical studies have focused only on the cell shape during migration [25], on continuum descriptions for cell populations [7,24] or on special short-term aspects of migration like migration speed [26] or effective adhesiveness [23].

In this paper we study the motility of mammalian epithelial-like cells. They are influenced by a variety of chemical and physical signals, in particular by different mechanical forces [27] and can show a very high efficiency in following all kinds of gradients [28]. Mammalian cells predominantly migrate by a crawling motion [29]. It consists of a cycle of five discrete steps carried out within about ten minutes [1,3,5,23,26,29–33]: 1) polarization of the cell yielding a defined leading and a defined lagging pole; 2) formation of protrusions (predominantly) at the leading pole and attachment of the lagging pole, see fig. 1(a); 3) stabilization of these protrusions by adhesion to the substrate or the extracellular matrix (ECM); 4) translocation of the cell body by myosin-mediated contraction; 5) retraction of the rear by loosening the adhesions at the lagging pole. This crawling is propelled by the active lamellipodium at the leading edge which pulls the passive cell body forward [34]. Cell motility depends on the stiffness of the substrate and the ECM. These effects are mediated indirectly via their impact on cell shape [35] and directly through so far unresolved mechanisms referred to as durotaxis [2,6,36–38].

The mechanical interaction with the substrate or the ECM can be thought of as a bilinear sequential binding [32], which affects the cell predominantly on the nanoscale through mechanosensing mechanisms [27,36]. However, cells can also chemically manipulate the ECM, e.g., in case of cancer, where tumor cells stiffen the surrounding ECM [27,37], and build a rigid stroma around the tumor. This step in ECM stiffness then promotes cells from the outside moving inside, but prevents cells from the inside to migrate outside [39]. In general, mammalian cells are not passive recipients of mechanical forces, but actively respond by pulling or pushing the ECM [6,27,31].
We introduce here a simple, generic model for environment-sensitive motion of fibroblast-like cells. It is solely based on mechanics and is applicable to chemotaxis, haptotaxis, and durotaxis as modes of motility. It provides the first theoretical explanation of the high efficiencies of mesenchymal-like motion independent of cellular morphology. Our model also covers the motility dynamics on large time scales. In particular, we can capture the statistical properties at environmental discontinuities, e.g., a step in substrate stiffness or a step in concentration of a chemoattractant. These results indicate that regulation of taxis might be based on mechanical forces.

In our model we represent the polarized cell body by an ellipse with major radius $R_I$ and minor radius $R_S$. The orientation with respect to the $x$-axis is measured by the angle $\theta$ that parameterizes rotation around the center $C$. To counterbalance the forces of the protrusions the cell is attached to the surface at an anchor point. Moving cells typically have a shape similar to the one shown in fig. 1(a) with a single elongated tail that appears when the cell body moves forward. This indicates that there is only a single anchor point. The motion occurs in such a way that the cell is effectively rotated around this anchor point, see fig. 1(b). In principle, cells could also fix the position of the tail with several anchor points. Then, the cell is effectively rotated around these fixed points. For simplicity, we restrict here the analysis to a single anchor point. We assume that the anchor point remains at a fixed position while the cell is not moving. Real cells might change their shape during motion that could lead to a change in the position of the anchor point. However, we do not take such effects into account.

In the following we assume that a molecular mechanism initially polarizes the cell along the $x$-axis (for example in the presence of a gradient as discussed below). For our purpose we do not explicitly model this process and assume that it leads to a normally distributed initial angle $\theta(t = 0)$ with mean $\mu_\theta = 0$. The standard deviation $\sigma_\theta$ was estimated by fitting a normal distribution to the polarization model shown in [40].

The protrusions by which the cells pull themselves forward are represented by adhesive arms that grow out of the ellipse at a random angle $\varphi$ with probability distributions given by Gaussian distributions centered around $\varphi = 0$ (for the leading pole) and $\varphi = \pi$ (for the lagging pole)

$$p(\varphi) = \frac{p_+}{\sqrt{2\pi}\sigma_+}e^{-\varphi^2/2\sigma_+^2} + \frac{p_-}{\sqrt{2\pi}\sigma_-}e^{-\left(\varphi-\pi\right)^2/2\sigma_-^2}.$$  

The weights $p_+$ of the leading pole and $p_- = 1 - p_+ < p_+$ of the lagging pole reflect the initial polarization of the cell and shift the arm distribution towards the leading pole.

Length and direction of the arms are random. For simplicity, we draw the angle $\gamma$ between growth direction and surface normal from a Gaussian distribution with mean $\mu_\gamma = 0$ and standard deviation $\sigma_\gamma$. Similarly, the arm length $l$ is distributed normally with mean $\mu_l$ and standard deviation $\sigma_l$.

Arm formation occurs at constant rate. Every arm applies a linear force on the cell that is proportional to the concentration of a chemoattractant or rigidity of the substrate. Thus, if at time $t$ there are $N$ arms that are attached to the cell body at position $x_i = x_i(\phi_i)$ pointing in direction $L_i$ (with $1 \leq i \leq N$) the total force on the cell is given by

$$F(r_{cell}, \theta, t) = \sum_{i=1}^{N} F_i = \sum_{i=1}^{N} k(c) L_i (r_{cell}, \theta, \phi_i),$$  

where $k(c) = k_0 c(r_{arm})$ depends on the chemoattractant concentration or the substrate rigidity $c(r)$. This results in a translocation of the cell. Furthermore, the arms exert a total torque $M(r_{cell}, \theta, t) = \sum_{i=1}^{N} (x_i \times F_i)_z$, that leads to a rotation of the cell that can be interpreted as a gradual repolarization of the cell.

For most of our simulations we used a linear gradient of fixed strength $c_0 = 1/R_I$. This results in a standard deviation of the initial angle of $\sigma_\theta \approx 1$.

To classify the planar cell motion (in the $x$-$y$ plane), we numerically calculated the moments $\langle x \rangle$, $\langle y \rangle$, $\langle x^2 \rangle$, and $\langle y^2 \rangle$, where $\langle \cdots \rangle$ denotes the average over 500 independent runs, see fig. 2. In these simulations we implemented the above-mentioned cycle of independent steps 1)-5). Starting from the polarized shape (step 1)) we grow $N$ new arms of lengths $l_i$ at angles $\phi_i$ in every iteration (steps 2) and 3)). The new position and orientation of the cell is then obtained by solving $F(r_{cell}, \theta, t) = 0$ and $M(r_{cell}, \theta, t) = 0$ independently (step 4)). Then, all arms are removed (step 5)), the time is increased by $\delta t$ and the iteration starts over. In the absence of a gradient the cells perform an isotropic random walk.
For $N = 10$ (for parameter values see [41]) the effective diffusion coefficients $D_x \approx D_y = 43.77 R_l^2 / \delta t$ were measured by fitting linear functions to $\langle x^2 \rangle$ and $\langle y^2 \rangle$.

In this limit the model can also be solved analytically. From the one-dimensional probability densities of $\varphi$, $\gamma$ and $l$ the two-dimensional probability density of the force induced by an arm can be calculated [41]. We have compared these analytical results with those obtained by direct numerical integration of the model and found excellent agreement. It is interesting to note that this force probability density resembles the shapes of migrating lamellipodial domains of keratocytes [25]. If one assumes that these shape deformations reflect the forces then the forces acting on the cell in our simple probabilistic model are remarkably similar to those exerted by the actin cytoskeleton of keratocytes.

Next, we consider substrates with gradients. For a linear gradient $c_0 = 1/R_l$ parallel to the $x$-axis, the cells perform a biased random walk in the gradient direction while the motion in the perpendicular direction is suppressed $\sim 135$-fold ($D_y = 0.32 R_l^2 / \delta t$ in the presence of a gradient compared to $D_y = 43.77 R_l^2 / \delta t$ in the absence of a gradient), see fig. 2. To quantify the efficiency of the motion in following the applied gradient we measured the chemotactic factor

$$CF = \left( \frac{L_{\text{grad}}}{L_{\text{tot}}} \right).$$

Here, $L_{\text{tot}}$ is the total path length and $L_{\text{grad}}$ the length of the projection in the direction of the gradient.

To investigate the robustness of our model to varying gradients $c_0$ and its behavior for small gradients we looked at the dependence of $CF$ on $c_0$, see fig. S4 in [41]. For small gradients we see a strong increase in efficiency with the gradient strength, but the efficiency saturates fast to its maximum value of around $CF = 95\%$.

An increase in number of arms results in a speedup of motion and an increase in efficiency, see fig. 3. However, there is saturation in efficiency and speed for large numbers of arms. If we take into account that 5 to 10 arms with an average length of 7 $\mu$m would roughly cover between 10 and 20\% of the surface of the cell (each arm has about 22 $\mu$m$^2$ surface area while the whole cell body has about 1000 $\mu$m membrane area [42,43]) we reach a good balance between increase of membrane area and gain of efficiency within this range of $N$. This number is also comparable to the number of arms found experimentally [6].

Next, we analyzed the influence of the cell geometry on the efficiency of motion. As fig. 4 shows, $CF$ depends on the geometry of the cell characterized by the ratio $R_l/R_c$. Thus, more elongated cells (with $R_l > R_c$) have a higher $CF$. This is somewhat surprising as these cells have a broader force distribution than less elongated cells. However, as we show in [41] $CF$ depends predominantly on the ability of the cell to align with the prescribed gradient. Thus, for more elongated cells this higher ability compensates for the broader force distribution. The $CF$ as determined by the ability to align with the gradient (characterized by a rotational rate $\alpha$) is given by [41]

$$CF = \exp(x^2) \left[ \text{erf} \left( \pi / (\sqrt{2} \sigma_\theta) - x \right) + \text{erf}(x) \right].$$

Here, $x = 2^{-1/2} \sigma_\theta \alpha / \chi$ and the opening angle of the force distribution $\chi = \arctan (R_l/R_c \tan \sigma_\varphi)$ parameterizes the dependence on cell geometry.

Lo et al. [6] have shown that non-moving cells grow longer protrusions on stiff substrates than they do on soft substrates. This implies a regulative effect of the substrate rigidity. To account for this effect in our model we assign
each arm $i$ the length

$$l_i + \delta l_i(c) = l_i + \frac{\delta l_{\text{max}}}{1 + c}, \quad (5)$$

where the regulated elongation $\delta l_i(c)$ depends on the stiffness (or concentration or adhesiveness) $c = c(r_{\text{arm}})$ at the position of the arm $r_{\text{arm}}$.

If we increase the average arm length, we see an increase in efficiency that saturates for longer arms, see fig. S5 in [41]. This rise in efficiency comes with an increase in speed. If we use the concentration-dependent regulation of arm lengths we see a 1% increase in maximum efficiency compared to the unregulated system, but the same efficiency is reached with an average arm length up to 40% shorter compared to the unregulated system.

The above results are robust with respect to variations in the standard deviations of the distributions for $\varphi$ and $\gamma$. $CF$ remains nearly constant for $\sigma_+\gamma$ and $\sigma_\gamma$ in a range from $\sim 0.01\pi$ to $0.2\pi$. For even broader distributions we see a decrease in efficiency as a result of insufficient polarization of the cell. Experimentally, it has been observed that the protrusions grow almost in the normal direction out of the surface of the cells, and that the protrusions are located around a narrow region at the leading pole [5,44–46]. We find a similar behavior (and the associated high efficiencies in motion) only for distributions with small standard deviations indicating that $\varphi$ and $\gamma$ are tightly regulated.

Furthermore, cell motion shows an interesting dependence on the position of the anchor point of the cell to the substrate quantified by the parameter $\delta$. If this point is shifted towards the leading pole, i.e. closer to the protrusions at the front, the torque exerted by these arms is reduced due to the shorter lever arm and the efficiency drops sharply to zero indicating that the cell is not able to follow the gradient at all (see fig. 5). This indicates that the efficiency in following a gradient is dominated by protrusions close to the leading pole.

Cells often encounter inhomogeneous substrates. As a general scenario, we analyze the movement towards a step in substrate rigidity where crawling cells show an interesting behavior. At these steps (that could represent the transition from a rigid stroma of a tumor to the softer surroundings [27]) cells tend to move from the softer substrate to the stiffer substrate [39]. Cells moving in this direction are only weakly influenced by the step. They keep moving but their trajectory bends towards the direction perpendicular to the step, see fig. 6. We observed that the relation between the angle of the cell before and after the step obeys a refraction law similar to that of light allowing us to characterize the motion by refraction indices, see fig. 6(a). The ratio of refraction indices $n_{\text{soft}}/n_{\text{stiff}}$ decreases with increasing step size $s = c_1/c_0$, where $c_0$ and $c_1$ are the stiffness of the softer and stiffer region, respectively.

On the other hand, a step from a stiff substrate to a softer substrate represents a barrier for cells. The passing probability depends on the step height. From the distribution of the minimal $x$-positions encountered by the cells during 500 iterations, we can calculate the probability of a cell moving across the step, which we define as transmission coefficient $T_C = \int_{-\infty}^{x_{\text{step}}} p(x) \, dx = p(x < x_{\text{step}})$, where $p(x)$ is the probability to find a cell that traveled to position $x$, and $x_{\text{step}}$ is the position of the step, see fig. 6(b). This coefficient decreases as the step size increases showing that the barrier effect becomes much stronger for larger steps, see fig. S7 in [41].

Finally, we wanted to compare our results with other types of cellular motion. However, there are neither theoretical or experimental data available for the efficiency of mammalian cell motion as a function of gradient
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motion of mammalian cells. Motion occurs by polarized growth of protrusions which push and rotate the cell. The description of molecular interactions occurs on a coarse-grained level effectively entering into the probability distribution for arm growth (eq. (1)). It is not the goal of this simplified model to achieve a detailed (molecular) description of cellular motility. Rather, we introduce it to analyze the influence of mechanical forces on the regulation of fibroblast motion.

The agreement of our findings with the experimental observations indicates that mechanical forces indeed play a significant role in this process. More specifically, the observed high efficiency in following a gradient is a robust feature of our model (see figs. 4 and 7). We find for a large parameter range chemotactic factors close to 1 as observed experimentally in [28]. Furthermore, the results on the motion in inhomogeneous environments are in good agreement with the experimental observations at steps in substrate rigidity [39].

Our analysis identifies two geometrical factors that have a significant impact on efficiency of cell motion: the position of the anchor point and the geometry of cell quantified by the ratio of major to minor radius. For both quantities we make specific theoretical predictions (figs. 4 and 5) that are experimentally testable.

Furthermore, the moments $\langle x \rangle$, $\langle x^2 \rangle$, $\langle y \rangle$ and $\langle y^2 \rangle$ can be easily measured for individual cells of different geometry for different gradients. By comparing these data with our theoretical predictions (fig. 7 and figs. S5 and S6 in [41]) information can be obtained about the concentration-dependent regulation of arm lengths. To check our results concerning steps in concentration, adhesiveness and stiffness, one could use the methods presented in [39] to produce flat substrates of different stiffness and measure the polarization axes of the cells before and after the interface as well as the transmission coefficients with time-lapse microscopy.

There are many possible extensions of our model. In future work we will take into account the mechanical effects that the cells have on the substrate. If cells attach protrusions to the substrate and contract, they locally stiffen the substrate. This local stiffening of the substrate might lead to an effective attraction between two cells in proximity, in this way promoting aggregation.

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