Fibroblast migration is essential to normal wound healing and pathological matrix deposition in fibrosis. This review summarizes our understanding of how fibroblasts navigate 2D and 3D extracellular matrices, how this behavior is influenced by the architecture and mechanical properties of the matrix, and how migration is integrated with the other principle functions of fibroblasts, including matrix deposition, contraction, and degradation.

Fibroblasts are cells of the mesenchyme, widely distributed throughout the organs of the human body, tasked primarily with secreting and maintaining the extracellular matrix. Despite their relatively homogeneous appearance, they can be characterized by systematic differences in gene expression patterns that correlate with their anatomical site of isolation (17, 112) and position along developmental anterior-posterior and proximal-distal axes, as well as their dermal vs. nondermal sites of origin (99). These findings suggest that fibroblasts take up their relative positions in the body during development and are confined to relatively local domains thereafter, although one cannot altogether exclude local influences that plastically alter fibroblast characteristics. The spatial differences in fibroblast gene expression function as a source of positional memory for neighboring epithelial cells, engaging in reciprocal interactions to ensure appropriate patterning during wound healing or to maintain homeostasis (100). In addition to resident fibroblasts, there is also evidence that cells with fibroblast-like characteristics can be derived from circulating cells (34) and from epithelial and endothelial sources (56, 118), particularly in disease or wound-healing contexts. However, there is strong evidence that locally derived resident mesenchymal cells are activated to proliferate and migrate during wound healing or fibrosis and that such locally derived cells play an important role in these processes (45, 48, 101, 118, 121). Thus understanding how fibroblasts navigate the extracellular matrix in their local tissue environment is a major question relevant to understanding injury responses, regenerative healing, and fibrosis.

In addition to spatial variations in gene expression, fibroblasts also exist along a differentiation continuum, and populations of cells likely include a variety of subclassifications. The best known of these is the myofibroblast, which is classically defined by the expression of the contractile protein α-smooth muscle actin (45). Definitive markers to positively identify and subclassify fibroblasts remain elusive, hence they are often defined by their absence of other definitive markers along with the presence of relatively nonspecific markers, such as vimentin and S100a4 (FSP-1) (114). More definitive insight into fibroblast heterogeneity and origin should come from lineage tracing studies (98), which are already identifying fibroblast subpopulations important in injury repair and fibrosis (18, 101).

Despite the variations in fibroblast subpopulations and the subtle differences in fibroblasts isolated from various organs and tissues, these cells exhibit many overriding similarities in appearance and function and are often studied interchangeably from across different tissues and sites of interest. Fibroblasts are easily isolated and grown in culture from many tissues, and the spontaneously immortalized 3T3 fibroblast cell line, originally derived from mouse embryo (120), is widely used in basic cell biology studies. Thus there is a long history of using cultured fibroblasts for routine investigation of cell and molecular biology, in particular their motile behaviors (e.g., Refs. 1, 2, 10, 41, 119, 123, 124). More recently, it has become apparent that the study of these cells in the artificial environment of the rigid 2D culture dish may strongly influence important behaviors of these cells and fail to capture some important aspects of how these cells behave within the tissues of the human body (27), echoing concepts first explored more than 30 years ago (9, 23, 40). This review highlights the differences in fibroblast function that emerge across extracellular matrices spanning simple (2D) to intact tissue matrices, using migration as widely studied and physiologically critical focus that illuminates the important interactions between the fibroblast and the extracellular matrix environment.

Fibroblast Migration in Two Dimensions

Although fibroblasts reside throughout many tissues of the human body, they still must be capable of motile function to fulfill their roles in tissue homeostasis and wound repair, traversing local
tissue environments as needed to degrade, repair, or remodel the extracellular matrix. In standard cell culture approaches, fibroblasts are grown on 2D surfaces, typically glass or plastic, with surfaces modified to encourage cell and protein attachment. Such an arrangement is optimal for a number of microscopic imaging techniques, allowing cells to be visualized and followed over time as they migrate, either spontaneously or in response to chemotactic gradients or other biochemical stimuli that perturb motility. However, such settings also impose nonphysiological constraints, restricting cell spreading and movement to an artificially flat 2D surface. Nevertheless, such systems proved instrumental in developing current concepts for understanding 2D cell motility. Briefly, fibroblast movement requires protrusion of cellular processes such as lamellelapodia or filopodia, adhesion to the underlying substrate, translocation of the cellular contents, and retraction of the cell at the trailing edge (62) (FIGURE 1). All of these steps rely on cell adhesion to the underlying matrix, principally through integrins, transmembrane proteins that bind specific epitopes on extracellular matrix proteins and cluster together in multiprotein adhesion complexes (29, 132). More than 100 additional proteins in the “integrin adhesome” facilitate signaling from such adhesions and connect the extracellular matrix to the intracellular cytoskeleton (29, 132). For this process to be integrated and result in net movement, the cell must exhibit polarity, or organization from front to back (97). Robust polarity results in persistent migration, while variations in polarity result in more random and less effective migration, despite high rates of instantaneous speed (90). Interestingly, migration rates are highly sensitive to adhesive strength (85), with an optimal adhesive environment supporting maximal migration, and too weak or too strong adhesive environments preventing formation of new adhesions or retraction of trailing adhesions, respectively. Thus it has been clear for some time that the rate of cell migration is not invariant but rather adapts to physical cues in the extracellular environment.

Substratum Mechanical Properties and 2D Migration

To move, the fibroblast must exert forces against the underlying matrix. Such physical transmission of force across the cell membrane was clearly demonstrated in early work using fibroblasts cultured on compliant silicone substrates, which revealed the ability of cells to mediate wrinkling of the surface of the underlying substrate (41). The scope and precision with which elastic cell culture substrates could be produced was expanded by the use of polyacrylamide hydrogels, which could be tuned over a large range of elastic modulus through variation in acrylamide and bis-acrylamide concentration (88). These methods helped to reveal the magnitude and localization of cell-generated forces present in migrating cells (10, 20, 89). But perhaps more importantly, they demonstrated that, not only do cells deform the substrate, but the adhesive structures of the cell are sensitive to the deformability of the substrate, resulting in vast differences in cell-matrix adhesion structure and dynamics, and alterations in cell morphology and cell migration as underlying substrate deformability changes (33, 66, 88) (FIGURE 2). Subsequent work showed that, not only do cells sense deformability of the underlying substrate, they also respond to spatial gradients in deformability in such a way that net cell migration is directed toward regions.

FIGURE 1. Schematic representation of the steps in 2D cell migration, including extension of a lamellapodium, formation of a new adhesion, translocation of the cell body, and de-adhesion and retraction at the trailing edge
Reproduced from Ref. 61 with permission from IOP Publishing.
of decreasing deformability (increasing elastic modulus), a process termed durotaxis (67). This behavior has subsequently been shown to depend critically on the steepness of the spatial gradient in matrix stiffness (50, 66). Recent studies have demonstrated that dynamic fluctuations in cell-matrix adhesion “tugging” forces are essential for sampling the local mechanical environment (93) and that phosphorylation and polarized function of nonmuscle myosin II is required for polarization and directed migration of cells across a stiffness gradient (94).

In addition to effects on migration, the mechanical properties of the substrate broadly alter the function of fibroblasts, including changes in the magnitude of traction forces generated (70), organization of α-smooth muscle actin into contractile stress fibers (33, 49) (FIGURE 2B), and the closely linked ability to activate TGF-β from a latent matrix-bound state (126), along with alterations in the overall transcriptional profile and factors secreted by the cells (65, 66). Increasing matrix stiffness also promotes fibroblast proliferation while limiting apoptosis (66), further amplifying the effect of durotaxis on the relative distribution of fibroblasts across a gradient in mechanical properties. All of these functional responses play out across a range of mechanical properties observed in normal and diseased tissues, including normal and fibrotic lung (12, 13, 66), liver (31), and intestinal (54) tissue, as well as in normal and malignant breast tissue (86), spanning shear modulus values from <100 Pa to >100 kPa. In addition to altering cell function, the changes in substrate mechanical properties also fundamentally alter cellular responses to biochemical perturbations (14, 70, 75, 76). Taken together, these observations suggest that tissue-specific differences in matrix mechanical properties may influence local fibroblast function and that pathological changes in matrix mechanical properties associated with impaired wound healing or fibrotic diseases may promote fibroblast activation, fibroproliferation, and net matrix accumulation (12, 49, 66, 133).

**FIGURE 2.** Changes in fibroblast morphology, adhesion, cytoskeleton, and motility as a function of underlying substrate stiffness

A: immunostaining of the focal adhesion protein vinculin in human lung fibroblasts cultured on collagen I-coated polyacrylamide gel surfaces of indicated shear modulus for 5 days showing gradual transition of focal adhesions from round (arrows) to elongated fibrillar shape (arrowheads). Bar = 10 μm. B: the cytoskeletal protein α-smooth muscle actin (α-SMA; green) is progressively incorporated into F-actin stress fibers (red), which themselves become increasingly distinct and organized as the underlying matrix stiffness increases (Liu F, Tschumperlin D, unpublished observations). Bar = 50 μm. C: fibroblast migration speed and persistence vary with underlying matrix stiffness, as measured with time-lapse video microscopy. Error bars indicate SD from 12 cells for each condition from two independent experiments. D: individual fibroblast migration tracks obtained from time-lapse video microscopy as in C. Digital images were taken every 2 min for a total of 5 h per experiment. Each wind rose plot shows centroid tracks from 7 to 10 representative cells from each indicated stiffness region, with the initial position of each track superimposed at a common origin. Bars = 50 μm. Reproduced from Ref. 66 with permission from Rockefeller University Press.
Fibroblast Migration in 3D Matrices

To study fibroblast migration and function within 3D matrices that better replicate aspects of the physiological environment, cells have been extensively studied in reconstituted ECM hydrogels, most commonly composed of purified type I collagen (37, 87). Collagen I can be acid- or pepsin-extracted from collagen-rich tissues, such as tendon, and stored in solution. At physiological temperature and pH, collagen spontaneously undergoes fibrillogenesis (formation of linear fibers from collagen monomers) and forms a hydrogel composed of a loose meshwork of collagen fibers, with the remainder of the structure occupied by fluid (23). The microstructure of the collagen gel is sensitive to the temperature, pH, ionic strength, ion stoichiometry, and monomer concentration used during gelation (95, 96, 102). To study cell interactions with such model matrices, fibroblasts can be added to hydrogels after gelation or they can be added during gelation to embed them within the 3D fibrillar meshwork.

Fibroblast migration within such 3D fibrous matrices has been shown to differ substantially from that across 2D surfaces. For instance, migration within 3D can be faster and more uniaxial than that observed across 2D surfaces (22). Intriguingly, these 3D migration characteristics can be recapitulated by patterning 2D surfaces such that adhesion and migration are constrained to thin straight lines, effectively reducing the surface interactions between cells and substrate from 2D to 1D (22). Similarly, microtopographical cues can modify migration patterns such that cells migrating across a complex (although not flat) 2D surface effectively behave as if in a 3D matrix (32). Such observations suggest that cellular migration through loosely organized 3D fibrillar matrices may effectively proceed by 1D migration along matrix fibers.

Controversy has emerged over whether cells migrating within 3D matrices completely avoid the formation of focal adhesions essential for 2D migration (26, 60). Although contradictory evidence for and against clustering of adhesion proteins such as paxillin can be seen in cells migrating within 3D matrices, the more striking finding is that knockdown of adhesion proteins such as talin and p130Cas, or genetic deficiency in the adhesion protein vinculin, can have opposite effects on migration speed and persistence in 3D vs. 2D contexts (26, 74). Moreover, cell speed in 3D migration strongly correlates with the growth rate of pseudopodial protrusions (26), a correlation that is absent in 2D migration (97). In addition, the polarity so important for 2D migration, including polarized activation of Rac, Cdc42, and phosphatidylinositol-3,4,5-trisphosphate, is absent in fibroblasts undergoing 3D migration (91). These results demonstrate that, not only is the character of 3D migration distinct from that on 2D, but so too is the functional effect of individual proteins and signaling cascades involved in cell-matrix adhesions. Physiological 3D collagen also promotes the formation of a newly described adhesion structure, the linear invadosome (55), which supports matrix invasion and shares some features with conventional invadosomes observed in 2D experimental culture models (21). As discussed below, these structures may be critical to orienting local proteolytic activity in support of migration through dense fibrillar matrices.

Although such findings hint at important distinctions in 2D and 3D migration, other observations document conserved principles that govern migration in both contexts. For instance, 3D collagen matrices can be fabricated with spatial gradients in both mechanical properties and ligand density, and, similar to effects observed in 2D systems, fibroblasts in these 3D gradients are also redistributed over time to regions of high stiffness and ligand density (38). Thus some core physical and mechanosensitive aspects of migration appear to be conserved across 2D and 3D matrices.

Although reconstituted 3D collagen matrices have been favored for their physiological relevance, the distinction between acid- and pepsin-extracted collagen as source material is an important one that has profound implications for our understanding of how cells migrate through fibrillar 3D matrices. Critically, pepsin extraction removes the nonhelical telopeptides situated at the NH2- and COOH-terminal ends of native collagen molecules. These telopeptides exert an important influence on the process of fibrillogenesis and support collagen cross-link formation that stabilizes the fiber architecture of collagen hydrogels (24, 30, 110, 128). Thus, although little or no difference can
be observed at the light microscope level in hydrogels formed from acid- and pepsin-extracted collagen, important differences in fibroblast invasion and migration can be observed. For instance, invasion of telopeptide-intact (acid-extracted) 3D collagen matrices by fibroblasts demonstrates an absolute requirement for matrix metalloprotease (MMP)-14 (46, 104–106), a cell-surface localized metalloprotease also known as membrane type (MT) 1-MMP (51), with potential compensatory roles for MT2-MMP and MT3-MMP in fibroblasts genetically deficient in MT1-MMP expression (47). In contrast, there is no need for MT1-MMP in migration through hydrogels formed from pepsin-extracted collagen (107), and in fact these matrices can be efficiently invaded by fibroblasts in the presence of a broad spectrum inhibitor of matrix metalloproteases, indicating that collagen extraction methods critically influence the need for proteolytic machinery. To understand these differences, it is useful to consider that, as the density of fibrillar matrices increases, the spaces available for migration drops below the size of invading cells. Thus the cells must either deform the architecture of the surrounding matrix fibrils to invade (78) or engage proteolytic machinery to degrade the matrix and generate paths suitable for invasion. The local need for matrix degradation has been confirmed in native cross-linked but not pepsin-extracted collagen matrices, using a molecular biosensor that fluoresces after cleavage (by MMP-2, -9, or -14) of a peptide site present in interstitial collagen (84). Such work confirms the localization of proteolytic activity at the leading edge of matrix invading cells and demonstrates the specific circumstances under which local matrix degrading activity is essential for 3D invasion and migration. Further testing of these concepts using invasion of the chicken chorioallantoic membrane (CAM), a type I collagen-rich ECM barrier commonly used to study invasive processes (6, 58), confirmed the necessary role for MT1-MMP in fibroblast invasion (106), expanding this concept to a true tissue-derived matrix. Interestingly, studies of tumor cell invasion have shown an alternative mode of amoeboid migration in fibrillar matrices, in which cells squeeze through small gaps by forming bleb-like structures that push fibers out of the way without the need for proteolytic activity (8, 92), although there is as yet no evidence for such amoeboid migration by fibroblasts navigating 3D matrices, supporting an essential role for local proteolysis in the navigation of dense, cross-linked fibrillar matrices.

Although matrices derived from acid-extracted collagen preserve the physiologically relevant dependency on local proteolytic activity, there are additional limitations that are important to consider with such systems. For instance, although these reconstituted matrices offer the experimentalist the advantage of being composed of a relatively homogenous distribution of fibrillar proteins, they fail to capture the molecular and topological complexity present in intact tissues (12). Strikingly, cells within complex matrices may navigate along preformed paths (107) or along prealigned bundles of ECM (108), features not typically observed in homogenous reconstituted ECMs upon polymerization. Although fibroblasts will reorganize the collagen fibrils in ECM hydrogels over time into oriented bundles, perhaps mimicking aspects of bundled fibers found in intact tissue, such cell-mediated changes also profoundly alter the local ligand density and matrix architecture (117), and likely the mechanical properties of the matrix as well (115), and do so in a spatially heterogeneous fashion that offsets the original advantage of a highly controlled starting condition present in such systems.

A second limitation revolves around the use of a single protein (e.g., collagen I) for matrix formation, which vastly understates the molecular complexity present in intact tissues, and may omit critical contributions from other matrix proteins, which can be present in normal tissues or increased in abundance in diseased tissues (27, 87). One intermediate step toward overcoming these limitations is to use cells themselves to generate 3D matrices for further study (4, 7, 19) (FIGURE 3). For instance, fibroblasts will produce their own pericellular matrix composed largely of glycosaminoglycan components hyaluronic acid and heparan sulfate and protein components fibronectin and collagen (43). Long-term culture of fibroblasts in the presence of ascorbic acid promotes cellular deposition and covalent cross-linking of a collagen-rich matrix (4, 35). Cell migration in such matrices is faster and more linear than in the random hydrogels of purified ECM (39). Micromechanical measurement techniques can also be used to measure the stiffness of cell-derived matrices (4, 91, 113), relating the studies of cell behavior on these matrices back to the concept of 2D mechanical stiffness responses summarized above. Intriguingly, the 3D mode of migration in such cell-derived matrices appears to vary depending on specific aspects of the mechanical environment, such as its linear or nonlinear elastic behavior (91).

Although cell-derived matrices offer potential for novel insights and physiological relevance, they also suffer from important limitations. Such limitations include local and batch variability in cell-derived matrices and challenges with maintenance and manipulation of such culture systems (27). In addition, these cultures are derived from a single cell type growing in isolation, omitting the influence...
of other cell types found in intact tissues, and the culture conditions for the formation of such cell-derived matrices typically begin with rigid plastic substrates as a starting point. Fibroblast-derived matrices synthesized under such culture conditions, including high levels of serum and omitting other paracrine signaling partners, may be most representative of matrices formed during wound healing, although improvements in defined culture methods may be able to offer superior control of the cell-derived matrix composition and architecture (4). On a positive note, the development of controlled methods for studying cell-derived matrices offers a unique opportunity to determine whether disease-derived cells produce matrices that are distinct from those produced by “normal” cells, as has been done for cancer-associated fibroblasts (5, 16).

Integrating 3D Migration with Matrix Contraction and Degradation

Although migration offers a clear example of how cell function is altered by matrix characteristics, other important fibroblast functions also diverge from their behavior on 2D surfaces when studied in 3D matrices. For instance, although fibroblasts typically proliferate rapidly in rigid 2D cultures, they are largely quiescent on collagen gels that are floating and allowed to freely contract (28, 103). Such differences in proliferation might reflect the altered density of ECM ligands in 3D hydrogels. Sparse hydrogels of reconstituted ECM are also highly deformable (63, 87), suggesting that limited proliferation may relate in part to observations from 2D studies that highly deformable matrices dramatically limit fibroblast spreading and proliferation (66, 75, 76). When 3D matrices are constrained by peripheral anchorage or attachment to a surface so that their outer boundary is maintained, rather than allowed to freely contract, cells compact the matrix over time in the free dimension. And although short-term culture leads to net matrix degradation in this setting, longer-term culture allows newly synthesized collagen to be deposited into and augment that matrix (81). Fibroblasts maintained in such “stressed” matrices take on a synthetic phenotype compared with cells in “relaxed” floating matrices (57), in a fashion similar to that seen with fibroblast phenotypes across matrices with variations in 2D matrix stiffness (66). Although challenging to quantify, the integrated effect of such cell-mediated effects on matrix mechanics can be measured in such 3D systems (63, 71, 72). Interestingly, allowing cells to remodel the matrix in a stressed state leads to a relatively organized ECM that promotes fibroblast growth factor responsiveness, whereas cell-mediated contraction of freely floating matrices results in a more disordered matrix and limits growth factor responsiveness of resident cells (80). Over time, with appropriate boundary conditions, fibroblasts locally reorient and align matrix fibrils, and fibroblast morphology and matrix adhesions will also reorient and mature, suggesting that the cells remodel the originally sparse matrix into dense aligned bundles, effectively altering their relationship

FIGURE 3. Comparing adhesion structures in 2D culture with those in 3D cell-derived matrices
3T3 mouse embryonic fibroblasts expressing GFP-paxillin migrating on a 2D fibronectin-coated rigid surface (A) or through a 3D cell-derived matrix (B). Insets: magnifications of GFP-paxillin localization and adhesion formation in squared areas. Fibroblasts in 3D adopt a more elongated morphology adapted to the fibrillar structure of the cell-derived matrix. Adhesive structures are located all around the cell body and are aligned with the fibers (B). Reproduced from Ref. 52 with permission from Elsevier.
with the matrix back toward that observed on stiffer 1D or 2D matrices (117).

How then do cells regulate the switch between migration through the matrix and contraction of the matrix? These processes employ largely overlapping components of the same machinery (37, 40, 73, 77, 111), although with subroutines specific to particular aspects of matrix remodeling (15). Such issues become critical in processes like wound healing, in which fibroblasts must first migrate into the provisional matrix of the wound bed but then transition to a contractile function to assist with wound closure and tissue regeneration (44, 45). One likely explanation is that alterations in cell-matrix adhesions are essential to this transition, shifting the cells from relatively weak adhesions that foster migration to strong adhesions required for generation of cytoskeletal tension and tissue remodeling. How such a transition in adhesion function is triggered is likely complex, but the physical state of the matrix may play an important role. For example, it has been postulated that the transition from migration to matrix contraction can be explained by the relative pliability of the matrix (37). By analogy, if the matrix moves under cell-mediated traction force, the cell remains stationary as on a treadmill, while the matrix is compacted. On the other hand, if the matrix fails to move under the influence of cellular tractions, these forces can translate into movement of the cell body.

Intriguingly, there is evidence that the tractions associated with migratory fibroblasts are indeed sufficient to contract floating collagen gels (37) and may be sufficient to close normal wounds, whereas conversion to the highly contractile myofibroblasts may be specific to late phase of certain wounds that do not heal easily (44) or to pathological fibrosis or scarring (3, 121) under the influence of an abnormally stiffened and cross-linked matrix (31, 33, 44, 66). In addition to such mechanical effects, it seems likely that some aspects of cell polarization remain key to 3D migration (91, 97), since a necessary step in migration is formation of new adhesions at the leading edge and release of adhesions at the trailing edge. Such polarity signals may come from the matrix itself (50, 67, 93, 94) or from the soluble environment (42). Moreover, specific soluble factors may also provide signals that shift fibroblasts between migration and contraction. For example, PDGF potently promotes migration in both 2D (42) and 3D (37, 53) contexts and cell spreading independent of matrix stiffness (36), whereas serum and serum components such as lysophosphatidic acid (LPA) and sphingosine-1-phosphate promote contraction of 3D collagen matrices (53). Although it is appealing to ascribe such definitive effects to soluble factors, it must be acknowledged that our understanding of these effects rests largely on model matrix systems, with in vivo relevance still in question. As a cautionary example, the “matrix contractile” agonist LPA has been shown to play a critical role in a mouse model of lung fibrosis (116) but appears to do so by promoting matrix invasion and migration; similarly, LPA also promotes collagen matrix invasion by HT1080 fibrosarcoma cells through MT1-MMP activation and collagen degradation leading to formation of single-cell invasion tunnels (25). Taken together, these experiments and concepts, while still fragmentary, indicate that changes in matrix density and deformability, along with changes in soluble signaling cues, likely engage a complex regulatory system to control fibroblast migration and matrix contraction. A fully integrated understanding of this critical cellular decision process remains elusive and will be essential in formulating strategies to modulate fibroblast invasion and function across a variety of applications, from tissue engineering to wound healing and therapies directed at scarring and fibrosis.

### Basement Membrane Invasion

One area of fibroblast migration that has received less attention but may be of great interest in pathological scarring and fibrosis is that of fibroblast invasion across basement membranes. For example, evidence is accumulating that the pathological remodeling of the lung ECM in pulmonary fibrosis requires a highly invasive fibroblast phenotype (64), with MT1-MMP again likely to play a prominent role in local degradation and invasion of the lung ECM (104). The constrained expression and proteolytic function of MT1-MMP at the cell’s leading edge helps to reconcile the invasive process with the overwhelming accumulation of abundant matrix proteins that characterize fibrosis. The process of translocation across the basement membrane, essential to the loss of fibroblast compartmentalization seen in lung fibrosis, may be specifically dependent on integrin ligation by fibronectin (125) or cellular interactions with hyaluronan (64), suggesting a prominent role for the matrix in promoting invasion, but evidence also exists that primary fibroblasts from humans with fibrotic lung disease may already possess an “invasive” phenotype (64). Such observation build on studies showing that normal growth limitations seen when cells are cultured in polymerized 3D collagen gels (109) are somewhat overcome in fibroblasts isolated from the lungs of individuals with pulmonary fibrosis (82, 129, 130). These findings suggest that fibroblasts harvested from fibrotic tissues may have a fundamentally altered relationship with the tissue matrix that promotes the
underlying pathology. In contrast to these findings, it has also been shown that several phenotypic traits of fibrotic tissue-derived fibroblasts can be reversed upon growing these cells on a physiologically compliant 2D ECM (69). Such observations, although limited, demonstrate that disease-derived cells retain elements of normal responsiveness to the mechanical environment (69). Clearly, more effort is needed to understand the regulation of basement membrane invasion by fibroblasts, and the relative importance of disease-associated changes in the matrix, and resident cells as drivers of normal and pathological basement membrane invasion. As in the case for 3D collagen gels, the model systems used for study of cell-basement membrane interactions are not without limitations. Such limitations include the use of tumor cells as the typical source of basement membrane preparations and the disorganized and noncross-linked nature of the reconstituted basement membrane hydrogel matrices (107), both of which limit the physiological recapitulation of typical basement membrane structure, composition, and mechanics. And as in the case with 3D collagen gel systems, the ultimate test of physiological insights obtained with such systems will rest on confirmation in intact tissues or organisms.

**Fibroblast Function within Tissue Matrices: Paving a Path to the Future?**

Although much progress has been made in understanding fibroblast migration and function in 2D and 3D model systems, the ultimate goal remains to decipher how migration occurs within physiological and pathological tissue contexts. No model system can yet recapitulate the molecular and architectural diversity and complexity present in tissue matrices. For example, recent mass spectrometry proteomics studies have illustrated the tremendous number of matrix proteins and matrix-associated proteins (the matrisome) present in normal and tumor-associated tissues (79), and underscored the complex changes that occur in the composition of the extracellular matrix in pathological conditions such as fibrosis (12). To move the study of migration toward physiological settings, intravital imaging approaches, which have been pioneered for the study of tumor cell motility, may provide a unique capability for in situ imaging of fibroblast migration within living tissues (8, 59, 68, 127). Such approaches are not without their own limitations, since they rely on animal models that often fail to replicate aspects of human pathophysiology. Nevertheless, the combination of such intravital approaches with more commonly employed 3D model systems could offer a compelling toolset as the field moves forward in understanding fibroblast interactions with physiological extracellular matrices.

In addition to these approaches, a middle ground is also rapidly emerging that is already providing important insights: the use of tissue-derived matrices for study of cell function. The past several years have seen robust progress in development of methods to study tissue explants (91, 131) and decellularize tissues as a way to isolate matrix preparations for the study of cell function (12, 134). These methods, although still under-development, appear to preserve important features of native tissue architecture and composition. And although such approaches also have limitations with regard to reintroducing cells artificially to the surface of matrices that are modified by processing, along with the loss of tissue tension associated with excision (27), they offer compelling and unique advantages. Chief among these is the capacity to study human tissue matrices and to compare cell function on normal and diseased matrices, which has potential to radically enhance our understanding of fibroblast-matrix interactions in normal and diseased tissues (122). Already, such approaches have demonstrated that fibrotic matrices possess intrinsic capacity to alter fibroblast migration and myofibroblast differentiation (12, 134). When such approaches are combined with already well established 2D and 3D model systems, and nascent efforts directed toward intravital imaging, the stage is set for a quantum advance in our understanding of fibroblast invasion and migration as they occur in physiological and pathophysiological tissue contexts. The integrated observations enabled by these approaches should provide a new understanding of the molecular and cellular processes that drive fibroblast motility through intact tissue matrices, and should ultimately allow new hypotheses to be generated and tested that are directed at controlling fibroblast migration and function in wound healing, fibrosis, and other physiologically relevant contexts.

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**References**


