Single-Cell Migration in Complex Microenvironments: Mechanics and Signaling Dynamics

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Cells are highly dynamic and mechanical automata powered by molecular motors that respond to external cues. Intracellular signaling pathways, either chemical or mechanical, can be activated and spatially coordinated to induce polarized cell states and directional migration. Physiologically, cells navigate through complex microenvironments, typically in three-dimensional (3D) fibrillar networks. In diseases, such as metastatic cancer, they invade across physiological barriers and remodel their local environments through force, matrix degradation, synthesis, and reorganization. Important external factors such as dimensionality, confinement, topographical cues, stiffness, and flow impact the behavior of migrating cells and can each regulate motility. Here, we review recent progress in our understanding of single-cell migration in complex microenvironments.
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1 Introduction

Cells are a fundamental unit responsible for enabling complex functions in living organisms. Their ability to migrate is critical in development, normal physiological functions, and disease. Cell migration is driven by intracellular biochemical and biomechanical organization that is sensitive to extracellular cues. In particular, mechanical signals have been implicated in cancer progression, stem cell differentiation, and tissue morphogenesis [1–3]. In vivo microenvironments, such as the extracellular matrix (ECM), are complex, with numerous mechanical features including 3D nature, fibrillar architecture, and flow that can influence cell polarization and motion. Here, we review recent findings and models of cell migration in complex microenvironments. We focus on two key areas of interest—(1) intracellular biochemical signaling pathways and their mathematical representations that drive polarized cell states and directed migration and (2) extracellular mechanical cues that modulate migratory behavior.

2 Intracellular Signaling

Cell migration is intrinsically a mechanical phenomenon involving internal molecular actuators displacing a complex material. The key mechanical machinery consists of myosin motors that enable contractile force generation and assist in cytoskeletal crosslinking, adhesion complexes that interface the cell to its external environment and enable force transmission, and polymerizing and depolymerizing actin filaments that drive forward protrusions and facilitate internal remodeling. The coordination and spatial organization of these mechanical parts are regulated by interconnected signaling pathways and feedback mechanisms, conferring cell polarity, and modulating cell migration modes and kinetics. Canonical signaling molecules include Rho GTPases that modulate the activity levels of the mechanical machinery.

2.1 The Internal Polarization of a Single Cell and Directed Cell Migration. To migrate persistently and in a directed manner in response to external stimuli, a migrating cell requires an internal machinery which defines the directionality of migration. This machinery consists of an interplay of biochemical and mechanical factors which polarize in such a way that forces are generated in a specific direction to move the cell forward. The classical picture from migration on substrates is that actin-driven protrusions happen primarily at the front, and myosin-driven contractile forces

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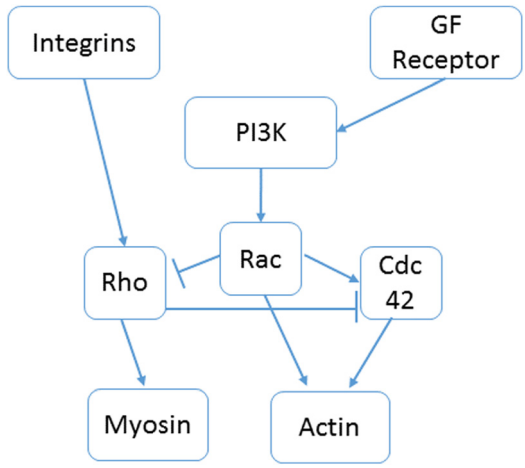

Fig. 1 Growth factors (GF), ECM ligand binding, or mechanical stimuli through integrins initiate an intracellular signaling cascade, which leads to regulation in actin and myosin activity. The molecules and interactions shown here are only a small selection of the full cascades, focusing on some key players which are necessary to regulate directed cell migration.

that lead to detachment are generated at the rear, causing the cell to move forward [4,5]. Typical scenarios tested in many in vitro experiments involve the exposure of cells to a gradient of a growth factor (chemotaxis) [6], a gradient of ECM ligands (haptotaxis) [7,8] or a stiffness gradient (durotaxis) [9,10]. For instance, if the cell is placed in a chemoattracting growth factor gradient, a typical response seen is the activation of molecules such as PI3K and PIP3 [11,12] or the GTPases Rac and Cdc42 [13] which drive actin polymerization at the front of the cell. Rac is associated with lamellipodia [14], which are broad, flat protrusions, and Cdc42 is associated with filopodia [15], which are finger-like protrusions. In migration on a two-dimensional substrate, Rho is upregulated at the back of the cell [13]. Rho will then activate ROCK and ultimately myosin motor activity [16], leading to contractions at the rear and subsequent detachment. The combined effect of protrusions and firm adhesions at the front and contractions and detachment at the rear is what makes the cell move forward. Cell migration guided through mechanical factors such as stiffness or geometry of the environment involves similar, but not necessarily identical molecular pathways; we will discuss some key work in this direction in Sec. 2.3 and Sec. 3. Figure 1 shows a schematic of such a signaling cascade. It is highly simplified and the precise details depend on the cell type and context. For instance, GTPases alone are regulated by dozens of GEF, GAP, and GDI molecules, leading to far more complex interactions between Rac, Rho, and Cdc42 than shown here [17]. Also, in migration in 3D, there are some important differences which we highlight in the following sections. Furthermore, in both 2D and 3D, the complete actin and myosin regulating pathways involve many more regulators than those discussed here, and we refer readers to Refs. [18–20] for excellent reviews of these topics.

Numerous mathematical models have been employed in recent years in an attempt to understand and predict gradient sensing, polarization and migration. Most of these models make use of techniques which have been successfully applied in many areas of biology, such as continuous, mixture theory partial differential equations [21–23], stochastic models [24–28], or agent based models [29–31]. The scope of many of the cell polarization models is to explain how the cell senses gradients or how it polarizes. Local-excitation-global inhibition models [32] explain how polarization can arise through an interplay of a fast diffusing inhibitor and slowly diffusing activator, and wave-pinning models [33] explain polarization in terms of the fixation of propagating waves of molecules involved in polarization. The general idea behind many of those models of cell polarization is that a receptor-mediated stimulus activates internal biochemical reactions. Then, diffusion mediates intracellular communication to weaken or enhance force-regulating molecules at the front or back, and hence define front and back states. Some models incorporate the dynamics of many of the molecules mentioned above [34–38]. Other modeling approaches focus on aspects such as thermodynamic considerations [39], the interplay of biochemistry and mechanics on polarization [34], or stochastic effects on cell polarization [40].

2.2 The Physical Intracellular Machinery. Signaling pathways ultimately lead to the activation and dynamic coordination of the physical machinery of the cell in order to drive mechanical motion. This machinery is primarily engaged in the actions of protrusions, adhesions, and contractions. Protrusions are driven by the polymerization of actin filaments, in which monomer subunits, G-actin, are added to the ends of existing filaments, F-actin. In particular, at the leading edge of cells, Cdc42 and Rac signaling activates actin nucleation promoting factors (WASP/Wave) and subsequently Arp2/3, which direct the branching and enhanced polymerization of F-actin preferentially on the "barbed end" of the filament [4,41]. Actin filaments are dynamic and can undergo turnover, with faster depolymerization at the "pointed end." Asymmetric polymerization and depolymerization lead to treadmilling of actin filaments [42], which along with other actin turnover processes such as severing [43,44] enables protrusions to occur dynamically.

At the leading edge, new adhesions form in order for cells to adhere to their new positions. These adhesions can be very dynamic and turn over quickly—nascent adhesions—so that cells may dynamically sense the environment without committing to those positions, or they can form complexes that become more stable—mature or focal adhesions—that enable cells to maintain firm attachments [45]. Adhesions are composed of transmembrane protein complexes, such as integrins, which bind to extracellular ligands, such as ECM or basement membrane proteins [46]. Inside the cell, these transmembrane adhesion complexes are connected to the actin cytoskeleton, enabling outside in and inside out signaling and force transmission. Tension generated in the cytoskeleton by myosin II motors can lead to the clustering of adhesion proteins, enhanced adhesion binding through catch bond behavior [47,48], and reinforcement of mature adhesions [49,50].

Finally, at the rear of the cell, contractile forces from myosin II motors, activated by RhoA, and the turnover of old adhesions lead to net forward movement [4]. Myosin II motors form minifilaments with many myosin heads that bind to actin filaments [49]. These myosin heads walk along actin filaments in different directions, generating contractile forces in the crosslinked actin network.

2.3 Impact of Dimensionality and Geometry on Cell Polarization. Most of the observations discussed in Secs. 2.1 and 2.2 were obtained from experiments where cells are plated on 2D substrates. There are similarities as well as key differences in the behavior of signaling molecules in cells embedded in 3D matrices. It was found that in 3D environments, Rac levels are generally lower than in 2D, and this reduced total level is associated with rapid, directional migration. Also, Cdc42 and its crosstalk with Rho are important in 3D migration [51]. However, the role of Rho-mediated contractility is more complex in 3D than in 2D. For instance, in fibrous matrices, contractility is important to align matrix fibers [52,53], and myosin-mediated blebs plays an important role in amoeboid migration through matrices [54,55].

In parallel with the development of 2D in vitro cell migration, computational models have initially mainly focused on 2D. However, even in 2D, the cell itself will have a complex shape, which can only be captured accurately by a 3D model of the cell. For

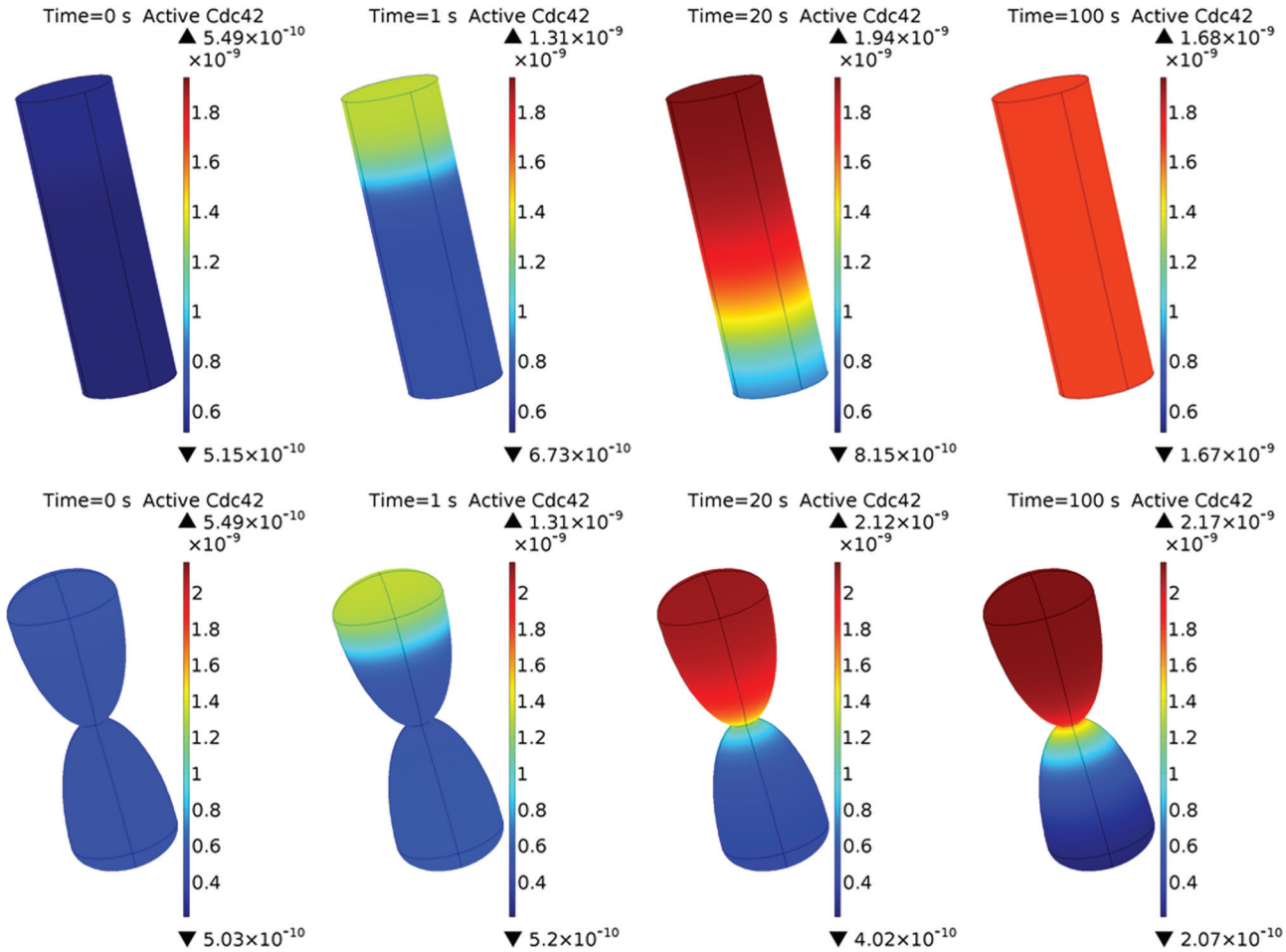

Fig. 2 Time evolution of active Cdc42 on the membrane for two cells. The top row shows a symmetric cell which loses polarization quickly after an initial stimulus at the front. The cell in the bottom row, which is very thin in the center, stays permanently in a polarized state. Both cells have the same length (40 μm) and would hence appear identical in a 1D model, confirming that their 3D shape plays an important role in determining their polarization state. All simulations were performed with the 3D reaction–diffusion model described by Spill et al. [57].

instance, Meyers et al. [56] argued that changes in the ratio of membrane surface area to cell volume can lead to differences in the signaling of membrane-bound signaling molecules. Using reaction–diffusion systems to simulate internal signaling molecules including Rho GTPases and Phosphatidylinositols in a 3D model of a cell, it was shown that cell shape can affect the response of the cell even to a purely 1D stimulus [57]. For instance, it is shown in Fig. 2 that cells which are thin in the center can maintain polarization whereas otherwise identical symmetric, cylindrical cells lose their polarization. This is due to the fact that the thinning prevents effective diffusion-mediated communication of signals between the two halves of the cell. Likewise, cell migration in vivo is influenced by 3D geometry effects even when the cells migrate on 2D surfaces, as these surfaces are typically not flat but curved, such as the lumens of endothelial cells or in the ECM, and this surface geometry can have a crucial effect on spreading and migration [58].

Cell geometry also characterizes different modes of migration. On the one hand, amoeboid cells, which are typically roundish, are known to migrate fast and adapt quickly to changes in stimuli directions [59]. On the other hand, mesenchymal cells, which are typically more elongated, are slow and migrate persistently through 3D matrices [60,61]. An evolving body of experimental studies explains differences in amoeboid and mesenchymal migration in terms of changes in biochemical signaling such as altered expression of matrix metalloproteinases (MMPs) [62] or Rho and ROCK [63], as well as changes in the mechanical environment due to shear stress [64]. Furthermore, confinement was shown to induce a transition from mesenchymal to amoeboid migration under conditions of low adhesion, and two modes of amoeboid migrations emerged [65]. The faster, more persistent mode of those two depends on myosin induced contractility and results in more elongated cell bodies than the slower of the two modes. Compatible with the above observations, theoretical work indicates that roundish cells adapt faster to new stimuli than elongated cells, even when their biochemical pathways are unaltered, and elongated cells can maintain their polarization against changes in stimuli direction [57].

These results are in line with a large number of recent results implicating an important role of cell shape in affecting cell behavior such as proliferation and apoptosis [66,67] or stem cell differentiation [2,68]. Interestingly, the signaling pathways leading to those different phenotypes also involve many of the key molecules regulating cell migration, such as Rho and its downstream effectors [69].

3 Extracellular Mechanical Cues

The previous section (Sec. 2) discussed the canonical pathways associated with cell polarization and migration, which have been confirmed mainly in 2D studies. Recent work in more physiological, 3D environments demonstrates that these pathways may be altered and migration mechanisms may be highly context dependent. In particular, mechanical features of the environment appear

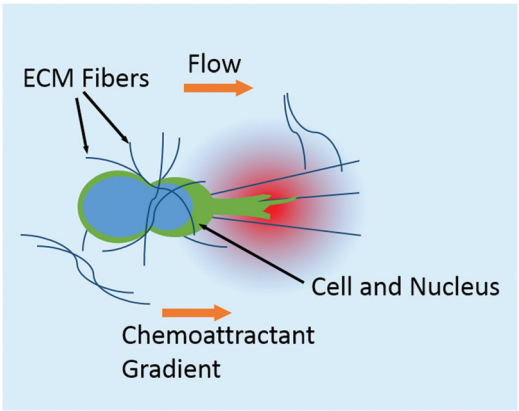

Fig. 3 Schematic of various signals in the 3D microenvironment. A cell migrating in physiological environments may be subject to numerous cues, including small pores that require deformation of the nucleus, aligned ECM fibers, interstitial flow through the porous ECM, and gradients of chemotactic factors (gradient profile).

to modulate cell migration in prominent ways and noncanonical mechanisms emerge. A schematic of various migratory signals in 3D matrices is illustrated in Fig. 3.

3.1 Dimensionality of the Environment and Mechanical Architecture of the ECM. A vast majority of studies on cell migration has been on 2D substrates, where model cells typically form well-defined structures including filopodia, lamellipodia, stress fibers, and focal adhesions. There is also distinct front to back asymmetry, where actin polymerization drives leading edge protrusion and myosin II contractility drives retraction of the cell rear [4,70]. Cells embedded in 3D ECM, however, appear to display morphological phenotypes distinct from 2D, as stress fibers and focal adhesions are less prominent [71,72]. Signaling and roles of adhesions are altered in 3D, and focal adhesion proteins modulate cell migration speeds via protrusion dynamics and matrix deformations [71]. Intracellular fluctuations driven by molecular motors are also altered in 3D due to a change in the cytoskeletal geometry [73]. Micropatterning and microfluidic methods have shown that dimensional cues and modulation can guide cell migration. On micropatterns interfacing 1D (thin adhesion sites $\sim 10\,\mu$m) and 2D (wide adhesion sites $\sim 50\,\mu$m) features, cells preferentially stayed on 2D regions in a myosin II-dependent manner [74]. In microfluidic paths, migrating cells preferred wider channels compared to subnucleus-scaled constrictions in a myosin II and path alignment-dependent manner [75].

In addition, the 3D physiological environment is highly complex, typically involving dense fibrillar matrices composed of proteins such as collagen and fibrin. The fibrillar architecture enables new physiologically relevant ways to interface with migrating cells. For instance, cells can migrate along matrix fibers. Along thin fibronectin lines mimicking 1D ECM fibers, cells have been shown to migrate at higher velocities than cells on uniformly coated 2D substrates and, with diminished velocities after disrupting myosin II and microtubules, in a manner comparable to cells embedded in 3D ECMs [76]. This has pathophysiological implications, as certain tumor microenvironments have increased alignment of matrix fibers [77,78]. Tumor invasion experiments in aligned 3D matrices show a strong preference of cells migrating along the aligned axis [52]. Tumors can align collagen through Rho kinase-mediated contractility, while this pathway becomes less prominent in driving tumor invasion in pre-aligned matrices [52]. Additionally, the fibrillar architecture of the ECM is conducive to long range force transmission by contractile cells [79,80], potentially leading to mechanotransduction in distal cells [81,82] and modulating binding kinetics of matrix proteins such as fibronectin [83,84].

The ECM can be proteolytically degraded via MMPs, which can lead to the generation of cell-scaled tracks [85]. Migration in intramatrix tracks has been shown to be enhanced, as in 1D migration in microchannels [86]. Cell phenotypes also appear distinct when migrating in tracks rather than through randomly oriented ECM networks [87,88]. For instance, cells are highly motile in tracks independent of collagen concentration, and actin- and myosin-targeting drugs more effectively reduce motile cell populations in random matrices than in tracks. In the absence of proteolytic activity, cells undergo drastic deformations in order to squeeze through small ECM pores in 3D matrices. This occurs in a Rho kinase-mediated, actomyosin, and β_1 integrin-dependent manner in which the ECM and cell nuclei are deformed via cell-generated forces [89,90]. The cell nucleus is the largest organelle in the cell and is stiffer than the cytoskeleton. In order for cell translocation to occur through tight spaces, the nucleus must deform substantially, which may rate limit invasion speeds [91–93]. Deformation of the nucleus has been shown to occur in collagen matrices, in highly confined microfluidic channels, and during transendothelial invasion [94]. During the squeezing process, it is shown that various mechanical strategies are employed. As the cell senses a constricted region in a subnucleus-scaled microchannel, it undergoes transition dynamics that can include initial slowdown, contraction, back extensions to deform the nucleus, and rotations of the nucleus as it squeezes through the physical barrier [93]. Extensions and protrusions are typically associated with actin polymerization activity [42,95], while contractions are typically affiliated with myosin II [96]. The actin cytoskeleton is linked to the nucleus via the LINC (linker of nucleoskeleton and cytoskeleton) complex, possibly enabling cell extensions and contractions to transmit force directly to the nucleus [97,98]. Furthermore, rotations of the nucleus have been shown to be regulated by dynein [99] and proteins of the LINC complex [100,101]. Further investigations are necessary to probe how different mechanical invasion strategies can be modulated via molecular targeting.

Interestingly, in confined environments, microtubules appear to play a prominent role in driving cell migration [93,102]. Stabilization of microtubules via paclitaxel reduces migratory persistence and abolishes the capability of cells to maneuver through tight constrictions, suggesting that persistent force generation is required to deform cells into contorted shapes. Furthermore, microtubules themselves might have propulsive capabilities in advancing cells through highly constrictive microchannels, while disruption of myosin, Rho, β_1 integrins, and actin does not fully suppress migration [102]. Recent work also shows that water permeation across the cell membrane can drive directional migration under confinement [103]. These findings suggest that canonical Rho GTPase signaling pathways discussed previously and studied heavily in 2D migration may not fully or accurately explain migration in 3D and 1D environments.

It is noteworthy, however, that in vivo environments are diverse, and 1D, 2D, and 3D model systems may each be relevant in different contexts. For example, nerve fibers are typically long and thin with high longitudinal-to-transverse aspect ratios in protrusion geometry and thus may be effectively modeled as 1D [104]. Additionally, cancer cells migrating along aligned tumor ECM fibers [52] or in proteolytically degraded ECM tracks [87,105] may also be better captured by 1D geometries. Endothelial cells and many types of epithelial cells typically form hollow tubes, which consist of a 2D planar geometry folded into a cylinder, and wound healing can be mimicked by 2D cell monolayers [106,107]. 3D geometries, in which cells are fully embedded in a hydrogel, are often more suitable for modeling cells migrating through interstitial space, e.g., invasion of individual cancer cells in the tumor stroma [108,109] or migration of immune cells in tissue [110].

3.2 Stiffness and Crosslinking. Substrate stiffness has been shown to guide stem cell differentiation in a manner that depends critically on nonmuscle myosin II activity [2], and stiffness gradients can induce directional migration via focal adhesion kinase-dependent durotaxis [9]. Furthermore, in 2D, substrate stiffness can drive malignant transformation in breast epithelial cells via integrin-mediated signaling and force generation [1]. In 3D, the role of stiffness is less clear, particularly due to coupled mechanical properties including pore size, ligand density, and matrix architecture. Recent studies developed ways of increasing matrix stiffness via crosslinking mechanisms, including glycation via ribose [111], adding an interpenetrating alginate network [112], and using transglutaminase [113]. Crosslinking via transglutaminase has been shown to be a characteristic feature in some cancers [114]. Lysyl oxidase-mediated crosslinking of collagen enhances cell invasion in 3D and tumor progression in vivo [115]. Crosslinking of fibrillar matrices can increase stiffness and also alters other properties such as strain stiffening, plasticity, and hysteresis [116]. Stiffer matrices may increase the difficulty for cells to deform the local pores, requiring larger cell deformations for MMP-independent translocation. While stiffness remains a fundamental property of any material, additional mechanical features need to be considered in 3D physiological environments in order to conclude that stiffness is the fundamental causal factor in any 3D cell phenomena.

3.3 Flow. Flow is a common feature in many physiological environments, from blood and lymphatic vessels to interstitial matrices and leaky tumor vasculatures [117]. For endothelial cells in 2D, shear flow has been shown to align cells, inducing stress fibers to form and the cells to elongate in the direction of flow, and altering the anisotropy of intracellular rheological properties [118,119]. Many sensing mechanisms exist, including integrins that cluster and receptor tyrosine kinases and G proteins that are activated under shear stress [119]. In 3D, interstitial flow can drive autologous chemotaxis in cancer cells, in which cell-secreted chemoattractant profiles of CCR7 ligands are biased by flow [120,121], and activate mechanotransduction and rheotaxis, leading to asymmetric distribution of cytoskeletal proteins, such as actin and adhesion complexes, and upstream migration [122]. Transmural and luminal flow in blood vessels can lead to endothelial cell sprouting into the ECM, dependent on a threshold shear stress and MMP upregulation [123]. Flow conditioning of microvascular networks also appears to decrease endothelial permeability and extravasation of tumor cells [124]. The physiological environment is conducive to fluid flow because of its porous nature or vessel networks, high fluid content, and actuating mechanisms such as heart beats and pressure gradients. This renders flow an important consideration in in vivo migration phenomena, particularly in metastasis, vascular formation, and diseases.

4 Conclusion

Cell migration is governed by a host of mechanochemical signaling mechanisms, including intracellular Rho GTPases that modulate actin and myosin activity, extracellular growth factor signaling that polarizes intracellular concentration profiles, interstitial, and shear flows that bias chemoattractant profiles and apply mechanotransductive stresses, ECM mechanical properties that modulate cell force generation, migratory persistence and speed, and cell–cell mechanocommunication. How all of these signals and mechanisms integrate in vivo to produce complex cell behavior is still largely unknown. Integrated computational models that take into account biochemical signaling, cytoskeletal mechanics and dynamics, and 3D ECM mechanics can help elucidate a more comprehensive and rigorous picture of the cell migration machinery. Recent modeling approaches of cell signaling and mechanics at multiple scales are reviewed in Refs. [84,125]. In particular, in vivo conditions often have a combination of biochemical and mechanical cues, but competing or synergistic effects from simultaneous cues are not well characterized. Combining models with experiments that explore these complex signals and responses can further reveal physiologically relevant cell migration phenomena.

Recent microfluidic experimental platforms have enabled the simultaneous generation of multiple cues to investigate the impact of multiple extracellular signals. For instance, stiffness gradients can be generated orthogonal to chemical gradients [126], or competing chemical gradients can be generated to gauge cell preferences [127]. Competing mechanical cues can also be generated, for instance dimensionality versus directionality of confined cell paths [75]. Computational models of cell movements are beginning to integrate multiple migration cues [128–130]. For instance in Ref. [129], bias from interstitial flow is incorporated with stiffness sensing of the environment. Future studies that controllably measure the relative sensitivity of cells to different mechanochemical cues can help guide the development of computational models that simulate and attempt to predict cell behavior in in vivo scenarios, which may have more complex boundary conditions, gradient profiles, or signaling content.

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