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Review

Phenomenological approaches to collective behavior in epithelial cell migration☆

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ABSTRACT

Collective cell migration in epithelial tissues resembles fluid-like behavior in time-lapse recordings. In the last years, hydrodynamic velocity fields in living matter have been studied intensely. The emergent properties were remarkably similar to phenomena known from active soft matter systems. Here, we review migration experiments of large cellular ensembles as well as of mesoscopic cohorts in micro-structured environments. Concepts such as diffusion, velocity correlations, swirl strength and polarization are metrics to quantify the cellular dynamics both in experiments as well as in computational simulations. We discuss challenges relating collective migration to single cell and oligocellular behavior as well as linking the phenotypic parameters to the underlying cytoskeleton dynamics and signaling networks. This article is part of a Special Issue entitled: Mechanobiology.

1. Introduction

During many physiologically relevant processes such as morphogenesis, wound healing and cancer invasion, cells migrate collectively in tightly connected groups. Epithelial cells are a prominent example of cells preferring not to migrate on their own. Instead, they are linked to their neighbors via various junctions, forming sheets, ducts, clusters or strands depending on the particular biological context they reside in [1–4]. Despite this, they still maintain the capability of remodeling their relative positions with time.

In this respect, the malleable epithelial cell sheets resemble two-dimensional complex fluids, which consist of interacting units that are not permanently linked and hence can be mutually displaced. The time scale for flow behavior in epithelial cell sheets is measured in hours or days. However, cellular matter differs from ordinary fluids in two important aspects. Each subunit consumes energy to propel itself and crucially, cells proliferate. In condensed matter theory such out-of-equilibrium systems are referred to as active matter and are known to exhibit unusual hydrodynamic properties and dynamic collective states such as swarming or turbulent swirling [5–10].

Collectively migrating cells indeed display intriguing features of soft active matter systems. In recent years, many efforts have been made to describe cellular motion in mathematical terms and to define rules that determine the apparent cellular flow behavior. This top-down approach is distinct from a cell-biological view on migration that accounts for molecular determinants of migration such as the cytoskeleton dynamics including actin polymerization and force generation via molecular motors, the molecular interaction with the substrate and extracellular matrix via the focal adhesion complex as well as extracellular stimuli via chemical signaling [11–15]. In a mechanistic biophysical view, the entire cell is modeled as an elastic body. Its shape is determined by cell substrate adhesion and the elasticity of the cellular cortex. In migrating epithelial monolayers, the cytoskeleton generates protrusions and exerts traction forces onto the substrate as well as onto adjacent cells via cadherins. Vast progress has been made on the mechanobiology of cells, cellular adhesion and migration in recent years [16–18]. Despite this, the relation of mechanical cell models to the observed motion in tissue at large scales is still challenging and subject of intense research. Collective migration can, to some extent, be analyzed and captured by mathematical equations parameterizing the underlying molecular and mechanical interactions in a coarse grained manner. The phenomenological description is obtained using image-based algorithms and the experimental sets of migration parameters can then be compared to parameter estimations in computational models.

In this review, we focus on the phenomenological analysis of cellular flow behavior, in particular in confining geometries of micro-structured surfaces. We address theoretical concepts capable of reproducing some of the generic properties of cellular matter and discuss the use of standardized micro-environments in cell migration experiments.

Much of this review will concentrate on experiments performed with layers of MDCK cells, as they represent a well-studied model

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system for epithelial sheets. Collective phenomena are also observed in other epithelial systems, such as the *Drosophila* wing disc, however, going into depth about complex biological phenomena like morphogenesis or tumor formation related to migration would go beyond the scope of this work.

2. A mechanical view on cell packing and growth

The outlines of epithelial cells in a sheet adhering to a surface show a striking resemblance to an accumulation of polygons (see Fig. 1). They appear to typically have either five or six corners and form a connected layer without gaps. In a first approximation, the contact lines between cells can be considered straight. In an early study, the average area of a cell in tissue was found to scale approximately linearly with the number of sides (and thus neighbors) it has [19], a relation known as Lewis's law. The pattern formed in experimentally observed cultured cell sheets or epithelial cells in the surface of tissues is captured well by a so called Voronoi construction that, starting from a set of cell center positions, divides the area into polygons corresponding to the regions closest to the cell centers [20] as illustrated in Fig. 1. Nowadays, this cell center is usually approximated from the position of the nucleus.

Voronoi constructions can also serve as a starting point for a theoretical approach to describing the packing geometry in epithelial monolayers. In the so-called vertex model the theory of foams, which assumes that the forces at each vertex have to vanish in the case of stable network configurations, is adapted. The theory introduces an energy function that needs to be minimized locally [21,22]. The energy function consists of three components describing the cells' elastic properties:

$$E(R_i) = \sum_{\alpha} \frac{K_{\alpha}}{2}\left(A_{\alpha} - A_{\alpha}^{0}\right)^2 + \sum_{\langle i,j\rangle} \Lambda_{i,j} l_{i,j} + \sum_{\alpha} \frac{\Gamma_{\alpha}}{2} L_{\alpha}^2. \quad (1)$$

The contributions are an area elasticity (K_α is the elastic constant, with A_α the area and A_α^0 the preferred area of cell α, respectively), the line tension at junctions between individual cells (Λ_{ij} is the line tension per unit length with l_{ij} the length of the junction between nodes i and j) and a term describing cell cortex contractility (L_α is the cell perimeter and Γ_α is a contractility-describing parameter).

The vertex model satisfactorily reproduces polygon class distribution, cell area variation and packing geometry found in the *Drosophila* wing disc, as well as its response to laser ablation [21]. In addition, it is capable of explaining how cell compartment boundaries can be maintained despite remodeling through cell division through increased tension along the boundaries [23].

While this mechanical cell model accounts for observed packing states, it still remains quasi-static, describing only the equilibrium states a system relaxes to after dynamic remodeling events such as cell divisions or disruption of cell-cell contacts. In order to study the perpetual remodeling of tissue as a function of time, Ranft et al. proposed a continuum description of tissue dynamics, showing that by inclusion of proliferation and apoptosis, cell sheets in essence behave like viscoelastic fluids [24]. One key point of this model is the existence of a homeostatic tissue pressure at which cell division and cell death are balanced. This state is reached autonomously if the growing tissue is confined to a fixed volume. If a pressure slightly larger or smaller than the homeostatic pressure is applied, the tissue will completely invade the surrounding area or vanish, respectively [24]. Experimentally, these fluid-like, out-of-equilibrium states are observed in wound healing assays [25–27], for migration on stripes or in channels [28,29], or for expanding patches and colonies of cells [30,31]. When cell density in such a colony is high enough to pose a mechanical constraint that causes following cell divisions to reduce the cell area, this initially leads to a drop in cell motility. Eventually, this “contact inhibition” leads to a static regime where a sharp transition in the rate of mitosis appears and cell rearrangement is completely limited to cell division [31].

In contrast to the compaction during growth in limited space, the average area of cells can also increase over time in the case of freely expanding cell groups. In previous work, we released small cohorts of MDCK cells by removing confining structures [30] created via a stencil-based technique similar to the one introduced by Poujade et al. [32]. Initially, cells are grown to high densities within the confinement.

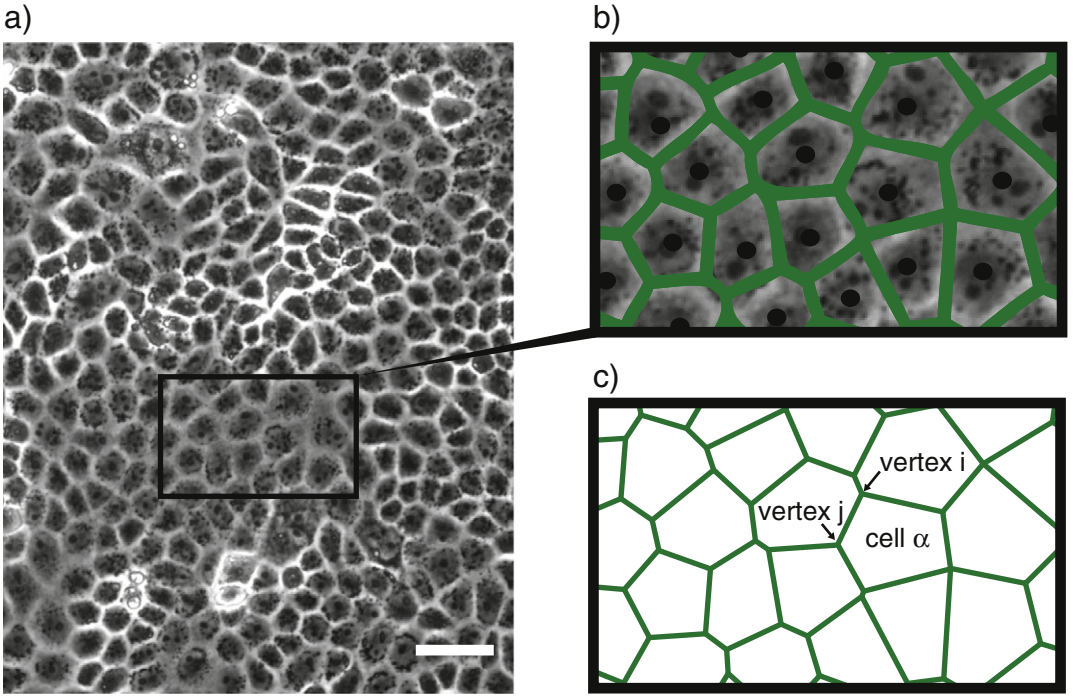

Fig. 1. Polygon-like cell shapes in an epithelial cell layer. a) Cells in an MDCK monolayer have nearly straight borders and close to 5 or 6 neighbors (scale bar corresponds to 50 μm). b) Subsection of the cell sheet with a polygonal meshwork resulting from a Voronoi construction overlain in green. Black dots mark the center points of the cells. c) Abstract vertex model of an epithelial cell layer.

Once the barrier is removed, the microculture of cells spontaneously expands (see Fig. 2), with its area $A(t)$ evolving according to

$$A(t) = a_0 + \Delta a \cdot \left(1 - e^{-t/\tau_r}\right) \cdot N_0 \cdot e^{\lambda t}. \quad (2)$$

In addition to the N_0 initial cells proliferating with a growth rate λ, the cell area relaxes exponentially from the compressed area a_0 at the time of lift-off by the difference Δa to the preferred area over a time τ_r [30]. Remarkably, the time scale of area relaxation was found to be on the order of the cell doubling time [30], in agreement with the result of Ranft et al. stating that dynamic stress relaxation and tissue remodeling should happen on the time scale of division and apoptosis [24].

3. Experimental recording of cellular flow fields

It is an intriguing notion to develop a hydrodynamic description of expanding cell cultures and to analyze the local displacement patterns in terms of flow fields. In recent years, flow vector fields were measured in migrating cell sheets using particle image velocimetry (PIV) [28,32–37]. PIV was originally developed to assess flow fields in fluids and gases. In the classical setup, tracer particles are seeded in the fluid. Displacement fields are then calculated from auto-correlation of lattices of small interrogation windows in successive recorded frames [38].

In migrating cell layers, in contrast to classical PIV, no tracer particles are necessary due to inherent structure of the cell sheets imaged in phase contrast microscopy [33]. Using PIV, the cellular flow of cells confined to stripes or channels has recently been studied [28,29]. Fig. 3 shows the velocity maps of cells invading channel-shaped microstructures. The vector fields show a net flow towards the cell free area, but at the same time contain considerable spatio-temporal noise. Nonetheless, a well-defined plug-flow type profile emerges over most of the channel width when the instantaneous flow fields are averaged in time and space. The average flow velocity increases from back to front of the channel, but does not depend on channel width for widths down to about 30 μm. On the other hand, noise patterns exhibits an interesting dependence on the geometric constraints. As noted by Vedula et al. the occurrence of transient swirls increases with channel width and in narrow channels with width below around 100 μm no swirls but a contraction–elongation type of motion are observed [28]. We will come back to the analysis of the spatio-temporal noise in cell sheets in the next section. The vector fields indeed open up several avenues of investigation, such as the possibility of extracting velocity correlations within the cell sheet [32–34]. Directionality in cell motion was found to be correlated over many cell body-lengths and over several hours in time. The dependence of the correlation functions on cell density was studied by Angelini et al. and the intriguing analogy to dynamics found in glassy materials was highlighted. In particular, the frequency of swirls decreases with increasing cell density [29,34].

Similar to the way the flow field can be obtained via PIV analysis from displacements in the cell sheet, the distribution of forces an epithelial layer exerts on a substrate can be obtained via traction force microscopy from displacements of fluorescent beads embedded in a polyacrylamide gel substrate [39]. Tambe et al. used this technique in an approach termed monolayer stress microscopy to map out the traction forces exerted throughout a sheet of MDCK cells in a spatially resolved manner [40]. In the process of this, they found that in the stress landscape local cellular migrations follow local orientations of maximal principal stress. This phenomenon was named plithotaxis. During monolayer expansion, traction forces appear in waves [41]. PIV analysis and traction force microcopy can also be used to obtain simultaneous velocity and force maps [42,43]

An alternative approach to assess migration patterns within cell sheets is analyzing motion of individual cells. Isolated cells are known to undergo random walk like motion in the 2D culture dish plane. The single cell motility is mostly analyzed in terms of a persistent random walk [44–46]. Tracking cell positions within the cell sheet from phase contrast images is possible ([37,47]), but not easy, especially when automation is desired. In contrast, stained nuclei can be used as a proxy for the cell center position and tracked by automated image analysis software. Hence, the mean-squared displacement and the speed distribution of single cells is readily analyzed [29,48]. In addition, nuclear staining allows for direct determination of cell densities by automated cell counting [29]. Fig. 4 shows, for instance, the average density profile of a cell sheet invading a microchannel, which will be discussed in the next section. In principle, the entirety of individual tracks represents the full data set of center of mass motions in collective migration.

These last examples shows that, in addition to advanced image analysis, the design of artificial boundaries for cell migration experiments is a powerful method for the study of cellular migration behavior. Micropatterning has emerged as an important tool, allowing experiments to be tailored for reproducibility and well defined initial conditions. Various different techniques, such as microcontact printing, micromolding in capillaries, UV chemistry and plasma-induced patterning can be used to structure a substrate with adhesive and cell-repelling areas [49]. In this way, cells have been confined to various geometries,

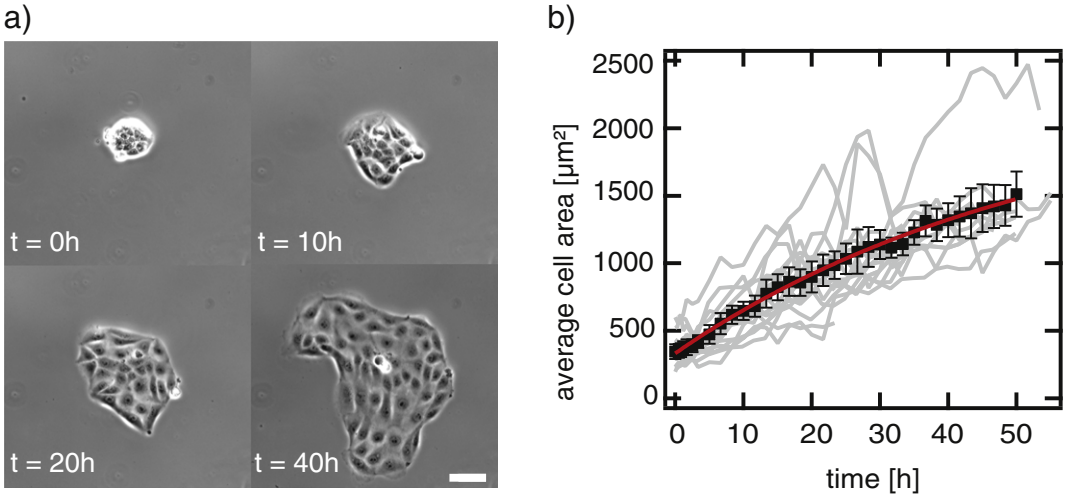

Fig. 2. Proliferation and expansion of a small cell patch. a) Cells are initially seeded into a circular cavity surrounded by PEG-DMA and grown to a high density. After removal of the confinement (t = 0 h), cells collectively move outwards. The patch remodels due to cell division and cell spreading (scale bar: 100 μm). b) Evolution of the average area per cell, A(t)/N(t) over time. Time courses of individual patches are shown in gray, the average is fitted by Eq. (2) (red line) [30].

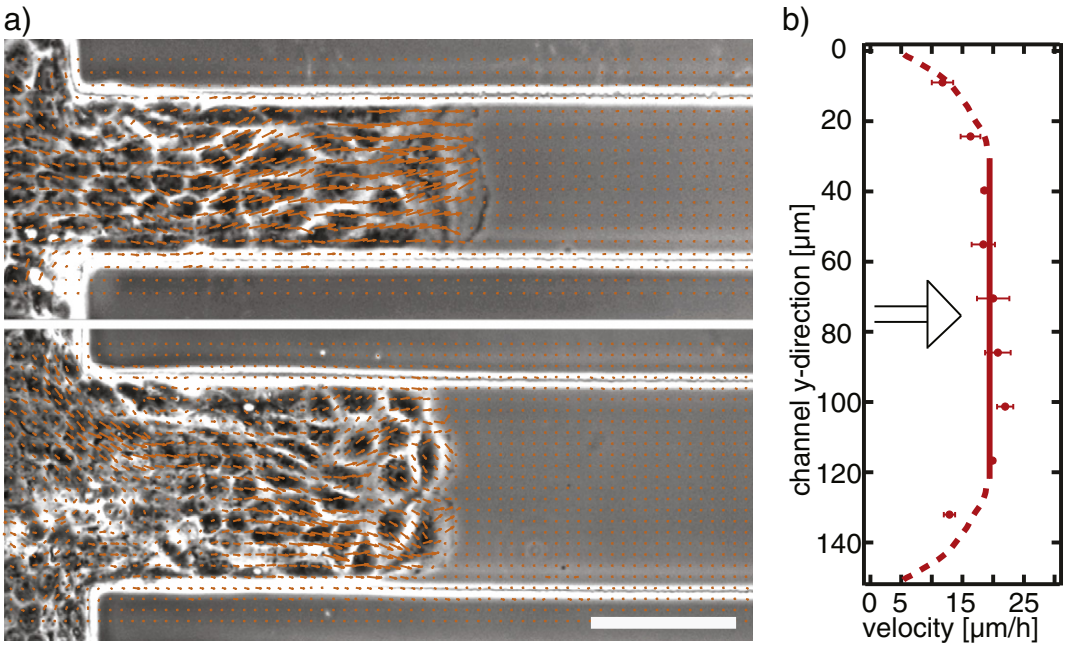

Fig. 3. Cellular flow field obtained from particle image velocimetry (PIV). a) Monolayer of MDCK cells invading microstructered channels of different widths with the velocity field overlain in orange (scale bar corresponds to 100 μm). b) Velocity profile averaged in time (2 h) and space (15 × 15 μm^2) showing a plug-flow like profile across the channel (data from [29]).

such as stripes or channels of different widths [28,29], as well as squares, circles or rings [50–52], but also more complex structures, such as hourglasses [53]. If the passivating agents used to confine cells to distinct geometries are bound via a photocleavable group it is even possible to create defined initial conditions and later switch the adhesiveness [54].

4. Phenomenological models of collective cell migration

There is a long-standing history of mathematical modeling of collective cell migration in wound healing, but also other scenarios such as angiogenesis [55–59]. The concepts of these models are borrowed from fluid mechanics and prove useful in explaining the observed cell kinetics at large scales. The main contributions are a) proliferation, b) diffusive migration and c) directional migration. As shown in Fig. 5, proliferation accounts for cells as a replicative system. Diffusive migration denotes the fact that in the presence of density gradients a net cellular flow arises which tends to balance cell density. In this analogy, the permanent cell activity plays the role of temperature in ordinary diffusion. Finally, directional migration describes active cell movement in a preferred direction due to planar polarization of cells. This polarization can couple between neighbors. If symmetry in the system is broken due to geometrical constraints, a preferential polarization direction can be introduced as opposed to all orientations being equal. These concepts provide some coarse-grained understanding of collective behavior at length scales beyond the single cell length. They are not linked in an obvious way to the biological models of cell growth, cell migration and cell–cell coupling. They do however provide measurable parameters that are distinct for various different cells lines and hence contribute to phenotypic characterization. The aforementioned techniques of analysis and experimental design allow a quantitative description of how cells migrate collectively.

A widely established method for studying collective cell migration is the wound healing assay (see Fig. 6). Originally, a confluent layer of cells was injured, either chemically or by scratching ([27,60]) and the closing of the wound observed over time. Micropatterning techniques nowadays

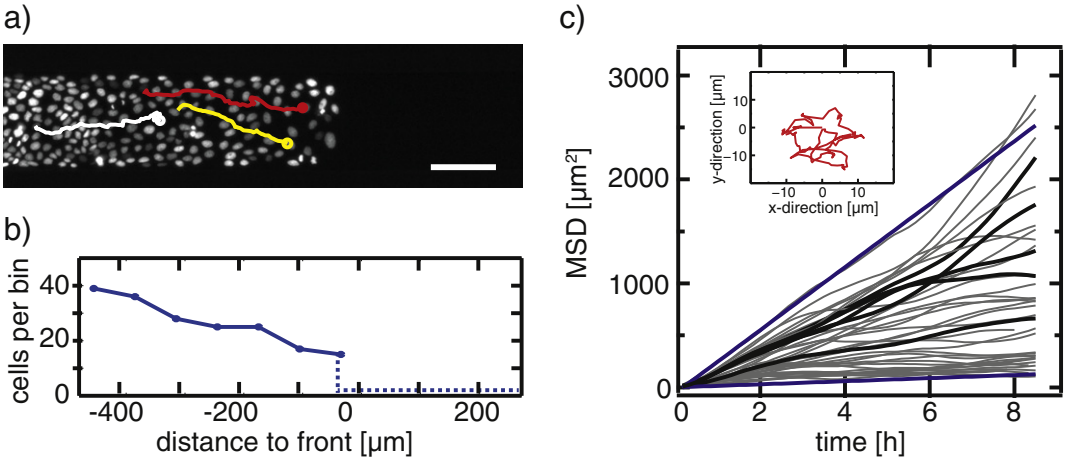

Fig. 4. Nuclear staining visualizes the position of each individual cell. a) Fluorescence image of cells invading a microchannel with tracks of individual cell movements overlaid (scale bar: 100 μm). b) Cell number density along the channel determined by automated cell counting (bin size 70 × 150 μm^2). c) Mean squared displacement of individual cells' motion relative to the invading cell sheet. Inset: Drift corrected time trace of a single cell (from tracks such as the colored ones shown in a).

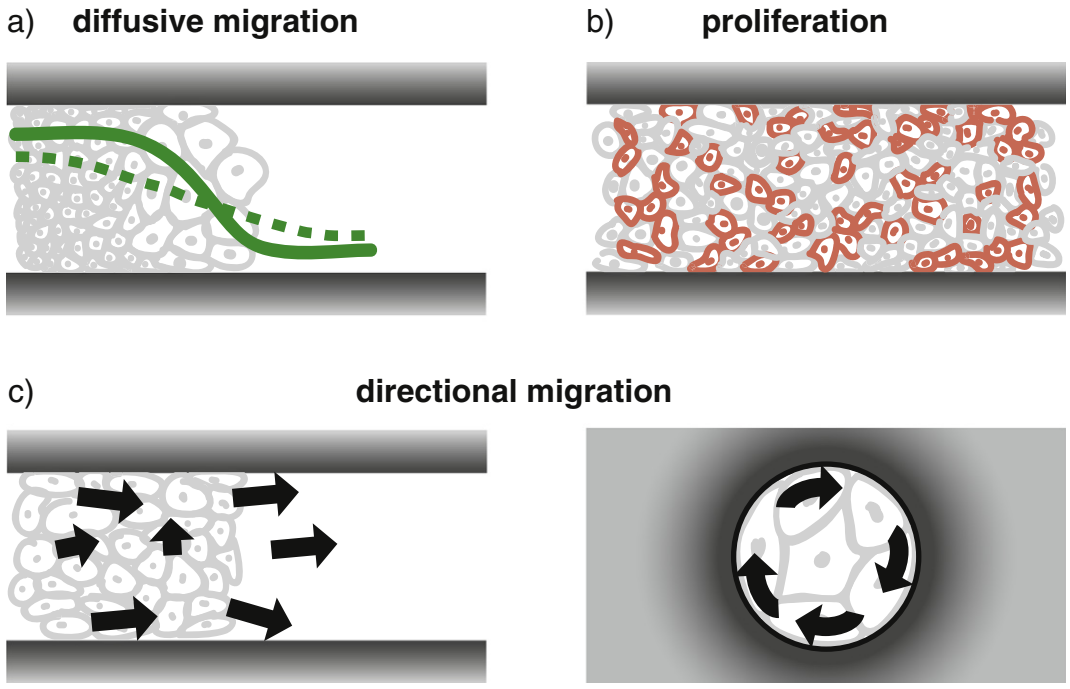

Fig. 5. Schematic cartoon of the phenomenological contributions to collective cell migration. a) Diffusive migration describing cell migration in response to a cell density gradient. Green lines indicates evolution of density profile. b) Proliferation (new cells depicted in red). c) Directional migration as a result of cell guidance induced by confinement.

allow producing stencils in cell sheets which can be removed without damaging surrounding cells [32]. Likewise circular patches can be prepared and the expansion studied as mentioned earlier. In both cases the manner of migration into the wound [61–63] or expansion from a patch [64] is reproduced by a continuum model that takes cell proliferation and an effective diffusive dynamics into account. The corresponding differential expression is known as the Fisher–Kolmogorov equation (Eq. (3)).

$$\frac{\partial c}{\partial t} = D_c \nabla^2 c + \lambda c\left(1 - \frac{c}{k}\right) \tag{3}$$

In this reaction–diffusion type equation, Ficks 2nd law of diffusion with collective diffusion coefficient D_c is combined with logistic growth defined by growth rate λ and carrying capacity k (i.e. the maximum density k possible in the cell layer) to describe the time evolution of the density profile $c(x,t)$. The Fisher–Kolmogorov equation has traveling wave solutions, which corresponds to the stationary density profile that propagates at a constant velocity $v = \sqrt{4\lambda D}$.

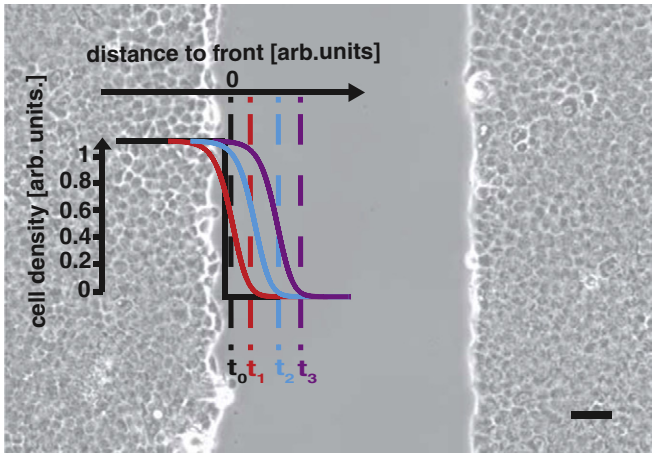

Fig. 6. Phase contrast micrograph of a wound healing assay overlaid by an illustration of the theoretical evolution of the density profile according to the reaction–diffusion equation Eq. (3). The scale bar corresponds to 50 μm.

In a previous publication, we studied the evolution of the density profile of cells invading 100–300 μm wide channels, which corresponds to a laterally confined wound-healing assay. We found that the addition of a constant drift term to the Fisher–Kolmogorov equation was required for a self-consistent description of the cell density profile. This drift term points to cell guidance by the straight geometric boundaries, which will be discussed later on. Angelini et al. studied the collective dynamics in confluent layers of MDCK cells as a function of cell density [35]. Using PIV and spectral analysis of the spontaneous density fluctuations they obtained similar values for diffusion at low cell densities as obtained by wound healing assays. The diffusive cell dynamics, however, decrease strongly at higher cell density and at a certain level, a glass-like transition occurs. Above this, relaxation processes within the epithelial layers take significantly longer and the spatial correlations decrease [35]. At the same time, force mapping revealed that with increasing density force cooperativity extended to greater distances [40]. The presence of glassy dynamics substantiates the importance of cell density as a critical parameter for determining the cooperative motion in cell migration. In agreement with this, Doxzen et al. found that at the lower limit, there is a critical density necessary for cells to migrate in a coordinated fashion [51].

The diffusive mechanism that balances out density gradients can be imagined as random bursts of correlated migration of cells. The bursts have a dimension on the order of the correlation length, l_c and duration of order of correlation time, t_c. Hence, the collective diffusion coefficient is well approximated by $D = l_c^2/4t_c$ [29]. MDCK cells, for instance, have been found to have a correlation length between around 100 μm to 200 μm [29,32,33,35] and a correlation time of about 1 h [29]. In this context it is worth mentioning that the self-diffusion coefficient obtained from the mean-squared displacement is smaller by a factor of 50 compared to the collective diffusion. Again in analogy to glassy systems, the freedom of motion of a single unit is restricted and cells can be taken as caged in by their neighbors. As a quintessence, the capability of tissue to equilibrate density gradients at long distances is intimately related to

correlated motion and hence cell–cell coupling rather than individually migrating single cells.

The reaction–diffusion model so far works well for describing the spreading front and the evolution of the average density profile. However, the analysis of flow pattern within resting cell sheets also reveals that transient swirls of collective rotation reminiscent of turbulent fluids form in the cell sheet. PIV analysis has been used to find that these vortices are on the order of 10 cells large [28,34] and have lifetimes of approximately 15 min [29]. Increasing cell density has been shown to both increase the swirl correlation length [34] and reduce their overall frequency [29]. Vortices appear frequently in migration experiments [28,34] which is consistent with findings that this phenomena generically emerges from simple models [6,10,65]. Notably, the appearance of swirls is reduced in migrating cell sheets compared to cell sheet of comparable density that are resting, i.e. in the absence of density gradients and open surface [29]. A migrating cell polarizes in plane with respect to its actin cytoskeleton and hence breeds a front and a rear end within the cell contour. Without external stimulus by cytokines or neighboring cells, a single cell has no preferential direction of polarization. If the polarization of connected cells is coupled via cell–cell contacts, however, long-ranged coordination of polarization emerges. Vortices are one manifestation likely resulting from such coordinated polarization. On circular geometries, collective rotation is observed even without an existing density gradient [51,52]. Planar polarization along the symmetry breaking circular boundary is likely the cause of the rotation in clockwise or counterclockwise direction. Boundary conditions have further been shown to play a crucial role by Vedula et al., who showed in the case of narrow stripes vortices disappear and collective migration behavior changes to a contraction–elongation mode [28].

Another source of local anisotropy is cell division. When one cell divides into two, a division axis can be defined, which for a single unbound cell has no preferred orientation. A cell embedded in connected tissue shows alignment of the cell division axis with the surrounding cellular flow [66].

Although phenomenological models such as the ones presented in this section are well suited to describe certain large scale characteristics of cell layers, it remains unclear how these phenomena emerge from the properties of the individual constituent cells. In order to approach this question, a variety of agent based simulations and cellular automata models have been developed over the last years. In such models, a single cell is implemented as an individual agent whose actions and interactions with other agents are determined by a specific set of predefined rules [47,67–71]. Therefore, in analogy to nature, the tissue scale properties emerge from the properties of the individual cells in such an approach. Accordingly, in order to achieve a high accuracy of such models, it is essential to assess these single cell properties and the rules of cell–cell interaction by highly specialized, tailored experiments.

5. From single cell to oligocellular systems

As described in the last section, an intriguing prospect for agent based theoretical modeling is to predict collective cell behavior from single cell properties. Such properties can be addressed and studied very specifically by techniques like micopatterning, which allow for direct control of the geometry and chemistry of the cell environment on a microscopic scale. Hence, in recent years, a large variety of micropatterns have been devised to study and even manipulate intrinsic cell properties at the single cell level.

Théry et al. showed, that the confinement of cells to specified geometries has influence on the orientation of the cell division axis [72] as well as the cells' internal polarization axis [73]. Pattern geometry is also known to guide the extension direction of cellular protrusions [74]. Furthermore, the shapes cells exhibit when spanned on arrays of small adhesion sites provided significant insight into the mechanic properties which guide cell shape formation [75].

Dynamic properties have likewise been studied on single cell level using micropatterning approaches. For instance, the migration of cells on parallel lanes was analyzed for a vast number of cell types by Maiuri et al.. They found a persistent random walk like migration pattern and parameters like cell speed and persistence were extracted [76]. In a slightly modified setup, cells migrating on lanes with diverging pattern walls or connected-triangle ratchets were found to migrate preferentially in one direction, showing that the symmetry of the random cell walk can be broken by predetermined geometry of the cells' environment [77,78].

Such setups can also be extended to an oligocellular level, meaning that the system is designed to study the interaction of only a small number of cells. Such oligocellular systems are mesoscopic in the sense that they represent an intermediate length scale between single cell and tissue-level phenomena. Cellular systems consisting of just a small number are surprisingly complex and exhibit rich scenarios of collective behavior arising from interaction of the individual cells. Despite this, such systems are simple enough that they readily provide access to the underlying single cell properties. Fig. 7a shows a time-lapse series of initially two cells on a micropatterned square. Cells are seen to divide and rearrange. The final geometric arrangement of four cells forming a T-junction in the square appears to be quasi-stable over an extended period of time.

Extending the concept of using micropatterns for studying static cell properties to oligocellular arrays, Tseng et al. used micropatterns to study static cell–cell arrangements in artificial geometries. Fibronectin micropatterns were used to constrain the location of cell–ECM adhesion. The observed spatial organization of intercellular junctions was found to align with the imposed geometry of the adhesion sites. The position of cell junctions is in accordance with predictions from vertex model described in Section 2, assuming that the overall cortical tension is minimized [53]. Complementing this, oligocellular assays more readily provide access to parameters such as the relation of cell contractility to cell–cell adhesion strength that dictate cell–cell interaction and are therefore key parameters for this kind of model (see Fig. 7a). The geometry of the spatial constraints of localized ECM–cell interaction leads to a quasi-static arrangement of cells which gives insight into relative strength of the competing forces in the system. Similar setups were also used to show that cell–cell adhesions can trigger cell polarization and orchestrate the direction of the cells' polarization axis [79].

In addition to static properties, oligocellular assays can also be used to study the dynamics of cells. In analogy to single cell experiments, Desai et al. used the straight lane setup to study cell–cell encounters and found that polarized collective cell motion can emerge from contact inhibition of locomotion of colliding cells [80]. Another prominent example for oligocellular migration was given by Ingber et al. [81], who described Yin-Yang type rotation of pairs of cells confined to circular adhesion islands. In this case, transiently rotating states with either left-handed or right-handed constant angular velocity emerged. Circular confinement also induces an elevated propensity for persistent rotation in larger cell groups [51]. Rotating cells are found to move much like a solid block in circles smaller than (or around the size of) the cells' velocity correlation length. In this case, the angular velocity of rotation depends on cell density as well as system size. The occurrence of rotation in such systems appears to be stochastic [82]. In a recent study, Segerer et al. carried this investigation further and studied the life time of the rotational state as a function of cell number. With density kept constant by varying the available area, the persistence of collective rotational states was found to increase with cell number but display a sharp discontinuity between 4 and 5 cells, where the geometric cell arrangement changes from a conformation without a cell in the system center to one including a centered cell. These findings indicate the critical role of local cell geometry and arrangement of neighboring cells on the internal planar polarization of cells and hence the stability of collective vortex states [52]. Fig. 7b exemplarily shows a rotating oligocellular state and illustrates that the dynamics of the systems are largely captured by the angular tracks of each individual cell.

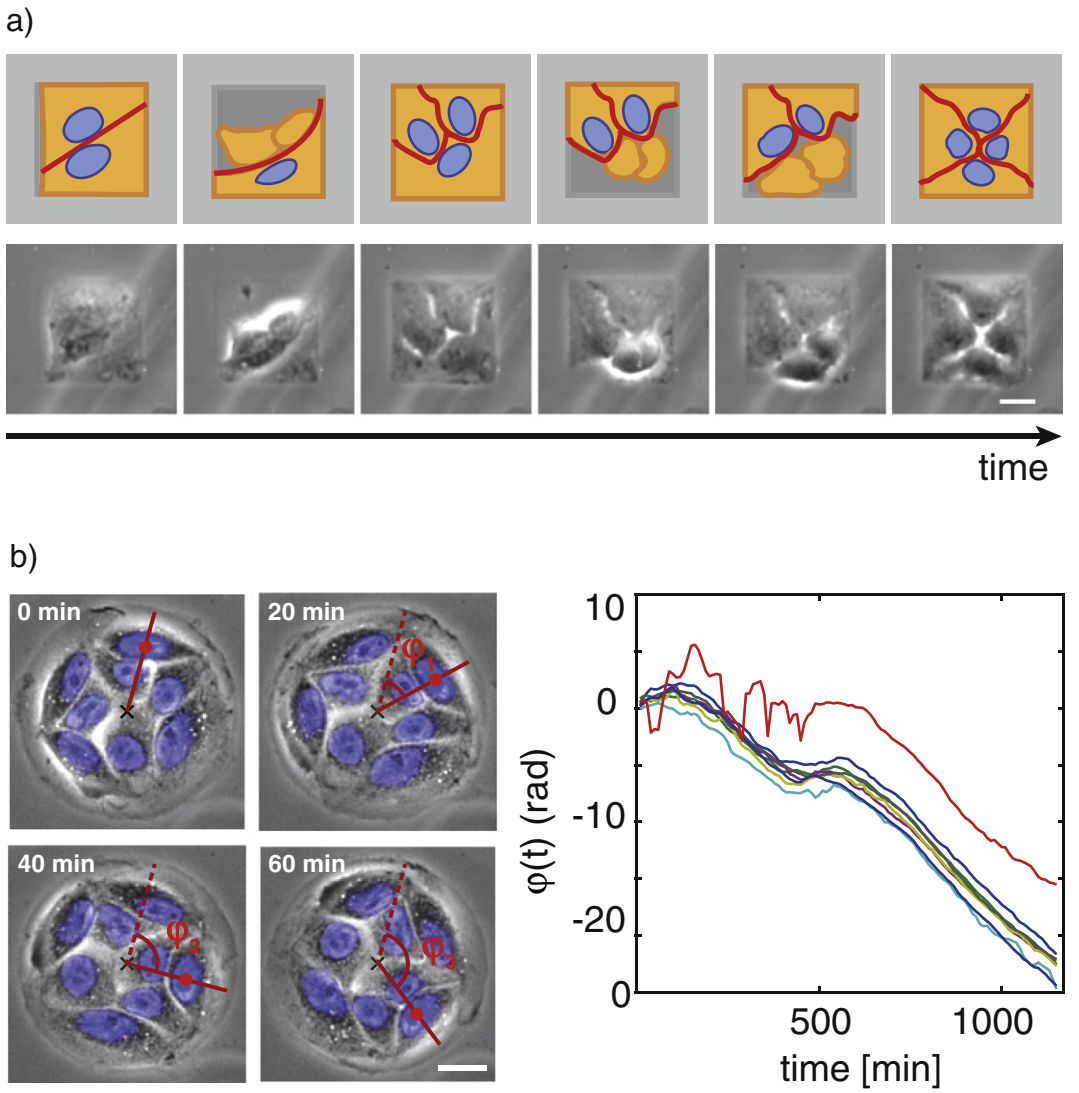

Fig. 7. Example of oligocellular assays (scale bars correspond to 20 μm). a) Time lapse sequence (18 h) of initially two cells. Cells divide and rearrange into a quasi-static packing state (image taken from [83]). b) Dynamic state of a group of cells collectively rotating in a disk pattern. Nuclei are stained and angles are plotted over time, revealing a coherent angular motion for some periods.

From such observations on single or oligocellular scale, specific single cell properties of individual cell types can be accessed systematically and in large, parallelized arrays. Consequently, highly specialized setups as discussed in this section are not only very suitable to asses, for instance, the effect of specific drugs on different cell properties, but also to extract the parameters essential for agent based modeling approaches as discussed in the last section. In this way, they provide important insights into the emergence of tissue scale phenomena from basic features of the constituent single cells.

6. Leader cells and roughening of the epithelial border

In the previous sections, the border of the cell layer was ignored, or in the case of the Fisher–Kolmogorov equation, assumed to be straight. Given the line tension between neighboring cells, it makes sense to expect such a relatively smooth interface. In reality, however, observed expanding epithelial sheets, such as MDCK cells or IAR2 rat liver cells, display the formation of finger-like protrusions that proceed the rim [32,84,85]. Within these fingers, migration seems to be highly correlated and very directional, and closer examination reveals a characteristic, multicellular actin belt running along its periphery [32,33]. Notably, each of these protrusions is distinguished by having a single leader cell at its tip that displays a very distinct and changed morphology. These leader cells, in addition to being significantly larger than the other cells within the sheet, exhibit highly developed lamellipodia (See Fig. 8). Leader cells are highly polarized and directional, never seem to divide while they are in the leading position and form many focal adhesions [32,86–88]. Typically, leader cells appear one hour after the surrounding surface becomes available for migration, remain in the guiding position for several hours but are transient and return to normal once they reach, for instance, the opposite monolayer in the wound-healing case [32,89,90].

Effectively, cells that become leader cells undergo a partial epithelial-to-mesenchymal transition [91], though the selection mechanism is still unclear. Both chemical signaling via growth factors [92,93] as well as a dynamic instability model which does not require any additional signaling [87] have been suggested. The former is known to play a role in the formation of branched ducts in Drosophila tracheal morphogenesis. Here, an initially not predetermined cell turns into a tip cell by local growth factor (FGF) signaling and then inhibits cytoskeletal activity in neighboring cells [94,95]. On the other hand, a purely physical model is supported by the fact that Rolli et al. found a dependence of the frequency with which leader cells appear on the curvature of the cell cluster's boundary [54]. While not applicable to monolayers

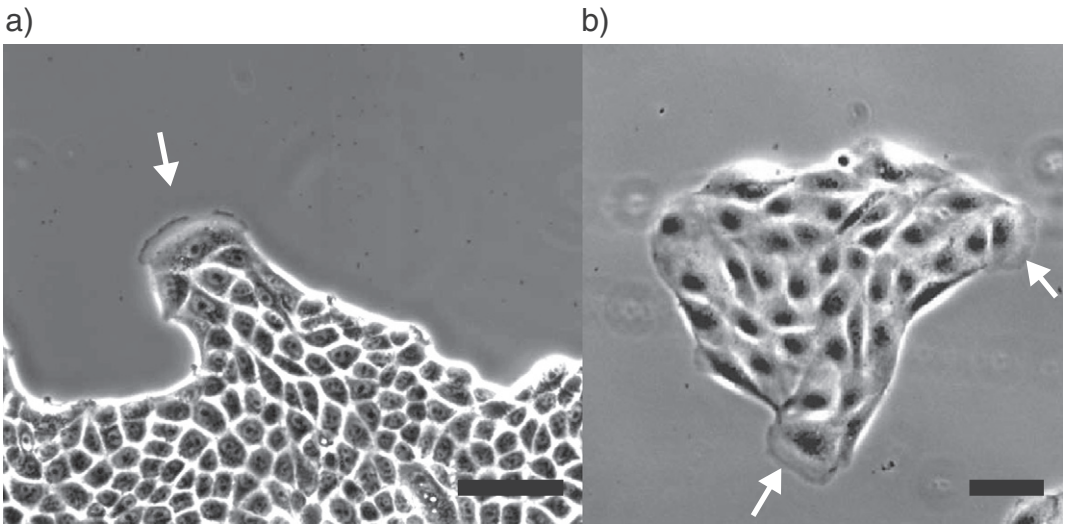

Fig. 8. Leader cells (indicated by white arrows) with distinct morphology and clearly visible lamellipodia in different scenarios of epithelial cell sheet expansion. a) MDCK cells sheet expanding predominantly in one direction form pronounced fingers of cells following behind leader cells. b) In small patches of MDCK cells, several leader cells appear, pulling in different directions. Scale bars correspond to 75 μm.

consisting of only a single cell type, collective invasion of epithelial cancer cells can occur via heterocellular-assistance, where the leading cells are from an entirely distinct cell lineage than the followers [96]. In the case of homogeneous epithelial sheets, leading cell function might simply be induced by the asymmetry the foremost cells experience, being connected via cell–cell junctions to neighbors in the rear, but not at their front ([97,98]), a state Khalil et al. term 'Intrinsic Polarity' [91]. Cell–cell contact is known to negatively affect migration as well as the formation of actin-driven protrusions [99], two of the apparent differences between leaders and followers. In addition, this intrinsic polarity is augmented by the fact that cells remodel the underlying substrate by depositing their own ECM proteins, meaning the following cells do not encounter the virginal surface the leader cells migrated over [100]. In this context it is noteworthy, however, that cells even hundreds of microns from the edge have in fact been found to extend cryptic lamellipodia beneath the cells in front of them [101]. Poujade et al., observe that leader cells in sheets of MDCK cells often arise from the second or third row and migrate to the leading edge without any apparent morphological changes, before undergoing the clear transition to a different phenotype [32]. While this seems to reinforce the notion that the position at the actual edge of the cell sheet is relevant for the transition, it also shows that cells need not originate there to become leaders. Thus, for the time being, the selection mechanism remains under debate.

Although the process that determines the cells 'differentiation' fate is still unclear, what role leader cells play has been elucidated to a certain degree. While it was originally suspected that leader cells might speed up wound closure [102] and that they are responsible for dragging the cell sheet behind them by excreting traction forces on the substrate ([32,85,102–104]), Trepat et al. mapped these forces throughout the cell sheet and showed that the leader cells' contribution to the overall force balance is small. Instead, traction forces of similar magnitude can arise throughout the cell sheet. Still, as the local average of the forces is about four times as high at the front as it is in the bulk of the sheet, they do not rule out this might be sufficient to direct the rows farther back [39]. The mechanical signaling between a leader cells and its followers is discussed to be supported by the observed peripheral, subcortical actin belt [32]. Recently, Reffay et al. showed that this contractile cable prevents the initiation of further leader cells in the finger. They proposed that both mechanical and biochemical cues determine new leader cells, with the role of both RhoA and Rac seemingly playing a role for collective migration [105,106]. Despite the biological complexity of leader cells, the phenomenon of finger formation and the accompanying flow pattern can be understood and reproduced in computational modeling [107]. These models need to arbitrarily invoke individual model "leader" cells, but the resulting digitated shape of the interface and the finger flow fields behind the seeded leader cells then emerge as a consequence of the cellular hydrodynamics of migrating cells described earlier.

7. Discussion

Collective cell migration is analyzable in terms of cellular velocity fields using PIV analysis, traction force microscopy and single cell tracking. At a coarse-grained level, migration phenomena, such as the behavior in a wound healing assay, are described by a phenomenological reaction diffusion type model. At an intermediate length scale, however, vortices, pulsation and waves expose a correlated motility of mutually interacting cells. Similar patterns of correlated motion are found in early phases of morphogenesis. These mesoscale properties are attractive to study as they reveal much about the intercellular coupling. During development, a collective of cells form defined shapes at larger scales, while at the individual cell level considerable stochastic behavior seems to counteract any ordering. While in earlier days it was generally believed that chemical signaling and morphogenetic fields are the dominant mechanism of pattern formation, it has become increasingly accepted over the last 10 years that mechanical forces play an important role. The central question is to understand how mechanical forces are transmitted to adjacent cells enforcing cooperative long-range cell motion. As shown here, long-range correlations account for the effective diffusive balancing of density gradients. Still more importantly, cooperative cell migration is the base of the formation of shape, functional organs and finally full organisms. In recent years, computational models with minimal requirements have elucidated and reproduced some key features of collective migration, such as sheet migration and swirl formation [51,82]. For the future development of the field it will be important to find ways to quantitatively and systematically compare experiments to computer simulations.

As shown in this review, confined geometries allow cell guidance and lead to novel cellular flow patterns. A multitude of data can be generated by variation of the guiding micropattern and flow fields, force fields and the localization of fluorescently labeled key protein components can be mapped out and compared to mathematical migration models. In the near future, the challenge will be to find models and

parameter settings that describe an increasingly large set of data in a self-consistent manner. In this context, an intriguing task will be to define standard migration assays and appropriate measurable quantities for the calibration as well as comparison of models. The wound healing assay is just one bench mark test. Other scenarios such as cellular rotation in circular confinement or directed migration in channels might deliver complementary information. The implementation of small system size assays will deliver easily reproducible experimental data with statistical measurements that will be corner points for calibrating experiments. A prominent example are quasi-static cell arrangements found in geometric ECM pattern, which are suitable to scrutinize cell–cell adhesion and cell–substrate interaction [53,108–110].

For a more intuitive understanding of how cell cooperativity manifests itself in a tissue, the introduction of "phenomenological rules" that describe collective behavior is valuable. Examples are the concept of glassy dynamics in collective migration [35] as well as the migration motives Plithotaxis and Kenotaxis introduced by the Trepat group [42,111]. The migration motive Plithotaxis describes the tendency for each individual cell within a monolayer to migrate along the local orientation of the maximal normal stress, or equivalently, minimal shear stress [111]. Kenotaxis denotes the finding that a cellular collective generates local tractions pulling systematically and cooperatively towards unfilled space [42]. Direct examination of these rules in cell monolayer requires analysis of average flow tendencies in a large ensemble with large degree of variability of cell constellations. Here, specialized oligocellular assays combined with computational modeling might be a promising approach to scrutinize flow motives in defined cell arrangements. Further advancement of cell adhesion and migration microassays to automated high throughput compatible platforms will allow screening of cell phenotypes and hence help to identify genes and proteins that play a role in the mechano-sensitive response of cells. The challenge remains to feed this molecular information back into future multiscale modeling. A mathematical framework of collective migration however, will be at the heart of a computational systems biology view in disciplines related to the dynamics of tissue, such as angiogenesis, cancer research or developmental biology.

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