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Cell migration in 3D matrix

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The ability of cells to migrate within the extracellular matrix and to remodel it depends as much on the physical and biochemical characteristics of a particular matrix as on cellular properties. Analyzing the different modes of migration of cells in matrices, and how cells switch between these modes, is vital for understanding a variety of physiological and pathological processes. Recent work provides new insights, but also raises some debates about the mechanisms and regulation of cell migration in three-dimensional matrices.

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Introduction

The mechanisms and regulation of cell migration have been studied extensively in two-dimensional (2D) cell culture models. However, discrepancies between the behavior of cells in culture and *in vivo* have led growing numbers of research groups to switch to three-dimensional (3D) models, which better represent the microenvironment of living tissues. In addition, the desire to look within living organisms has led to the development of advanced real-time *in vivo* imaging technologies that reveal some novel processes, but not without limitations. Although some would argue that nothing rivals viewing the real thing *in vivo*, the necessity of defining mechanisms by isolating and defining specific contributions of single factors to the overall process makes *in vitro* models indispensable.

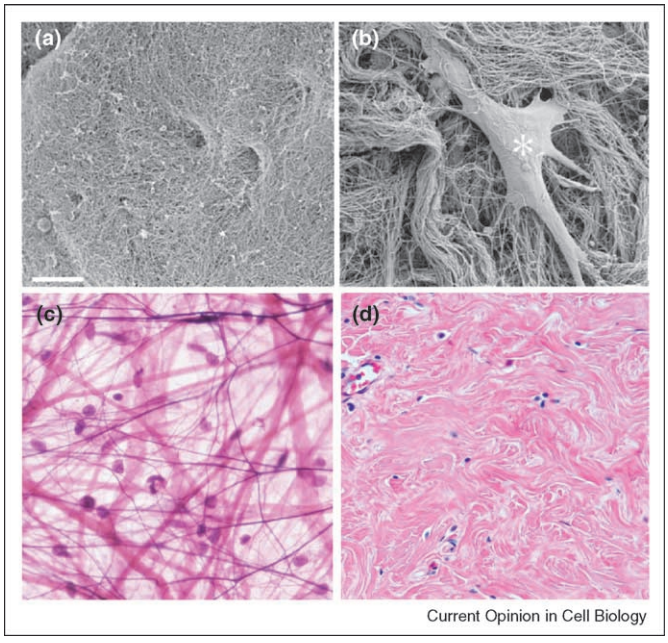
Cells *in vivo* migrate through three major types of extracellular matrix (ECM): dense connective tissue (Figure 1b,d), loose connective tissue (Figure 1c) or tightly packed basement membrane organized as a thin, dense acellular layer (Figure 1a) [1]. 3D models that attempt to mimic these environments have many variations that can affect the ability of a cell to migrate and its mode of migration. Previous reviews have summarized extensive work on the importance of polarity and the basement membrane for epithelial cell function [2], as well as the use of 3D collagen gels to study fibroblasts in pure collagenous environments [3,4]. We will focus on recent advances and some unanswered questions.

Dimensionality, signaling and directionality of migration

Ligands on 2D surfaces are normally evenly distributed at relatively low concentrations per unit area, whereas 3D matrix models often consist of fibrillar, tightly packed clusters of ligands. In cells on 2D substrates, lamellipodia or filopodia carry out exploratory activity at the leading edge, while stronger adhesion to the substrate and actomyosin contractility in the lamella contribute to forward movement of the cell. One question is whether the lamellipodium really exists in 3D environments. Wang and colleagues used fluorescence and time-lapse microscopy to show that under conditions where both dorsal and ventral ECM support is provided to migrating fibroblasts in place of the artificial dorsal–ventral polarity associated with 2D substrates, the cells acquire a bipolar elongated morphology that resembles fibroblasts *in vivo*. They report that these cells lack lamellipodia [5]. However, an earlier study using electron microscopy and computer-assisted reconstruction showed that even though fibroblasts in collagen gels indeed exhibit *in vivo*-like cylindrical cell bodies and major pseudopodial processes, the leading edges of advancing pseudopodia do have small, flat extensions similar to the lamellipodia seen in cells on glass [6]. This finding suggests that exploratory activity in 3D environments may involve a structure of the same type, although less exaggerated in appearance. This situation is typical: because of the very thin, elongated morphology of cells in 3D, many cellular structures can appear less obvious than familiar structures in flattened cells on 2D substrates. There is a need for better optical resolution in time-lapse microscopy to visualize such morphological details during migration in 3D.

Moderately high resolution *in vivo* imaging by two-photon microscopy has revealed yet another interesting function: exploratory motile activity by processes of presumably resting cells. Microglia in the brain are immune cells that perform surveillance by continuously sampling their environment using extremely motile, thin processes and protrusions. Disruption of the blood–brain barrier provokes immediate activation of microglia, switching their behavior from patrolling via filopodia to shielding the injured site [7••].

Figure 1



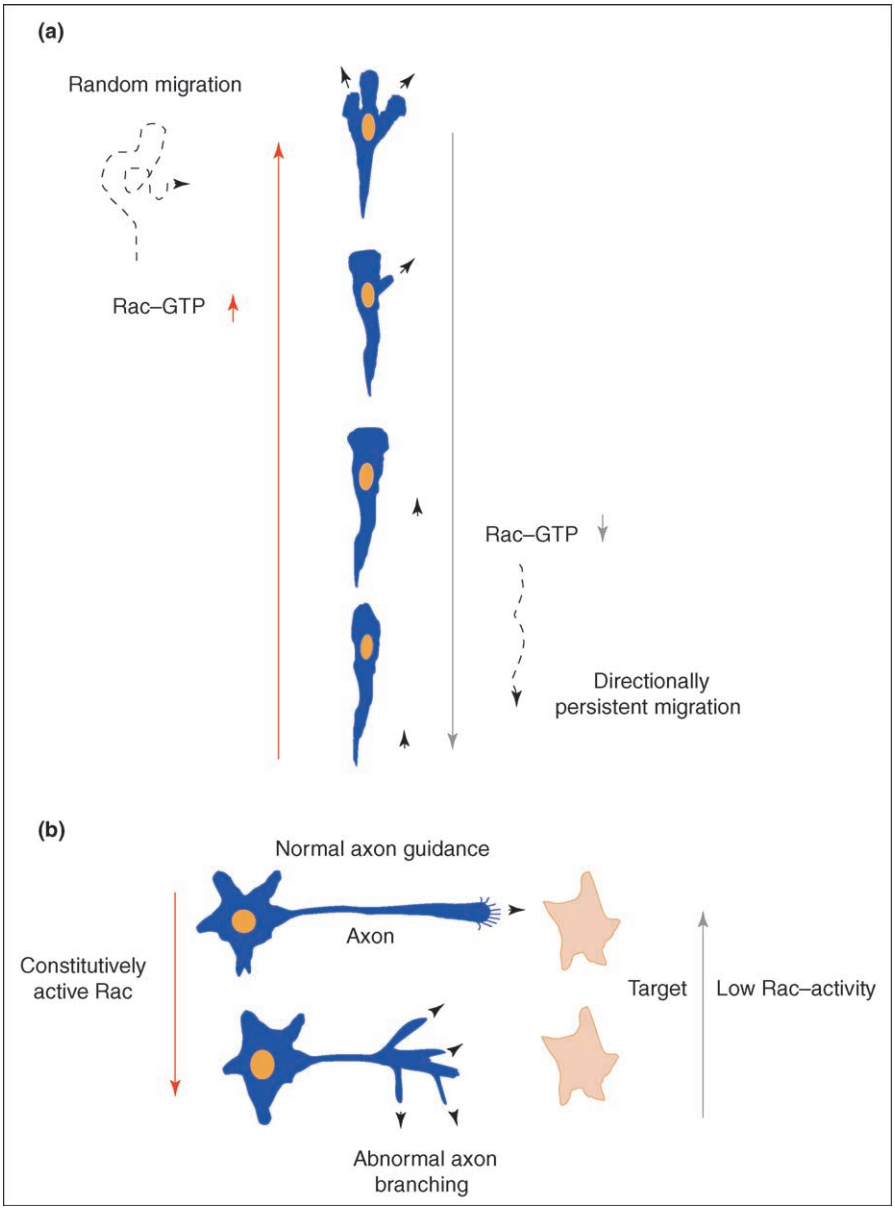
In vivo ECM architecture. Visualization of the dense collagen meshwork of **(a)** the basement membrane and **(b)** the dermis. After enzymatic removal of the epidermis, the basement membrane, adherent to the dermis, was visualized by scanning electron microscopy. The different packing densities and patterns of the collagen meshwork in the basement membrane (a) and the dermis (b) are evident in these pictures. Note that in the dermis a fibroblast (asterisk) is visible in the collagen meshwork. Scale bar: 10 μm. Images by P Stoitzner [1]. **(c)** Loose areolar connective tissue of mesentery (stained for elastin, x40), showing collagen fibers, elastic fibers and fibroblasts. Note the irregular orientation of the fibers and the large spaces in between. **(d)** Irregular dense connective tissue of nipple skin (hematoxylin and eosin staining, x20). Collagen fibers are oriented irregularly but are densely packed. Images modified from [47•].

3D environments provide surrounding support for cells *in vivo*. For epithelial cells, this allows organization of cells into polarized epithelia and higher-order structures such as acini. For fibroblasts, it corrects the artificially imposed dorsal–ventral polarization of cells plated on 2D substrates, where adhesions are only formed at the ventral surface. Cells in 3D exhibit matrix adhesions all over their surface. The matrix not only physically supports cells, but also provides additional information, which can induce certain differentiation pathways. A recent analysis of fibroblasts from tumor-associated stroma reveals that they assemble a highly oriented 3D matrix *in vitro* that resembles tumor stroma and differs markedly from normal fibroblast-derived matrix [8]. The matrices deposited by tumor-associated fibroblasts can induce morphological and gene-expression changes in normal fibroblasts that are characteristic of tumor-associated fibroblasts. This work also provides a nice example of contact guidance, where the well-oriented organization of tumor fibroblast matrices clearly directs the alignment of cells parallel to the fibers.

Small GTPases have gained increased prominence as regulators of the directionality of migration. It is well-established that Rac1 is required for cell spreading and lamellar ruffling, whereas RhoA is required for intracellular contractility and tension [9]. Cells on 2D surfaces often spontaneously change their direction of migration; these changes are associated with relatively high Rac activity. Cells in a 3D matrix are much more persistent in direction and show lower levels of Rac activity. Reducing the levels of active Rac in fibroblasts or epithelial cells migrating on 2D surfaces results in similar directional persistence of migration [10] (Figure 2a). An optimal level of Rac activity appears to be important for directional migration in neuronal guidance. For example, studies in *C. elegans* show that neuron-specific expression of constitutively active Rac results in formation of ectopic lamellipodia and filopodia and excessive axon branching *in vivo* (Figure 2b). Normal axon guidance involves interaction of Rac with semaphorin–plexin complexes; the cytoplasmic tail of plexin binds directly to Rac–GTP and inhibits Rac activity by sequestering Rac–GTP away from its effector, p21-activated kinase (PAK) [11]. This finding is consistent with the idea that lower Rac levels promote directionality.

Another factor affecting cells in 3D matrices is that these substrates are more pliable than 2D substrates; cells have the ability to sense areas of increased stiffness and move preferentially towards them (durotaxis) [12]. Changes in substrate compliance alter the distribution of integrins

Figure 2



Small GTPases and directionality. **(a)** Increased levels of GTP–Rac confer random migration by inducing formation of peripheral lamellar protrusions. These protrusions initiate turns of the cells in other directions. By contrast, lower levels of active Rac induce persistent directional migration by maintaining a stable, single lamella in the direction of migration. **(b)** A neuronal-specific example is the interruption of axon guidance by abnormally extensive branching upon expression of constitutively active Rac.

and several related signaling molecules; integrins display conformational activation in response to rigidity [13]. The focal adhesion proteins talin, paxillin and $p130^{cas}$ were downregulated in cells on gels by a mechanism that requires functional $\alpha_2\beta_1$ [14]. Interestingly, $\alpha_V\beta_3$ was not detected in 3D-matrix adhesions of fibroblasts [15], but it is a central constituent of focal adhesions on 2D substrates or in stiff matrices. Focal adhesion kinase (FAK) was also found to be less phosphorylated at residue Y397 in fibroblasts in a 3D matrix than on a 2D substrate [15] and in breast epithelial cells on highly compliant substrates, but phosphorylation levels were elevated with increasing rigidity [16]. These findings underscore the importance of the physical properties of the 3D matrix in signaling.

Migration modes in 3D environments

Different cell types employ different mechanisms to migrate into and within the ECM. Single migrating cells can either be native tissue residents or circulating cells

that have infiltrated the tissue. Permanent residents usually express a specific integrin profile dependent on the type of ECM, but they can alter their integrin expression pattern and activation state as they change location. Infiltrating cells, on the other hand, have few active integrin adhesion receptors while in the circulation but show progressive integrin changes as they migrate through different tissues and ECMs [17,18].

Tissue fibroblasts are quiescent cells that will migrate in response to either mechanical or chemical cues. Such mesenchymal cell migration is characterized by specific steps: exploration by the leading edge, attachment, maturation of adhesions, advancement of the cell body, and release of adhesions to pull the rear forward. In this relatively slow crawling mode, cells migrate at ~0.5–1 micron/minute in a cell-derived 3D matrix [15]. Immune system cells can migrate 10–40 times faster [19]. In rapidly moving cells, adhesion formation at the front and release at the rear occur more quickly than in naturally adherent cells. Leukocytes tend to polarize, forming a leading edge and a trailing uropod [20]. Originally, leukocytes were thought to advance in an integrin-independent manner. However, subsequent studies indicated that integrins play a crucial role in leukocyte migration. The orchestrated expression of different integrins concomitant with the changes in the ECM along the migration route is crucial for the proper functioning of leukocytes throughout migration and tissue colonization. β_2 integrins seem to be involved in extravasation, whereas β_1 integrins function at a later stage [21,22]. Slow, crawling migration is β_1-integrin-dependent [19]. Reduction of β_1 integrin clustering stimulates migration [23], probably by ablating strong adhesions such as $\alpha_5\beta_1$ matrix adhesions.

Proteolytic versus non-proteolytic migration

A current debate in the field concerns whether cells are capable of migrating through 3D ECMs without using proteolytic degradation. Despite extensive evidence that migrating cells, particularly tumor cells, express various proteases that are able to degrade ECM and basement membrane components, it is not clear whether migration in a 3D environment absolutely depends on proteolytic events. Although multiple proteases have collagenolytic activity, the most studied and debated are the metalloproteinase family members. Both secreted and membrane-type matrix metalloproteinases (MMPs) have been reported to affect normal and cancer cell migration. However, it is still unclear whether human epithelial tumors actually express MT1-MMP (membrane-type-1 MMP), since normal epithelial cells rarely, if ever, express MT1-MMP *in vivo* even though many cells express it artificially when cultured *in vitro* [24]. Highly aggressive tumor cells were found to express MT1-MMP *in vivo*, predominantly at the tumor–stroma border, with functionally active MMP-2 restricted to this invasive front [25]. Despite the limited expression of MT1-MMP, it was able to elicit proteolytic activity by surrounding stromal cells [26], which contributed to ECM remodeling.

Weiss and colleagues recently demonstrated that migrating tumor cells and fibroblasts have pericellular collagenolytic activity that allows them to traverse the ECM, and that MT1-MMP serves as the major cell-associated protease that confers normal or neoplastic invasive activity [27••]. Surprisingly, MMP-2 and MMP-9 were not required for migration through 3D collagen gels or dermis explants. MT1-MMP deficiency also impaired sprouting and collagen degradation by endothelial cells [28•]. The idea that MMP-9 is dispensable for migration appears to conflict with results from MMP-$9^{-/-}$ smooth muscle cells, which exhibited decreased migration and contraction of collagen gels [29]. It has also been shown that in MMP-9-deficient mice, Langerhans cell migration from skin explants is strikingly reduced [30••] and cerebellar granule cell migration is defective [31•]. A possible explanation for this discrepancy may be cell-type specificity: smooth muscle, Langerhans and granule cells *in vivo* may not express MMPs other than MMP-9, whereas MMP-$9^{-/-}$ fibroblasts do.

The major debate, however, is not just about which protease is required for migration through the ECM, but concerns whether proteases are required at all. Disappointing results from clinical trials of matrix protease inhibitors as anti-cancer drugs — some inhibitors even reduced patient survival — have challenged the scientific community to re-evaluate the role of proteases in cell migration and invasion. Two groups proposed a new concept: that tumor cells use a plasticity mechanism that enables them to maintain migration in a proteolysis-independent manner. Friedl and colleagues used both an *in vitro* 3D collagen gel model and intravital microscopy to show that tumor cells maintain their ability to migrate after chemical inhibition of proteases [32••]. They propose that cells can continue to migrate by switching their mode of migration: instead of using an integrin-dependent mesenchymal type of migration along matrix fibers, they adopt an ameboid type of migration similar to what is seen in migrating leukocytes. This switching could explain the intriguing resistance of tumor cells to the effects of protease-inhibiting drugs. A study by another group tested four different cells lines that initially exhibited different morphologies [33]. They, too, showed that the motility of rounded cells does not require proteolytic activity. Instead, it was associated with active Rho and ROCK. The hypothesis is that Rho-induced contractility generates hydrostatic pressure to form protrusions and facilitate motility. Elongated cells, however, were more sensitive to protease inhibition, suggesting that although some tumor cells have the ability to switch modes upon protease inhibition, others cannot. Morphological changes were observed by yet another group who examined tumor cell migration in collagen gels for

extended periods of time [34•]. They described a multistep mode of migration with alternating stationary and migratory events and showed a decrease in stationary periods and faster pseudopodia formation following stimulation, but disagreed with the concept of mode switching. They too, reported that an MMP inhibitor did not affect migration, but that, unlike in the previous studies, a cocktail of protease inhibitors did suppress migration.

Discrepancies or debates such as these may stem from technical or conceptual factors. Similar models can have small but significant differences in matrix composition, organization or testing procedures. For example, a cross-linked 3D collagen gel may prevent protease-dependent migration, whereas non-cross-linked collagen may not (compare [27••] and [32••]). Also important, however, may be conceptual issues: ECM is a very broad term that covers many different forms of acellular material in connective tissues and basement membranes. Thus, the question whether a given cell can migrate in a proteolysis-independent manner must be presented in the relevant tissue context. Loose (areolar) connective tissue, composed of random collagen and elastic fibers, is probably much more permissive to cell migration, and the presence of abundant fibroblasts, macrophages, plasma cells and clusters of lymphocytes in this tissue may support this idea (Figure 1c). On the other hand, irregular dense connective tissue such as in the dermis typically contains sparse quiescent fibroblasts aligned with the collagen fibers but does not contain as many other cells (Figure 1d). The tightly packed collagen fibers may impede the passage of cells unless the collagen can be degraded.

Crucial technical points

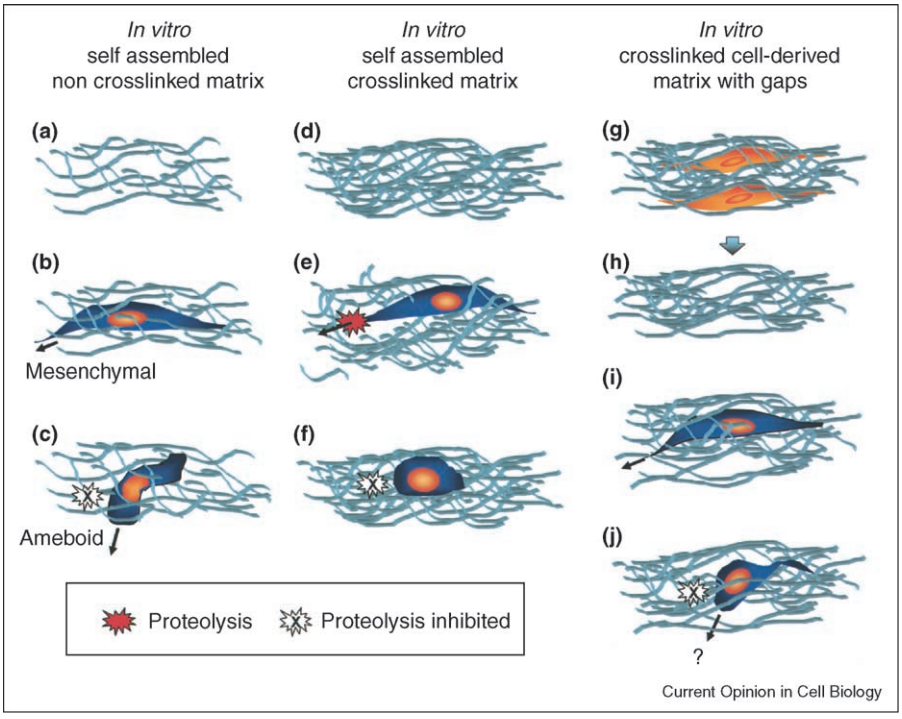
3D models have obvious advantages over 2D cultures in mimicking *in vivo* conditions, and they allow us to study specific factors under more physiological conditions with respect to dimensionality, architecture and cell polarity. At the same time, however, the complexity and diversity of *in vivo* ECM organization and molecular composition cannot be easily mimicked *in vitro*. Although collagens are the dominant component of both *in vivo* and *in vitro* ECM, other ECM proteins are equally important. Tenascin, for example, is an ECM protein that has a restraining effect on migrating lymphocytes. It is present at sites where lymphocyte migration needs to halt, including lymphoid tissues and inflammation sites, and also tumors [35]. The interaction of $\alpha_5\beta_1$ with tenascin has a restraining effect on lymphocyte migration [36]. Fibronectin, on the other hand, promotes migration in 3D matrices. Addition of fibronectin to 3D collagen type I substrates markedly augments the migration of infiltrating T-cell lines into the matrix [37]. Fibroblasts also show enhanced migration in fibronectin-rich matrix compared to collagen gels [15].

The architecture of an ECM is crucial in studying the ability of cells to migrate within a 3D milieu. It is governed by molecular composition and concentration, macromolecular orientation, and the degree of cross-linking (Figures 3 and 4). Collagens and laminins have an intrinsic capacity to polymerize and form a 3D gel spontaneously, whereas fibronectin fibrillogenesis is an active, cell-dependent process involving $\alpha_5\beta_1$ translocation [38]. Collagen fibrils are stabilized *in vivo* by covalent cross-links. Enzymatic cross-linking is initiated by lysyl-oxidase on lysyl or hydroxy-lysyl residues at N- and C-terminal non-helical regions of the collagen molecule. Collagens I, II and III, the fibrillar collagens, align laterally to permit cross-linking. Collagen IV, the scaffold of basement membranes, forms an open lattice structure that undergoes extensive disulfide and lysine-derived cross-linking. Collagen cross-linking results in tighter packing, elevated melting temperature and marked resistance to proteolysis [39]. Consequently, pepsin-treated collagen (atelocollagen such as Vitrogen) that lacks the telopeptides that contain the cross-linking residues will polymerize to form a loose non-cross-linked substrate. From the point of view of a migrating cell, this matrix is likely to be much more permissive for penetration than an *in vivo* cross-linked collagenous ECM (Figure 3); hence, assessment of migration and invasion capabilities in such 3D gels may not be fully representative of migration *in vivo*. The second factor is the concentration of an ECM. *In vitro* preparations of collagen are limited by the maximal concentration of the stock solution, typically 3–5 mg/ml. Yet the collagen content of a normal epidermis is ~70% of the dry weight, in other words 700 mg of collagen per 1 g dry tissue [40] or about 140 mg/ml collagen in wet weight, which is nearly 100 times the concentration normally used *in vitro*.

Similarly, the characteristics of basement membrane extracts should also be carefully considered when using them as a matrix model (Figure 4). The tumor basement membrane extract produced by Engelbrecht-Holm-Swarm sarcoma cells (Matrigel) differs from normal basement membranes *in vivo*: it is significantly less cross-linked and is therefore more susceptible to proteolysis, remodeling and turnover [41], and it also contains substantial quantities of growth factors. Basement membranes are tightly woven, flat substrates that, we suggest, may be perceived locally as 2D by epithelial cells, which polarize and establish contact only at their basal surface on these flat surfaces. By contrast, Matrigel forms thick, loosely cross-linked gels. Although it can promote 3D tissue organization, Matrigel does not present cells with a 2D structure analogous to basement membrane *in vivo*.

Fibrin gels are also commonly used substrates for fibroblast and tumor cell migration studies. Thrombin cleavage of fibrinogen will generate fibrin, which self-assembles to form a tight meshwork of fibers. Plasma concentrations of fibrinogen are 25–30 mg/ml, but a typical fibrin gel only contains 0.3–2 mg/ml. To migrate into

Figure 3



Differences in matrix architecture govern the ability of cells to migrate through ECM, with or without the assistance of proteolytic degradation. **(a)** Collagen ECM models prepared from pepsin-treated collagen (atelocollagen) are non-cross-linked. Spaces between the fibers depend on concentration. **(b)** Cells show typical mesenchymal migration along the fibers. **(c)** In the presence of protease inhibitors, the cells switch to an ameboid migration mode and squeeze through the spaces between fibers. **(d)** Cross-linked ECM limits cell migration as a result of the small spaces between fibers. **(e)** Degradation of the matrix is required for cell migration. **(f)** Protease inhibition causes cell rounding but migration cannot proceed. **(g,h)** Cell-derived matrices maintain some of the gaps that originally accommodated cells. **(i)** The gaps are detected by migrating cells and permit the mesenchymal type of migration within the matrix, although it is not yet clear whether inhibition of proteolysis will stop migration **(j)**.

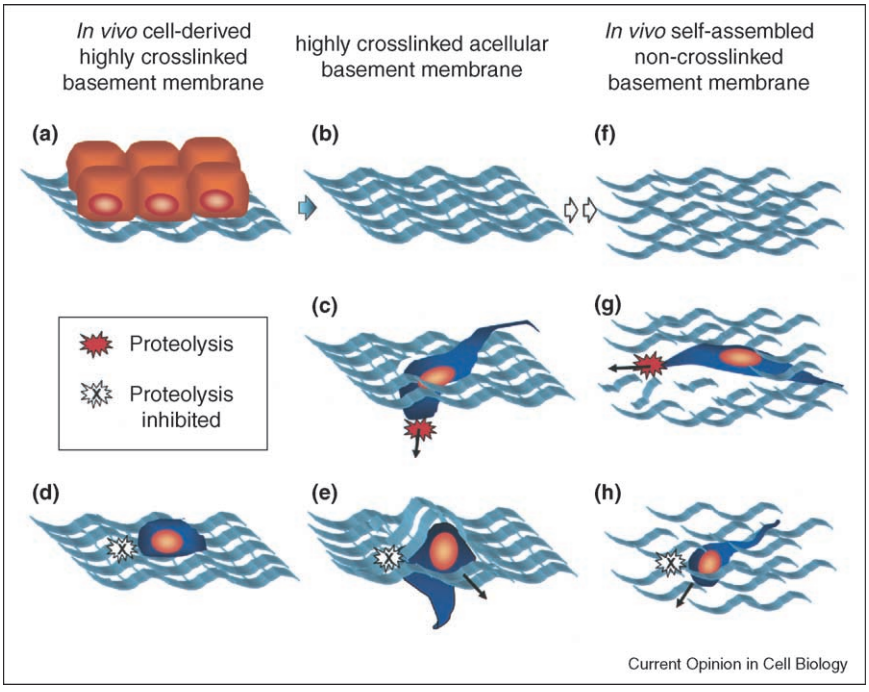
or within a fibrin clot, cells must be capable of degrading the tight barrier.

Another concern is the omission of minor constituents that are significant in determining architecture or in facilitating adhesion or repulsion. The importance of the dermatan sulfate-CD44 proteoglycan in the migration of fibroblasts from collagen to fibrin/fibronectin clots was demonstrated [42]. Older work [43] showed by scanning electron microscopy how the addition of fibronectin, laminin or heparin alters meshwork structure, spacing and neutrophil motility within the ECM.

Self-polymerizing ECM models are convenient to use, but they also provide a uniform matrix that lacks discontinuities and spaces that may normally be present *in vivo* (Figure 3). For example, matrices through which leading cells have migrated during embryonic development or that have been invaded by tumor cells are likely to have spaces that are usable by other cells that follow. These spaces would be detected by the probing activity of a migrating cell, and their presence might affect cell behavior, for example enabling the cell to invade without using proteases. By contrast, basement membranes are formed *in vivo* as dense, interconnected, acellular lattices and seem to be barriers to migrating cells lacking proteolytic activity (Figure 4).

In vivo models, with their physiological components and structures, are theoretically ideal for examining concepts of migration. However, they can have built-in disadvantages. First, the depth of imaging of fine structures, such as putative lamellipodia, only allows a working distance of several hundred microns, even with advanced two-photon microscopy. For clear visualization, native cells often need to be labeled by fluorophores (or express GFP-chimeras), or alternatively, fluorescent protein-labeled or dye-loaded cells need to be added ectopically. Yet isolating cells for labeling can quickly change their phenotype in terms of adhesion, tension, signaling and morphology. To study cell interactions with surrounding tissue, ECM components also need to be labeled; alternatively they may be detected by their inherent light scattering, but this does not allow imaging of the finest fibers. Second, opening a window to look into living tissue involves wounding the animal and the resultant inflammatory processes may change the microenvironment,

Figure 4



Migration through basement membrane. **(a)** The basement membrane type of ECM is deposited by cells in a polarized manner and is dense and highly cross-linked. **(b)** The basement membrane is acellular and densely packed. **(c)** Cells with proteolytic capability can degrade and traverse this barrier. **(d)** Protease inhibitors cause a switch to a rounded-ameboid morphology, but this change does not allow cells to squeeze in between the tightly packed fibrils. **(e)** Some cells may be able to mechanically disrupt the basement membrane locally to allow migration without degradation. **(f)** Extraction of a basement membrane type ECM and self-assembly into a gel disrupts the tight structure. **(g)** A migrating cell can degrade its way across the matrix, or alternatively, rounded-ameboid morphology induced by protease inhibitors may help cells squeeze between non-cross-linked fibrils and migrate through the basement membrane **(h)**, but if prepared at a high concentration, basement membrane extracts are impermeable to cells without proteolysis [48].

triggering, for example, protease activation, chemokine release or recruitment of immune cells. Such variables that might affect migration should not be ignored.

Another issue is whether the use of chemical protease inhibitors or of knockout cells or tissues is conceptually safer for determining definitive roles. A cocktail of inhibitors can cover numerous candidate molecules, whereas knockout cells can only eliminate a single gene or two at a time. Elimination of a gene product by knockout is definitive, but it can in turn produce a compensating response, such as increased expression of functionally overlapping family members. An increasingly popular alternative involves gene knockdown approaches using RNA interference, but 'off-target' effects are a potential concern with this approach. Typical chemical cocktails contain inhibitors for common proteases, such as serine-, cysteine- and metalloproteinases, but not for less abundant or less studied ones like ADAMs (a disintegrin and metalloproteinase) or heparanase. The effective concentrations of inhibitors can also vary between *in vitro* models and *in vivo* studies. While a single dose may be sufficient in culture, the dilution, clearance and bioavailability need to be considered for effective *in vivo* treatment.

Conclusions and future directions

Matrix organization and composition and the biological activity of each component are all likely to modulate both normal and pathological cell migration and invasion [44]. Differences in these properties may help to explain the different propensities of tissues to support tumors and the selective targeting of metastases to specific locations. The cellular and matrix density of a tissue, its susceptibility to degradation and release of matrix fragments and its ability to bind and sequester growth factors may all play an important role in tumorigenesis. Micro-heterogeneity in matrix composition and architecture may also shed light on the entry points and homing sites of immune cells and circulating stem cells.

3D ECM models together with advanced imaging techniques are contributing a great deal to our understanding of physiological and pathological motility and migratory processes in their correct context. 3D models of self-polymerizing ECM proteins, such as collagen or fibrin gels, allow us to isolate and study the contribution of single components to the process of cell migration and to define molecular mechanisms without compromising the effect of dimensionality. However, such 'clean' systems can have

limited relevance to cell migration *in vivo* for the very same reason: they oversimplify the complex microenvironment in terms of structure, content and cellular interactions. The route taken by a cancer cell from the moment of detachment from the tumor mass until it establishes a metastasis or the more limited and better-controlled migration of an immune-surveillance lymphocyte both involve crossing numerous tissues with differing densities of fibrillar material, amorphous substance and cells.

General issues, such as the importance of migration-mode switching and the functional dependency on specific proteases, should theoretically be tested in many different models, each representing a different phase and local microenvironment. There is a strong need for a range of such complex, physiologically relevant 3D models. Although not yet rigorously tested, it seems likely that different 3D matrices will induce different cellular responses and that progress will await the development of multiple sophisticated 3D models in the future.

Intravital imaging of living organisms will complement the data from 3D model systems. Using special settings to accommodate a live animal in a confocal/two-photon microscope reveals real-time events [45]. Nonetheless, tracking cells in a living organism by light microscopy is depth-limited by the working distance of available objectives and fluorescent labeling *in vivo* is also more challenging. Newly emerging developments using MRI and other imaging approaches may offer deep-tissue imaging with sufficient resolution. The development of labeled probes with greater sensitivity and stability would also provide the field with powerful tools to study migration processes *in vivo*.

A useful *in vitro* approach to obtaining tissue-like 3D architecture and composition has been to produce cell-derived 3D matrices and *in vitro*-produced tissue substitutes. They can be grown to a thin, microscopy-compatible thickness and can be labeled more easily than *in vivo* matrices, and hence permit high-resolution imaging in a 3D context. Unlike ECM gels, these models cannot be assembled from a bottle, and their production requires skilled and careful preparation, but they should gradually become commercially available [46]. Grown by different primary cells, these 3D ECM models will have complexity and diversity in their molecular and structural organization and should therefore better represent the wide range of *in vivo* matrices.

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- •• of outstanding interest

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