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The mechanobiology of fibroblast activation in disease

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ABSTRACT

Fibroblasts play crucial roles in wound healing, cancer, and fibrosis. Many aspects of these roles are driven by the process known as fibroblast activation. The generally accepted definition of fibroblast activation is the transition from a quiescent state to a state in which fibroblasts participate in a number of active processes, including extracellular matrix (ECM) production and remodeling, elevated contractility, and enhanced migratory capacity, although there is no universal consensus on what exactly constitutes "activation." Interestingly, the time scale of activation is not consistent across tissues and disease states; some fibroblasts quickly return to quiescence after activation (e.g., in wound healing), others undergo apoptosis, while a subset become persistently activated. This activation, both acute and persistent, is inherently a mechanical process, given the increase in ECM production and remodeling and the enhanced traction force generation. Thus, there exists a dynamic reciprocity, or cell-ECM feedback, in which activated fibroblasts produce a mechanical microenvironment that in turn supports persistent activation. This has a wide variety of implications for disease, most notably fibrosis and cancer, as the fibroblasts that become persistently activated in connection with these conditions can contribute to disease state progression. Like other mechanosensitive processes, this mechanically induced persistent fibroblast activation is driven by a number of mechanotransduction signaling pathways. Thus, an opportunity exists in which the mechanosensitive underpinning of fibroblast activation can be leveraged to improve clinical outcomes. Here, we highlight these opportunities and make a call to the field to consider the mechanosensitive pathways governing fibroblast activation as an important frontier in mechanomedicine.

THE WHO: FIBROBLAST IDENTITY, FUNCTIONS, AND NICHES

Fibroblasts are highly dynamic, multifunctional cells with diverse origins and complex molecular signatures, enabling them to play pivotal roles in tissue maintenance, repair, and pathological processes like fibrogenesis and cancer.[1,2] They can arise from multiple developmental pathways, including mesenchymal cells during embryonic development, bone-marrow-derived hematopoietic stem cells, endothelial-to-mesenchymal transition (EndMT), and epithelial-to-mesenchymal transition (EMT) in adult or maturing tissues.[3,4] It has been shown that secondary epithelial and endothelial cells can undergo a cell state transition and be driven toward a fibroblast state in response to biochemical stimuli.[3,5] While the precise mechanisms of fibroblast development are still not fully understood, significant progress has been made in recent years to uncover the key mechanical and biochemical processes and signals involved.

In vivo, fibroblasts are widely distributed throughout the body and primarily reside within interstitial spaces in connective tissues and organs, where they are often closely associated with the basement membrane.[6,7] In this niche, they are primarily responsible for the production of extracellular matrix (ECM), which forms most of the extracellular tissue space and has various important structural and biochemical functions.[1,2] Fibroblasts also actively remodel the ECM, exert forces on it, and secrete cytokines, growth factors, and adipokines into it, all of which influence cell behavior and tissue homeostasis.[1] Beyond their role in ECM dynamics, fibroblasts have also been shown to regulate interstitial fluid volume and pressure and modulate local immune responses in chronic infection, inflammation, and cancer in a tissue- and disease-specific fashion.[7,8] Due to their functional adaptability *in vivo*, many have recognized their enormous potential to be employed as therapeutic agents that may one day be deployed against conditions as diverse as lung fibrosis, myocardial infarction, and

invasive metastasis.[9] This progress is currently hindered by the fact that despite the high degree of experimental interest that fibroblasts attract in the field, there are currently no universal biomarkers that identify them, further complicating both fundamental research and interdisciplinary studies that attempt to study and leverage fibroblasts for biomedical and tissue engineering purpose.[10,11]

One major contributor to this is the inherent heterogeneity of fibroblasts. While it has been shown that fibroblasts have a common precursor that gives rise to specialized fibroblasts in various tissues, variations in morphology and cell surface markers exist both between and within tissues.[12] Across various tissue types, fibroblast morphology is heterogeneous, as cells within even the same tissue can exhibit either spindle-shaped or stellate appearances.[6,7,13,14] As morphology alone is generally not sufficient to identify fibroblast populations, numerous sets of fibroblast-specific surface markers have been proposed, including fibroblast activation protein (FAP), vimentin, and fibroblast-specific protein 1 (FSP1).[15,16] However, these markers are not completely unique and universal to fibroblasts, as epithelial cells, hematopoietic stem cells, and myocardial endothelial cells, among others, also express these markers to varying extents.[17,18] In addition to surface markers, alpha 1 and alpha 2 chains of type I collagen have become generally accepted as fibroblast markers due to consistent levels of elevated collagen expression in fibroblasts across various tissues, although it should be noted that several other cell types, including bone-marrow-derived mesenchymal stem cells, have also been found to express these surface markers, which underscores the inherent difficulty in fibroblast identification and isolation.[19–22] While there is currently no accepted pan-tissue specific fibroblast marker, some tissue-resident fibroblasts can be identified *in situ* using highly specific cell surface markers, such as CD26, PDGFRa, and LRIG1 in papillary dermal fibroblasts.[1,23] Surface marker heterogeneity has also been shown to correlate with function, as fibroblasts expressing high levels of fibroblast activation protein (FAP) play a more dominant role in ECM turnover, while fibroblasts with high levels of alpha-smooth muscle actin (α-SMA) tend to be more contractile and proliferative.[24] Fibroblasts from distinct anatomical locations also exhibit unique expression patterns of Homeobox (HOX) genes, showing that they retain positional memory. This site-specific programming gives rise to fibroblasts with location-specific activities, enhancing fibroblast diversity.[13,25] In recent years, great importance has been placed on finding unique and universal biomarkers to identify these fibroblasts due to their critical biological role in the body and their importance in therapeutic medicine.

THE WHAT: THE CONTINUUM OF FIBROBLAST PLASTICITY AND ACTIVATION

Fibroblast plasticity, or the ability to adapt phenotype and function in response to environmental cues, underlies the heterogeneous and specialized roles they play both within and across different organs.[21] These cues can include acute tissue damage, immune response, or extracellular signals from transformed cancer cells.[26,27] Perhaps, the most notable example of fibroblast plasticity is their ability to "activate" in response to biochemical and mechanical changes in the microenvironment.[28,29] One of the best studied locales in which this occurs are tissue injury sites, where immune cells detect the wound and release a cascade of cytokines, including fibroblast growth factor (FGF), transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF).[30] Cytokine secretion forms a chemical gradient that drives chemotactic fibroblast migration to the wound site,[31] where fibroblasts sense elevated cytokine levels via transmembrane receptors and the disrupted ECM, which stimulates a transition into the activated state via intracellular signaling cascades.[28,32] This activation is essential for wound healing, as only activated fibroblasts can effectively remodel the ECM and facilitate wound closure.[33] Once tissue integrity is re-established and repair is complete, activated fibroblasts typically undergo apoptosis or revert to a quiescent state.[34] This resolution phase is critical to prevent excessive scarring and fibrosis, highlighting the finely tuned balance of fibroblast activation and deactivation to maintain tissue homeostasis.

In the past, this fibroblast activation step was understood to be a binary shift from a "quiescent" state to an "activated" state. However, in recent years, the concept of activation has been shown to be far more complex, involving a dynamic spectrum of phenotypic changes including alterations in cellular morphology, cytoskeletal reorganization, and the acquisition of contractile properties.[35] This activation is often referred to as the fibroblast-to-myofibroblast transition (FMT) (Fig. 1). Although this transition occurs along a continuum, it can be broadly divided into three cell states: quiescent fibroblasts, proto-myofibroblasts, and myofibroblasts.[36] During FMT, the functional role of the fibroblast evolves from a quiescent, matrix-maintaining state to an active, contractile, and secretory phenotype. These functional changes are accompanied by distinct phenotypic alterations, which can be visualized via the upregulation of α-smooth muscle actin (α-SMA), an increase in stress fiber formation, and quantifiable changes in cell morphology.[37]

During the quiescent phase, fibroblasts reside within the interstitial spaces of tissues, showing minimal migratory activity, limited proliferation, and low levels of ECM synthesis. They are smaller in size, exhibit a spindle-shaped morphology, and express few actin stress fibers, with minimal or no expression of α-SMA.[38] Their focal adhesions are smaller and weaker, reflecting limited interaction with the ECM.[39] In response to sufficient mechanical and biochemical stimulation, fibroblasts start to activate. In the skin, this process begins during fibroblast migration to damage sites, where they play a critical role in initiating tissue repair. Upon initial wound closure, fibroblasts align parallel to mechanical stress. At this stage, they adopt an intermediate phenotype known as proto-myofibroblasts, which are primed to contract the wound and begin synthesizing essential ECM components, including collagen and fibronectin, to support the repair process.[33,36] They increase in size, adopt a more spread morphology, and begin to express α-SMA alongside increased stress fiber expression. Concurrently, they develop mature focal adhesions, strengthening their adhesion to the ECM.[39,40] As the FMT is a gradual and dynamic process, it is important to note that it is inherently challenging to define a proto-myofibroblast, as it represents an intermediate state within this continuum. As tissue repair progresses, proto-myofibroblasts mature into fully differentiated myofibroblasts, often referred to simply as activated fibroblasts, acquiring the ability to generate strong contractile forces in the process.[36,41] These forces are essential for completing ECM remodeling and achieving full wound closure. At this stage, α-SMA incorporates into actin stress fibers, and fibroblasts fully transition into myofibroblasts. They become significantly larger with a more compressed nucleus, a consequence of mechanical forces transmitted from the actin cytoskeleton to the nuclear envelope.[42,43] Myofibroblasts also form supermature focal

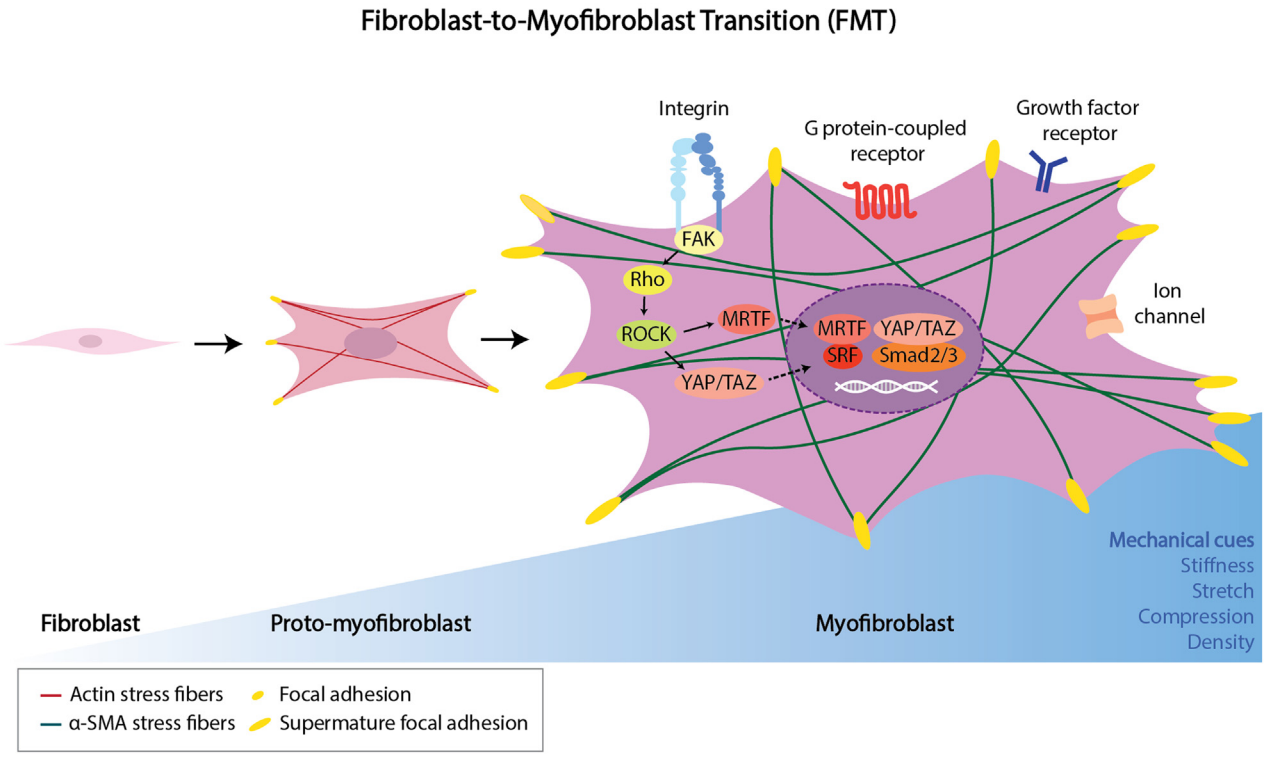

FIG. 1. Phenotypic changes in fibroblast activation during fibroblast-to-myofibroblast transition (FMT). In response to biochemical signaling, fibroblasts undergo activation. While this activation occurs along a continuous spectrum of phenotypic changes, it can be broadly categorized into three main stages. In the first stage, fibroblasts are quiescent and do not actively remodel the ECM. As they activate, they grow bigger, exhibit more robust stress fibers, and develop mature focal adhesions. Fibroblasts at this stage are categorized as proto-myofibroblasts. Fully activated fibroblasts form supermature focal adhesions and express α-SMA. As the ECM is remodeled by the fibroblasts, mechanical cues including stiffness, stretch, compression, and density increase, further influencing fibroblast activation. Fibroblasts sense and respond to these changes via integrins, G protein-coupled receptors, growth factor receptors, and ion channels. One of the key mechanotransduction pathways underlying fibroblast activation is the integrin-FAK-ROCK-MRTF-YAP-TAZ signaling axis.

adhesions, which are substantially larger and more robust than those observed in quiescent fibroblasts.[33] These focal adhesions are also more irregular in shape, reflecting the dynamic reorganization of the cytoskeleton and enhanced cell–ECM interactions.[44] This complex transition underscores the nuanced nature of fibroblast activation and highlights the need for further research to fully elucidate the mechanisms governing this transition.

Further complicating the picture is the presence of so-called "fibrocytes," which are bone-marrow-derived cells that originate from monocytes that exhibit characteristics of both immune cells and fibroblasts.[45,46] These cells are phenotypically similar to fibroblasts and are also capable of differentiation into myofibroblasts. Although they exist in low numbers in peripheral blood, they participate in wound healing by migrating to wound sites and differentiating into activated fibroblasts.[47–49] Their ability to transition into activated fibroblasts and contribute to tissue repair introduces additional complexity in distinguishing the origins and complete roles of activated fibroblasts. As a result of the fibrocyte contribution to the activated fibroblast population, it is difficult to investigate the precise molecular pathways driving fibroblast activation in both physiological and pathological contexts.

THE WHERE: MECHANICAL CUES DRIVING FIBROBLAST FATE

While biochemical cues such as cytokines and growth factors are well-established drivers of fibroblast activation, the role of mechanical forces in this process remains less explored, despite their profound influence on fibroblast behavior and phenotype. The field of mechanobiology investigates the interplay between cells and mechanical forces, usually focusing on the phenomena of mechanotransduction, which enables cells to convert mechanical stimuli into biochemical signals via receptor–ligand interactions, the triggering of conformational changes in force-sensitive proteins, and the initiation of mechanosensitive intracellular signal cascades.[50] The mechanobiology of fibroblasts is especially important due to their localization within the interstitial spaces of the ECM,[51] placing them in direct and continuous contact with a wide variety of mechanical stimuli and making them particularly responsive to the changes in ECM mechanics.[52] Fibroblasts have been found to sense and activate in response to different forms of mechanical stress within the ECM, including substrate stiffness, stretch, topography, density, and compression.[53] Matrix stiffness is perhaps the most extensively studied mechanical cue, as it has been found to play a direct and critical role in regulating fibroblast activation.

Fibroblast activation in response to mechanical cues involves a complex network of mechanotransduction pathways.[54] Even within the context of stiffness sensing alone, multiple mechanosignaling pathways have been identified. One of the key mechanotransduction pathways that ECM stiffness activates is the integrin-FAK-ROCK-MRTF-YAP-TAZ signaling cascade.[34] Stiffness sensing begins with the recruitment of focal adhesion kinase (FAK) by integrin β1, a process that has been shown to promote fibroblast migration and enhance collagen deposition.[28,55,56] This leads to the formation of a denser matrix with larger and thicker scars. In addition to FAK recruitment, increased ECM stiffness elevates actomyosin contractility, which drives nuclear translocation of myocardin-related transcription (MRTF) as well as the transcriptional co-activators yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ).[34] The nuclear translocation of these mechanosensitive regulators is a critical step in linking matrix stiffness to fibroblast activation and changes in gene expression. Once in the nucleus, YAP and TAZ interact with Smad2/3, while MRTF binds to and activates transcription factors like serum response factor (SRF), which increases expression of the fibroblast activation hallmark α-SMA.[34,57–59] The expression and nuclear localization of YAP/TAZ are well-documented in the field of mechanobiology, with studies demonstrating their role in fibroblast activation across multiple organs including the heart, the kidney, and the lungs,[57,60,61] underscoring its importance in mediating stiffness-sensitive fibroblast activation.

In addition to integrin-mediated signaling, a variety of mechanosensitive pathways have been found to contribute to fibroblast activation in response to ECM stiffness, including ion channel activation, G protein-coupled receptor stimulation, and the binding of cell surface membrane proteins like fibroblast activation protein alpha (FAPα).[62] For instance, the calcium channel protein TRPV4 exhibits increased activity in fibroblasts on stiff substrates, leading to cytoskeletal remodeling and the nuclear translocation of MRTF-A.[63] Similarly, FAPα is upregulated in response to stiffness, activating P13K and upregulating pAKT/AKT, both of which have been shown to contribute to fibroblast activation.[62,64] Furthermore, stiff substrates have been shown to upregulate G protein-coupled receptors like angiotensin II receptor type 1 (AT1R), stimulating the upregulation and release of transforming growth factor-beta 1 (TGF-β1), a potent inducer of fibroblast activation.[65] More importantly, changes in stiffness have been shown to lead to a positive feedback loop between integrin β1 and the mechanosensitive ion channel Piezo1, ultimately leading to YAP/TAZ overexpression.[66]

These observations have primarily been made on 2D substrates, which may not fully recapitulate the complexity of the *in vivo* microenvironment, as fibroblasts seeded in 3D substrates have been found to behave differently.[67,68] A notable example of this is the observation that fibroblasts exhibit increased spreading, focal adhesion formation, and activation in soft and deformable 3D fibrous matrices compared to those in stiffer 3D matrices with identical architecture.[69] This discrepancy highlights the importance of studying stiffness and its role in fibroblast activation within more physiologically relevant 3D contexts.

To model tensile forces in the cellular microenvironment, mechanical stretch is commonly applied to systems containing cells. The resultant strain creates resistance against fibroblast-driven contractile forces, modulating fibroblast activity and mechanosensing.[70] Fibroblasts have been found to display a nonlinear response to mechanical strain, with moderate levels promoting Rho-ROCK signaling-driven actomyosin contractility and the upregulation of mechanosensitive transcriptional pathways, ultimately enhancing fibroblast activation. Excessive strain can lead to cellular detachment and reduced activation.[71]

In addition to the various mechanical cues presented, surface topography, or the nanoscale and microscale architecture of the ECM, is another factor that influences fibroblast activation. Aligned topographies have been shown to direct fibroblast migration via anisotropic alignment along the topographic pattern.[72] However, the effects of fiber arrangement on fibroblast activation remain complex and sometimes contradictory. Randomly aligned nanofibers have been shown to promote fibroblast activation due to increased surface tension.[73,74] Other work has found that aligned collagen fibers can lead to higher tension at focal adhesions, leading to fibroblast activation. This topography-induced activation has been linked to enhanced nuclear translocation of p38, YAP, and TEAD, as well as enhanced p38-YAP-TEAD interactions, highlighting the role of topographic alignment in mechanotransduction.[75] The influence of topographic gradients, such as wavelike features with varying hill heights and spacing, adds further complexity. One study found that fibroblast migration speed increases with shorter hill heights and reduced spacing between hills and valleys, likely due to decreased focal adhesion area,[76] while another found that wider and taller microchannels enhance activation.[77] These diverse outcomes may be attributed to variations in focal adhesion dynamics, which in turn modulate intracellular tension and mechanosensitive signaling. In general, a larger focal adhesion area promotes focal adhesion maturation, causing FAK-mediated phosphorylation and stress fiber tension and leading to enhanced α-SMA expression and incorporation into stress fibers.[78] Topographical transition regions also have been shown to modulate fibroblast behavior by altering focal adhesion dynamics.[79]

Another mechanical cue influencing fibroblast activation is ECM density, a feature closely related to ECM fiber orientation. Dense collagen networks are often associated with enhanced fibroblast activation, as activated fibroblasts produce and remodel the ECM, leading to the formation of fibrotic scars.[80] However, the relationship between ECM density and fibroblast activation is not straightforward. Interestingly, less dense matrices have been shown to activate fibroblasts as low density promotes a spread morphology with enhanced α-SMA expression and elevated intercellular tension. In contrast, denser matrices have been found to induce spindle-shaped morphologies, which are more representative of quiescent fibroblasts.[81]

While matrix density can influence fibroblast activation via morphological changes, it can also exert compressive forces on the cells in conjunction with the surrounding tissue. While the role of mechanical compression in fibroblast activation has been relatively understudied, it has been established that it can increase fibroblast activation as measured by α-SMA expression and collagen production.[82,83] However, newer research shows that this increase in fibroblast activation is likely due to tension anisotropy, as aligned collagen fibers create directional cues that can lead to an amplification of anisotropic tension.[84] Parallel to these two concepts, fibroblasts also experience high degrees of confinement as they migrate through narrow interstitial spaces in response to soluble cues.[85] During this process, fibroblasts experience both compressive forces and spatial constraints, which can alter their cytoskeletal organization, mechanosensitive signaling, and ECM remodeling.[86–88] In addition, confined migration has been shown to result in a

decrease in nuclear volume and stimulate osteogenic differentiation in human mesenchymal stem cells.[89] Thus, a more complete understanding of how fibroblasts respond to confined migration in terms of activation will likely provide critical insights into their role in tissue repair and disease.

Fibroblasts experience significant nuclear compression and deformation in high degrees of confinement and intracellular tension due to various mechanical forces, including substrate stiffness, matrix density, and stretching forces.[90] The nucleus plays an important role in the mechanobiology of fibroblast activation by acting as a mechanical "ruler" for sampling spaces in the microenvironment, utilizing a network of connections beginning at focal adhesions, extending through the cytoskeleton, and transmitted via the linker of nucleoskeleton and cytoskeleton (LINC) complex to the chromatin.[91,92] This integrated pathway allows the nucleus to translate mechanical cues into changes in cellular behavior by causing alterations in chromatin organization, gene expression, and mechanosensitive pathways.[93,94] These mechanical cues and mechanotransduction pathways may operate simultaneously or independently, depending on the cellular context and microenvironmental conditions, highlighting the multifaceted nature of fibroblast mechanosensing.

THE WHEN: FIBROBLAST ACTIVATION IN DISEASE

As fibroblasts play such a key role in homeostasis, it is of little surprise that their response to disease is of critical importance. The most significant microenvironment alterations that fibroblasts are exposed to occur during chronic wound dysregulation, which is a hallmark of both cancer and fibrosis.[80] Disease-associated fibroblasts exhibit elevated contractility, increased α-SMA expression, enhanced ECM production, and are perpetually activated, unlike myofibroblasts present during wound healing, which ultimately undergo apoptosis.[95–98] This sustained activation has been attributed to epigenetic changes, including DNA methylation, histone modifications, and dysregulated non-coding RNA expression.[99] As a result, disease-associated fibroblasts drive the aberrant overproduction and deposition of ECM components, further contributing to the dysregulated mechanical microenvironment found in both diseases.[100,101]

The interplay between cancer and fibrosis has been studied extensively. The phenomenon of desmoplasia, a hallmark of cancer involving excessive ECM deposition and remodeling, creates a mechanical environment strikingly similar to what is seen in fibrosis.[102] In both conditions, the enhanced deposition of ECM components and increased cross-linking lead to significant changes in the mechanical microenvironment, including heightened stiffness, and accumulation of solid stress that generates both compressive and tensile forces. Additionally, the denser matrix due to increased cross-linking further amplifies these mechanical alterations.[103–105] These mechanical changes drive a positive feedback loop that leads to even further fibroblast activation measured by elevated α-SMA expression, enhanced fiber formation, and nuclear translocation of YAP/TAZ (Fig. 2). Ultimately, these mechanosensitive responses in fibroblasts can drive progressive scarring in various organs and cancers.[106–110]

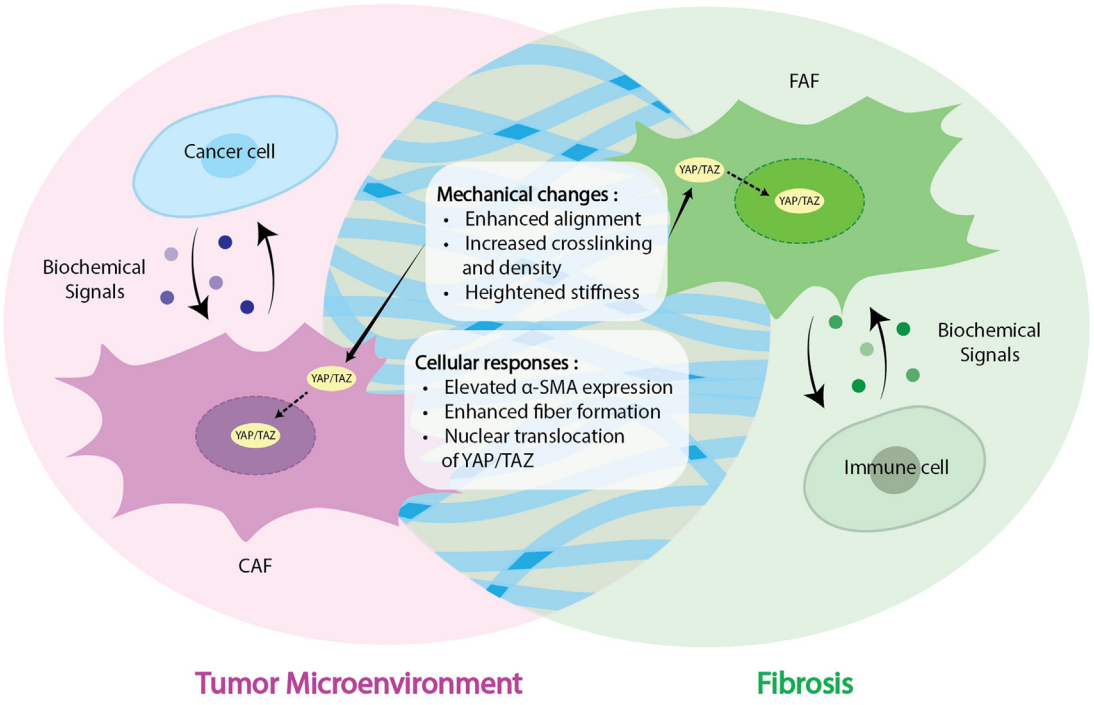

FIG. 2. Similarities in the mechanical environments of cancer and fibrosis. Although the biochemical environments found in cancer and fibrosis are different, fibroblasts experience similar mechanical changes in both cancer and fibrosis, including enhanced alignment of the ECM and increased cross-linking, which can modulate the density and stiffness of the substrate. These mechanical changes result in similar cellular responses in CAFs and FAFs, including elevated α-SMA expression, enhanced ECM production, and nuclear translocation of YAP/TAZ which has been shown to contribute to the persistent activation of fibroblasts.

In tumors, fibrotic remodeling of the ECM is a key driver of cancer progression and metastasis.[111–115] Beyond solid tumors, fibrosis also contributes to blood cancers, with hematologic malignancies often causing bone-marrow fibrosis.[116,117] Within the tumor microenvironment, fibroblasts, which are referred to as cancer-associated fibroblasts (CAFs), exhibit reduced capacity for apoptosis, enabling their persistence and continuous support of tumor growth.[118–120] Compared to normal fibroblasts, CAFs display enhanced migratory and invasive potential, express higher levels of activation markers including FAP, α-SMA, and platelet-derived growth factor receptor (PDGFR),[96,121,122] and actively promote cancer cell proliferation, angiogenesis, creating a supportive microenvironment for further cancer growth.[123–125] Additionally, CAFs remodel the ECM that guides cancer cell invasion and secrete chemokines and growth factors that facilitate EMT in cancer cells.[126–130] Despite significant efforts to fully understand the role of CAFs in cancer, major knowledge gaps exist, which prevent the development of mechanomedicine strategies for mitigating the CAF-cancer feedback loop. This is especially evident in studies that have found conflicting roles for CAFs, including tumor-suppressive roles, or worsened cancer progression following CAF removal.[131,132] Given the strong yet complex observations of the roles CAFs play in cancer progression, it is imperative to not only study the biochemical factors that influence the CAF-cancer relationship but also to provide insight into the mechanical factors that might contribute to the development and persistence of the CAF state.[133]

Beyond biochemical factors, the CAF phenotype is strongly reinforced by mechanical cues present in the tumor microenvironment. A key feature enabling CAFs to sense and propagate mechanical signals is their altered focal adhesion dynamics, which elevate RhoA and Rac1 levels.[134] Less dense extracellular environments increase cell spread area via more active focal adhesion dynamics that lead to higher intercellular tension activating the AKT/mTOR pathway and upregulating the CAF markers FAP, FSP-1, and PDGFR-β.[81] Notably, tensile forces further contribute to this process by physically stretching fibroblasts, and this promotes the secretion of fibronectin in a more aligned, linear network, facilitating directional cancer cell migration, and upregulates PDGFRα, a marker associated with reactive tumor stroma.[135] The CAF state is sustained by both increased stiffness of the tumor microenvironment and compressive forces from solid stress, via distinct mechanisms.[106–108] While matrix stiffness promotes CAF activation primarily through the nuclear translocation of YAP/TAZ, compressive stress induces DNMT3A-dependent promoter methylation, leading to downregulation of miR-9 and subsequent regulation of its target genes, including VEGFA, thereby reinforcing the pro-tumorigenic functions of CAFs.[136] Additionally, compressive forces stimulate fibroblasts to secrete growth differentiation factor 15 (GDF15), which in turn promotes cancer cell migration and invasion, further driving tumor progression.[82] Collectively, these mechanical factors contribute to the transformation of normal fibroblasts into CAFs and sustain their tumor-promoting functions, underscoring the key role of mechanobiology in cancer progression.

While fibrosis and cancer are deeply intertwined, fibrosis also occurs independently of cancer in several different diseases. In these contexts, the key drivers are fibrosis-associated fibroblasts (FAFs), which, like CAFs, are persistently activated and produce excessive ECM components, leading to progressive tissue stiffening and remodeling.[137–139] This is particularly evident in systemic sclerosis, as increased expression of α1(I) collagen exacerbates fibrotic changes.[140–142] Unlike myofibroblasts involved in normal wound healing, FAFs resist apoptosis and become unresponsive to regulatory signals such as TGF-β inhibitors, contributing to the chronic, irreversible nature of fibrosis.[34,143] This persistent activation is sustained not only by biochemical cues like TGF-β but also by mechanical factors.[144,145] ECM remodeling driven by FAFs drives fibrosis progression, as their sustained contraction increases ECM strain and stiffness, mechanically activating pro-fibrotic TGF-β1 and further fibroblast activation.[34] Specifically, contraction of the stiffened matrix engages α(v)-containing integrins bound to the latency-associated peptide (LAP), triggering the release of active TGF-β.[146] This creates a vicious positive feedback loop in which FAFs produce excessive ECM, causing an increase in matrix stiffness and ligand density, and sustains FAF activation, driving progressive fibrotic remodeling.[147–149]

As this cycle progresses, several new mechanical forces come into play. Similar to tumors, collagen bundles in fibrosis are often densely packed and aligned in a parallel configuration.[150,151] This response is mediated by several common mechanotransduction pathways, including the integrin-FAK-ROCK-MRTF-YAP-TAZ signaling cascade.[152] Additionally, increased stiffness results in the overexpression of the mechanosensitive ion channel Piezo1, leading to an upregulation of Wnt2/Wnt11 expression and establishing a positive feedback loop that promotes ECM synthesis, α-SMA production, cellular contractility, and the secretion of inflammatory cytokines.[153] Piezo1 also triggers an increase in YAP/TAZ nuclear localization and, together with integrin β1, results in sustained YAP/TAZ overexpression, reinforcing ECM stiffening and contributing to the positive feedback loop.[66]

Downstream of these cytoskeletal mechanosensitive signaling pathways, FAFs exhibit increased histone deacetylases (HDAC) activity and altered chromatin structures within the nucleus, making the chromatin landscape less accessible compared to normal activated fibroblasts due to increased tension on the nuclear envelope.[43] For example, in response to stiff ECM, keloid fibroblasts deform their nuclei, causing nuclear lamina reorganization. This results in nuclear softening due to reduced lamin A/C expression, causing partial detachment of lamin-associated heterochromatin and allowing for enhanced migration through confining ECM.[59]

While both CAFs and FAFs have been extensively studied individually, recognition of the commonalities between these fibroblast populations, especially with respect to their mechanobiology, could unlock new translational opportunities. Mechanosensitive pathways discovered in FAFs could inform mechanomedicine approaches for cancer, particularly since fibrosis is often more accessible to intervention than cancer due to its less invasive and destructive nature. By leveraging insights from fibrosis research, novel strategies can be developed to target CAFs and disrupt their tumor-promoting functions.

THE HOW: ENGINEERING MECHANOMEDICINE APPROACHES TO FIBROBLAST ACTIVATION

Understanding the *in vivo* relationship between the ECM and fibroblast activation is crucial for the development of effective therapeutics to address both cancer and fibrosis.[146] To explore this relationship *in vitro*, biomedical engineers have employed a variety of experimental systems, ranging from simple 2D platforms to complex 3D models to investigate the mechanobiology of fibroblast activation in both physiological and pathological contexts (Fig. 3).

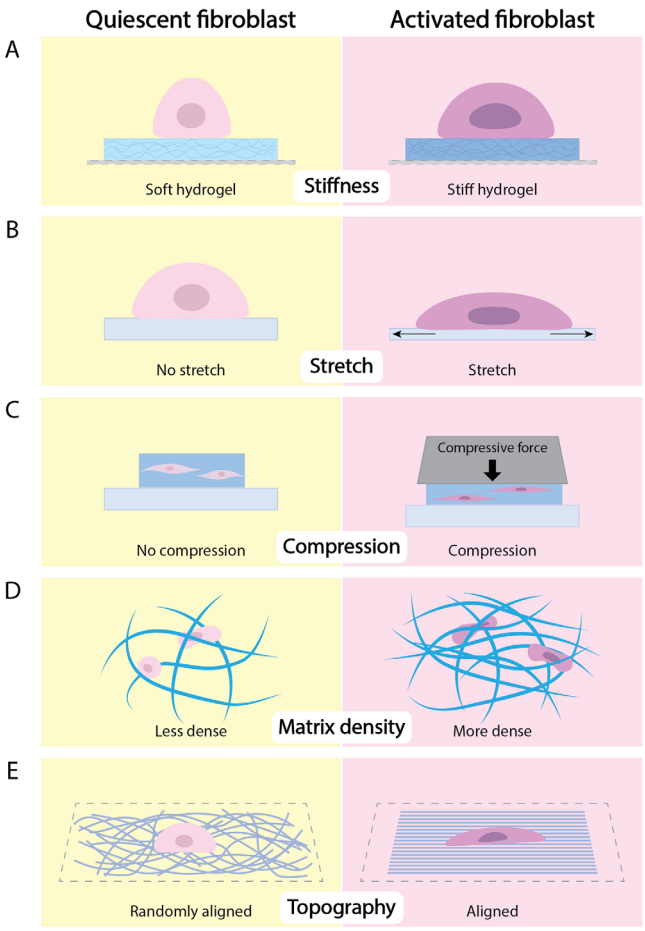

FIG. 3. Engineering techniques to study the mechanobiology of fibroblast activation. Fibroblasts experience diverse mechanical stresses in cancer and fibrosis. These mechanical stresses have been investigated through 2D and 3D systems. Engineering techniques that have been used to study fibroblast activation include the use of (a) hydrogels to vary stiffness, (b) PDMS to stretch fibroblasts, (c) weight to compress fibroblasts, (d) electrospun fibers to create matrices with varied density, and (e) micropatterned surfaces to create random and aligned topographies. These platforms have shown that dynamic changes in stiffness, stretch, compression, density, and alignment can promote fibroblast activation.

2D systems have provided a strong foundation from which the field has been able to assess the role of individual mechanical factors in isolation, including matrix topography, substrate stiffness, dynamic compression, and cyclic stretching. Cells rely on focal adhesions to sense their mechanical microenvironment and activate downstream signaling pathways accordingly. By engineering micropatterned surfaces with defined topographical features, focal adhesion dynamics can be manipulated, allowing for consequential changes in fibroblast behavior to be investigated[72,75,76] [Fig. 3(e)]. Focal adhesion size, distribution, and maturation can be modulated by altering the spacing, alignment, and geometry of micropatterns, and fibroblast activation in response to changes in focal adhesion dynamics can be studied. More specifically, microscale topographies, including microgrooves with widths ranging from 2 to 100 μm, can be patterned precisely onto various hydrogels to study fibroblast behavior.[72] These patterns can be random or aligned, with aligned topography more effectively promoting fibroblast activation.[75] Additionally, periodic features with tunable wavelength and amplitude have been used to study fibroblast migration.[76]

While matrix topography provides valuable insights into how fibroblasts sense and respond to mechanical cues, the mechanical environment *in vivo* is complex, involving dynamic forces such as stiffness, compression, and stretching. These forces have been studied individually to understand their distinct effects on fibroblast behavior.

Soft, biocompatible hydrogels coated with integrin-binding ligands have become a cornerstone in fibroblast mechanobiology studies [Fig. 3(a)]. These substrates can be precisely tuned to specific Young's modulus levels, allowing researchers to mimic the mechanical properties of both healthy and diseased tissues. High stiffness levels ranging from 30 to 50 kPa (consistent with the mechanical properties of the fibrotic microenvironment) have been shown to activate fibroblasts.[62,65,154] Their versatility makes them compatible with a wide range of experimental approaches and microscopy techniques. A variety of materials have been employed to study fibroblast activation, including synthetic polymers such as polyacrylamide (PA) and polyethylene glycol (PEG), and natural materials like gelatin methacryloyl (GelMA) and silk.[57] These platforms allow researchers to study fibroblast behavior when exposed to varying stiffnesses, providing valuable insights into mechanosensitive cell phenotypes and behavior.

To more accurately capture how cells respond to mechanical changes in the body, dynamic systems have been developed. In the body, mechanical changes are dynamic rather than static, and cellular behavior in response to gradual changes in stiffness can differ from behavior under static conditions.[59] Dynamic systems help mimic compression and stretching, which are forces present in interstitial spaces where fibroblasts reside. These forces arise from both internal sources, including muscle contraction, blood flow, and organ movement, and external sources like pressure or impacts. Cyclic compression systems apply controlled compressive forces to fibroblasts, mimicking the dynamic loading experienced in the tissues[136] [Fig. 3(c)]. Under pathological levels of compression (15%–20%), cyclic compression was sufficient to activate and maintain the activated phenotype.[83] Similarly, stretchable substrates, like the polydimethylsiloxane (PDMS) used in microfluidic devices, allow for the application of tensile forces to fibroblasts [Fig. 3(b)]. Vacuum-driven PDMS systems producing 4% strain, which matches strain levels found in tissues during movement or growth, have been used to show that fibroblasts become activated in response to mechanical stretch.[135]

As organisms exist in three dimensions, the field has advanced toward more complex *in vitro* systems containing multiple controlled mechanical cues in a 3D form factor. Like 2D systems, these platforms can also incorporate tunable stiffness, compression, and density as experimental variables, allowing for the study of fibroblast activation in environments that better mimic the *in vivo* microenvironment. 3D matrices can be created using various methods, including multicomponent hydrogels, electrospun fibers, alginate beads, and GelMA hydrogels[81,83,136] [Fig. 3(d)]. Using electrospun dextran vinyl sulfone suspended in the hydrogel, fibroblasts in denser matrices showed altered morphology, migration, and activation due to higher levels of resistance exerted on fibroblasts.[155] Similarly, stiffness-tunable hybrid hydrogels composed of type I collagen and alginate, with mechanical

properties that can be altered with calcium chloride and sodium citrate across a range of 1–21 kPa, demonstrate that matrix stiffening and softening mediates fibroblast activation.[149] This approach replicates the mechanical stress encountered in tissue, providing insights into how cells adapt to a dynamic mechanical environment.

One understudied aspect of the microenvironment that the field should consider in the future is confinement, as it plays an important role in the mechanobiology of fibroblast activation, as discussed in the previous section. There are various systems that can be utilized to study confined migration, including PDMS microchannels, grooved substrates, micropatterned surfaces, vertical confinement systems, and hydrogels[86,156] (Fig. 4). These platforms can have dimensions as narrow as 2 μm wide and can vary in channel length from shorter than the diameter of a spread cell to longer than a fully spread cell.[90,157,158] These systems are particularly valuable as they allow for the study of cellular behavior and migration dynamics before, during, and after confinement.

Some of the most advanced systems for studying fibroblast activation are *in vitro* microtissues, which offer a high degree of physiological relevance. These systems recapitulate the complex 3D architecture and mechanical microenvironment of tissues, allowing for the investigation of fibroblast behavior in conditions that closely mimic *in vivo* tissues. Microtissues can be created with fibroblasts embedded in ECM, cultured on flat surfaces, or within engineered clefts, all of which allow for the formation of de novo tissues.[159] This system allows researchers to observe the organization of ECM and study the dynamic relationship between fibroblasts and the ECM.[71,160,161] Microtissues have been used to model fibrosis, revealing how mechanical cues drive fibroblast activation and ECM deposition in a self-reinforcing cycle.[161] Similarly, they have been employed to study wound healing, demonstrating how fibroblasts remodel the ECM to restore tissue integrity.[70,159,160] This ability to precisely control mechanical and biochemical cues and investigate how fibroblasts respond makes them invaluable for advancing our understanding of fibroblast mechanobiology and developing targeted mechanomedicine therapies for disease. To incorporate more mechanical cues and cell types, fibrosis-encapsulated tumoroid (FET) models were developed, in which fibroblasts encapsulate a tumoroid, forming a fibrotic shell through ECM deposition and generating solid stress, allowing for the study of the relationship between fibroblasts and cancer cells under the influences of mechanical compression.[162] These advanced models serve as powerful platforms for investigating the complex relationship between fibroblast activation and the ECM in both healthy and diseased states and allow for the development of translational solutions to combat cancer and fibrosis.

THE WHY: FUTURE DIRECTIONS IN FIBROBLAST MECHANOMEDICINE

The nascent field of mechano-therapeutics has just started to explore new ways to target the mechanical environment and mechanobiology of fibroblasts for clinical applications. Therapeutic strategies to combat disease commonly target a subset of five general phenomena: ECM cross-linking, mechanosensitive fibroblast activation, integrin-mediated activation, cell–cell interactions, and epigenetic modulation.[163,164] Although these therapeutics have been developed and showed promising results in animal trials, many have not found clinical success in treating human disease. One of the major barriers to

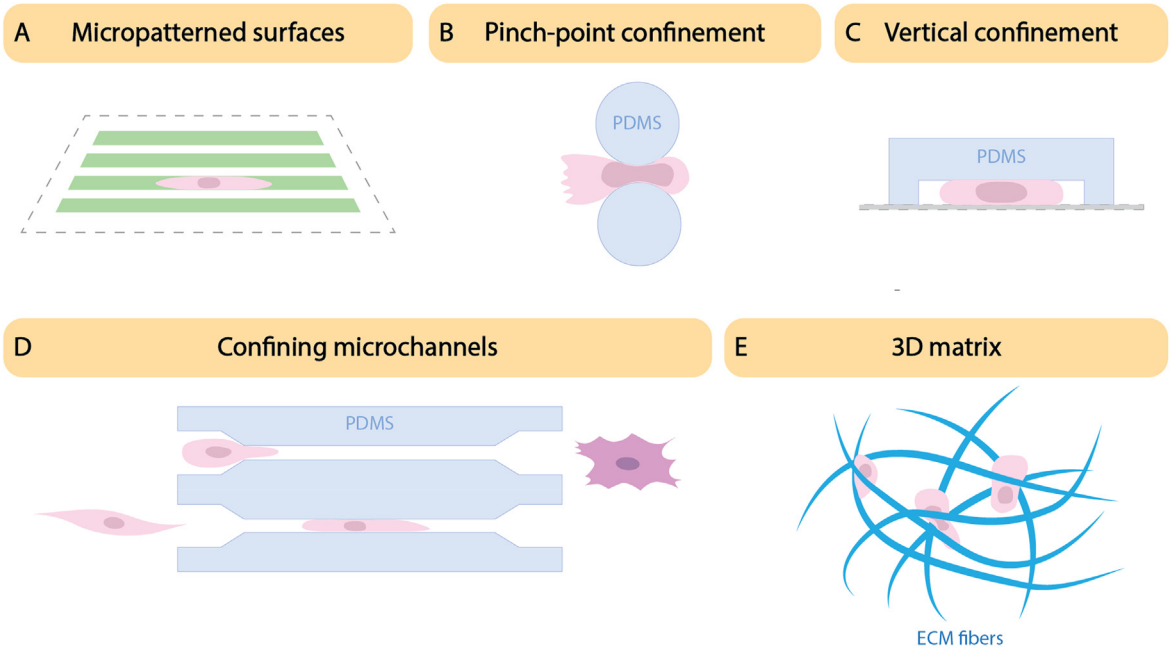

FIG. 4. Overview of key techniques to study confined migration *in vitro*. (a) Linear micropatterned surfaces guide cell alignment and migration within the defined pattern, resulting in 2D confined migration. In addition, PDMS-based platforms are used to study more physiologically relevant types of confinement, including (b) pinch-point confinement using micropillars to study intravasation and extravasation, (c) vertical confinement, and (d) long microchannels to investigate sustained confinement *in vitro*. (e) 3D ECM fiber networks are used to study the complex interplay between confinement and matrix composition.

advancing mechanomedicine is the difficulty of targeting the ECM without compromising the structural integrity and function of a given organ. Nonetheless, as there is strong evidence showing that the ECM regulates fibroblast activation and persistence in disease, targeting the mechanosensors and mechanotransduction of fibroblasts offers a promising strategy to disrupt persistent fibroblast activation.

Research to date has explored the identity and magnitude of mechanical stresses that drive fibroblast activation in various pathological contexts, including ECM stiffness, ligand density, mechanical compression, substrate strain, and topological cues. Moving forward, a critical yet understudied aspect of fibroblast mechanobiology is the role of mechanical confinement and its role in fibroblast activation during migration through interstitial spaces. Studying cell migration in a physiological context is crucial because cells continuously sense and adapt to their changing microenvironment as they move.[165] During fibroblast migration, the requirement to navigate dense and constricted ECM results in significant nuclear deformation and cytoskeletal adaptation, processes likely mediated by mechanosensitive pathways.

Future research should prioritize understanding how mechanical cues, including physical confinement, can activate fibroblasts in the context of wound healing and diseases. Mechanical stimuli present in the wound environment may amplify intracellular tension and activate mechanosensitive transcription factors, further perpetuating fibroblast activation and ECM remodeling. Interestingly, fibroblasts have been shown to retain mechanical memory, which influences their behavior long after the mechanical stimulus is removed.[166–168] Understanding both how and for how long fibroblasts retain this mechanical memory after migration through physiologically relevant microenvironments will likely yield new therapeutic opportunities in disease and wound healing. Confinement-primed fibroblasts could be harnessed for translational applications in wound healing by promoting controlled ECM remodeling to reduce fibrotic scarring and stimulating ECM production in chronic wounds.

Given the significant parallels between fibrosis and cancer progression, insights from fibrosis will continue to provide valuable insights into the behavior of CAFs. By studying the mechanisms driving FAFs, we can identify shared pathways and therapeutic targets that may also apply to CAFs, offering new strategies to disrupt their tumor-promoting functions. Understanding the mechanotransduction of fibroblast activation could lead to innovative approaches for reprogramming activated fibroblasts back to a quiescent state or programming them to undergo apoptosis, both of which would be new avenues for treating fibrosis and cancer. While significant progress has been made in understanding the mechanobiology of fibroblast activation, the continued development of 3D systems that present physiologically relevant confinement is needed. These developments hold great potential for addressing the persistent fibroblast activation that is seen in fibrosis and cancer and offer one of the best opportunities for growing the field of mechanomedicine by bridging fundamental work with clinical intervention.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Ethics Approval

Ethics approval was not required for this work.

Author Contributions

Yeji Chang: Conceptualization (equal); Writing – original draft (equal); Writing – review & editing (equal). **Jia Wen Nicole Lee:** Writing – review & editing (supporting). **Andrew W. Holle:** Conceptualization (equal); Supervision (equal); Writing – original draft (equal); Writing – review & editing (equal).

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

REFERENCES

[1]M. V. Plikus *et al.*, "Fibroblasts: Origins, definitions, and functions in health and disease," Cell **184**, 3852–3872 (2021).
[2]V. S. LeBleu and E. G. Neilson, "Origin and functional heterogeneity of fibroblasts," FASEB J. **34**, 3519–3536 (2020).
[3]M. Zeisberg and E. G. Neilson, "Biomarkers for epithelial-mesenchymal transitions," J. Clin. Invest. **119**, 1429 (2009).
[4]A. Salminen, "The role of immunosuppressive myofibroblasts in the aging process and age-related diseases," J. Mol. Med. **101**, 1169 (2023).
[5]R. Kalluri and E. G. Neilson, "Epithelial-mesenchymal transition and its implications for fibrosis," J. Clin. Invest. **112**, 1776 (2003).
[6]H. Z. Movat and N. V. P. Fernando, "The fine structure of connective tissue: I. The fibroblast," Exp. Mol. Pathol. **1**, 509–534 (1962).
[7]R. J. McAnulty, "Fibroblasts and myofibroblasts: Their source, function and role in disease," Int. J. Biochem. Cell Biol. **39**, 666–671 (2007).
[8]S. Davidson *et al.*, "Fibroblasts as immune regulators in infection, inflammation and cancer," Nat. Rev. Immunol. **21**, 704–718 (2021).
[9]K. Y. DeLeon-Pennell, T. H. Barker, and M. L. Lindsey, "Fibroblasts: The arbiters of extracellular matrix remodeling," Matrix Biol. **91–92**, 1–7 (2020).
[10]U. Lendahl, L. Muhl, and C. Betsholtz, "Identification, discrimination and heterogeneity of fibroblasts," Nat. Commun. **13**, 3409 (2022).
[11]G. Apodaca, "Defining the molecular fingerprint of bladder and kidney fibroblasts," Am. J. Physiol. Renal Physiol. **325**, F826–F856 (2023).
[12]M. B. Buechler *et al.*, "Cross-tissue organization of the fibroblast lineage," Nature **593**, 575–579 (2021).
[13]H. Y. Chang *et al.*, "Diversity, topographic differentiation, and positional memory in human fibroblasts," Proc. Natl. Acad. Sci. U. S. A. **99**, 12877–12882 (2002).
[14]S. H. Phan, "Biology of fibroblasts and myofibroblasts," Proc. Am. Thorac. Soc. **5**, 334 (2008).
[15]F. Strutz *et al.*, "Identification and characterization of a fibroblast marker: FSP1," J. Cell Biol. **130**, 393–405 (1995).
[16]J. D. Lajiness and S. J. Conway, "Origin, development, and differentiation of cardiac fibroblasts," J. Mol. Cell. Cardiol. **70**, 2 (2014).
[17]P. Kong, P. Christia, A. Saxena, Y. Su, and N. G. Frangogiannis, "Lack of specificity of fibroblast-specific protein 1 in cardiac remodeling and fibrosis," Am. J. Physiol. Heart Circ. Physiol. **305**, H1363–H1372 (2013).
[18]Z. Kahounová *et al.*, "The fibroblast surface markers FAP, anti-fibroblast, and FSP are expressed by cells of epithelial origin and may be altered during epithelial-to-mesenchymal transition," Cytometry, Part A **93**, 941–951 (2018).
[19]E. C. Goldsmith, A. D. Bradshaw, M. R. Zile, and F. G. Spinale, "Myocardial fibroblast-matrix interactions and potential therapeutic targets," J. Mol. Cell. Cardiol. **70**, 92–99 (2014).

[20]T. Tabib, C. Morse, T. Wang, W. Chen, and R. Lafyatis, "SFRP2/DPP4 and FMO1/LSP1 define major fibroblast populations in human skin," J. Invest. Dermatol. **138**, 802–810 (2018).
[21]L. Muhl *et al.*, "Single-cell analysis uncovers fibroblast heterogeneity and criteria for fibroblast and mural cell identification and discrimination," Nat. Commun. **11**, 3953 (2020).
[22]Y. Chen *et al.*, "Type-I collagen produced by distinct fibroblast lineages reveals specific function during embryogenesis and Osteogenesis Imperfecta," Nat. Commun. **12**, 7199 (2021).
[23]M. D. Lynch and F. M. Watt, "Fibroblast heterogeneity: Implications for human disease," J. Clin. Invest. **128**, 26 (2018).
[24]D. Avery *et al.*, "Extracellular matrix directs phenotypic heterogeneity of activated fibroblasts," Matrix Biol. **67**, 90–106 (2018).
[25]J. L. Rinn, C. Bondre, H. B. Gladstone, P. O. Brown, and H. Y. Chang, "Anatomic demarcation by positional variation in fibroblast gene expression programs," PLoS Genet. **2**, e119 (2006).
[26]A. Salminen, K. Kaarniranta, and A. Kauppinen, "Tissue fibroblasts are versatile immune regulators: An evaluation of their impact on the aging process," Ageing Res. Rev. **97**, 102296 (2024).
[27]F. Boraldi *et al.*, "The role of fibroblasts in skin homeostasis and repair," Biomedicines **12**, 1586 (2024).
[28]D. S. Foster *et al.*, "Integrated spatial multiomics reveals fibroblast fate during tissue repair," Proc. Natl. Acad. Sci. **118**, e2110025118 (2021).
[29]I. A. Darby, B. Laverdet, F. Bonté, and A. Desmoulière, "Fibroblasts and myofibroblasts in wound healing," Clin. Cosmet. Invest. Dermatol. **7**, 301 (2014).
[30]T. Schreier, E. Degen, and W. Baschong, "Fibroblast migration and proliferation during in vitro wound healing," Res. Exp. Med. **193**, 195–205 (1993).
[31]A. T. Melvin, E. S. Welf, Y. Wang, D. J. Irvine, and J. M. Haugh, "In chemotaxing fibroblasts, both high-fidelity and weakly biased cell movements track the localization of PI3K signaling," Biophys. J. **100**, 1893–1901 (2011).
[32]M. I. Suwara *et al.*, "IL-1α released from damaged epithelial cells is sufficient and essential to trigger inflammatory responses in human lung fibroblasts," Mucosal Immunol. **7**, 684–693 (2014).
[33]B. Hinz *et al.*, "The myofibroblast: One function, multiple origins," Am. J. Pathol. **170**, 1807 (2007).
[34]B. Hinz and D. Lagares, "Evasion of apoptosis by myofibroblasts: A hallmark of fibrotic diseases," Nat. Rev. Rheumatol. **16**, 11–31 (2020).
[35]A. Hillsley *et al.*, "A strategy to quantify myofibroblast activation on a continuous spectrum," Sci. Rep. **12**, 12239 (2022).
[36]J. J. Tomasek, G. Gabbiani, B. Hinz, C. Chaponnier, and R. A. Brown, "Myofibroblasts and mechano-regulation of connective tissue remodelling," Nat. Rev. Mol. Cell Biol. **3**, 349–363 (2002).
[37]B. Hinz and G. Gabbiani, "Cell-matrix and cell-cell contacts of myofibroblasts: Role in connective tissue remodeling," Thromb. Haemost. **90**, 993–1002 (2003).
[38]H. Zhang *et al.*, "Generation of quiescent cardiac fibroblasts from human induced pluripotent stem cells for in vitro modeling of cardiac fibrosis," Circ. Res. **125**, 552 (2019).
[39]V. Dugina, L. Fontao, C. Chaponnier, J. Vasiliev, and G. Gabbiani, "Focal adhesion features during myofibroblastic differentiation are controlled by intracellular and extracellular factors," J. Cell Sci. **114**, 3285–3296 (2001).
[40]B. Hinz, V. Dugina, C. Ballestrem, B. Wehrle-Haller, and C. Chaponnier, "α-Smooth muscle actin is crucial for focal adhesion maturation in myofibroblasts," Mol. Biol. Cell **14**, 2508–2519 (2003).
[41]E. L. Elson, H. Qian, J. A. Fee, and T. Wakatsuki, "A model for positive feedback control of the transformation of fibroblasts to myofibroblasts," Prog. Biophys. Mol. Biol. **144**, 30–40 (2019).
[42]C. K. Nagaraju *et al.*, "Myofibroblast modulation of cardiac myocyte structure and function," Sci. Rep. **9**, 8879 (2019).
[43]C. J. Walker *et al.*, "Nuclear mechanosensing drives chromatin remodelling in persistently activated fibroblasts," Nat. Biomed. Eng. **5**, 1485–1499 (2021).
[44]M. D'Urso, I. Jorba, A. van der Pol, C. V. C. Bouten, and N. A. Kurniawan, "Spatial regulation of substrate adhesion directs fibroblast morphotype and phenotype," PNAS Nexus **3**, pgae289 (2024).
[45]G. Grieb, G. Steffens, N. Pallua, J. Bernhagen, and R. Bucala, "Circulating fibrocytes—Biology and mechanisms in wound healing and scar formation," in *International Review of Cell and Molecular Biology* (Elsevier, 2011), Vol. 291, pp. 1–19.
[46]D. Pilling, T. Fan, D. Huang, B. Kaul, and R. Gomer, "Identification of markers that distinguish monocyte-derived fibrocytes from monocytes, macrophages, and fibroblasts," PLoS One **4**, e7475 (2009).
[47]H. Suga *et al.*, "Tracking the elusive fibrocyte: Identification and characterization of collagen producing hematopoietic lineage cells during murine wound healing," Stem Cells **32**, 1347–1360 (2014).
[48]R. A. Reilkoff, R. Bucala, and E. L. Herzog, "Fibrocytes: Emerging effector cells in chronic inflammation," Nat. Rev. Immunol. **11**, 427–435 (2011).
[49]C. Ling *et al.*, "Differentiated fibrocytes assume a functional mesenchymal phenotype with regenerative potential," Sci. Adv. **5**, eaav7384 (2019).
[50]D. E. Jaalouk and J. Lammerding, "Mechanotransduction gone awry," Nat. Rev. Mol. Cell Biol. **10**, 63–73 (2009).
[51]M. G. Ushakumary, M. Riccetti, and A.-K. T. Perl, "Resident interstitial lung fibroblasts and their role in alveolar stem cell niche development, homeostasis, injury, and regeneration," Stem Cells Transl. Med. **10**, 1021–1032 (2021).
[52]F. S. Younesi, A. E. Miller, T. H. Barker, F. M. V. Rossi, and B. Hinz, "Fibroblast and myofibroblast activation in normal tissue repair and fibrosis," Nat. Rev. Mol. Cell Biol. **25**, 617–638 (2024).
[53]A. Saraswathibhatla, D. Indana, and O. Chaudhuri, "Cell–extracellular matrix mechanotransduction in 3D," Nat. Rev. Mol. Cell Biol. **24**, 495 (2023).
[54]R. G. Wells, "Tissue mechanics and fibrosis," Biochim. Biophys. Acta BBA **1832**, 884–890 (2013).
[55]X.-K. Zhao *et al.*, "Focal adhesion kinase regulates fibroblast migration via integrin beta-1 and plays a central role in fibrosis," Sci. Rep. **6**, 19276 (2016).
[56]Y. Tai *et al.*, "Myofibroblasts: Function, formation, and scope of molecular therapies for skin fibrosis," Biomolecules **11**, 1095 (2021).
[57]S. G. Szeto *et al.*, "YAP/TAZ are mechanoregulators of TGF-β-Smad signaling and renal fibrogenesis," J. Am. Soc. Nephrol. **27**, 3117–3128 (2016).
[58]K. M. Herum, J. Choppe, A. Kumar, A. J. Engler, and A. D. McCulloch, "Mechanical regulation of cardiac fibroblast profibrotic phenotypes," Mol. Biol. Cell **28**, 1871–1882 (2017).
[59]X. Fu *et al.*, "Targeting nuclear mechanics mitigates the fibroblast invasiveness in pathological dermal scars induced by matrix stiffening," Adv. Sci. **11**, 2308253 (2024).
[60]L. Niu *et al.*, "Matrix stiffness controls cardiac fibroblast activation through regulating YAP via AT1R," J. Cell. Physiol. **235**, 8345–8357 (2020).
[61]A. Aravamudhan *et al.*, "TBK1 regulates YAP/TAZ and fibrogenic fibroblast activation," Am. J. Physiol. Lung Cell. Mol. Physiol. **318**, L852–L863 (2020).
[62]J. He, B. Fang, S. Shan, and Q. Li, "Mechanical stiffness promotes skin fibrosis through FAPα-AKT signaling pathway," J. Dermatol. Sci. **113**, 51–61 (2024).
[63]S. O. Rahaman *et al.*, "TRPV4 mediates myofibroblast differentiation and pulmonary fibrosis in mice," J. Clin. Invest. **124**, 5225–5238 (2014).
[64]H. Wang, M. W. Tibbitt, S. J. Langer, L. A. Leinwand, and K. S. Anseth, "Hydrogels preserve native phenotypes of valvular fibroblasts through an elasticity-regulated PI3K/AKT pathway," Proc. Natl. Acad. Sci. U. S. A. **110**, 19336–19341 (2013).
[65]K. W. Yong *et al.*, "Paracrine effects of adipose-derived stem cells on matrix stiffness-induced cardiac myofibroblast differentiation via angiotensin II type 1 receptor and Smad7," Sci. Rep. **6**, 33067 (2016).
[66]L. Niu *et al.*, "A positive mechanobiological feedback loop controls bistable switching of cardiac fibroblast phenotype," Cell Discovery **8**, 84 (2022).
[67]K. M. Hakkinen, J. S. Harunaga, A. D. Doyle, and K. M. Yamada, "Direct comparisons of the morphology, migration, cell adhesions, and actin cytoskeleton of fibroblasts in four different three-dimensional extracellular matrices," Tissue Eng. Part A **17**, 713–724 (2011).
[68]J. P. Woodley, D. W. Lambert, and I. O. Asencio, "Understanding fibroblast behavior in 3D biomaterials," Tissue Eng. Part B **28**, 569–578 (2022).
[69]C. D. Davidson *et al.*, "Myofibroblast activation in synthetic fibrous matrices composed of dextran vinyl sulfone," Acta Biomater. **105**, 78–86 (2020).
[70]M. C. Benn *et al.*, "How the mechanobiology orchestrates the iterative and reciprocal ECM-cell cross-talk that drives microtissue growth," Sci. Adv. **9**, eadd9275 (2023).
[71]Y. Hong *et al.*, "Cell–matrix feedback controls stretch-induced cellular memory and fibroblast activation," Proc. Natl. Acad. Sci. **122**, e2322762122 (2025).

[72]M. Bril *et al.*, "Digital photoinduced topographical microsculpting of hydrogels," Adv. Mater. Technol. **9**, 2400721 (2024).
[73]Y. Li *et al.*, "Controlling the multiscale network structure of fibers to stimulate wound matrix rebuilding by fibroblast differentiation," ACS Appl. Mater. Interfaces **11**, 28377–28386 (2019).
[74]Y. Xu *et al.*, "ECM-inspired micro/nanofibers for modulating cell function and tissue generation," Sci. Adv. **6**, eabc2036 (2020).
[75]D. Bugg *et al.*, "Infarct collagen topography regulates fibroblast fate via p38-yes-associated protein transcriptional enhanced associate domain signals," Circ. Res. **127**, 1306–1322 (2020).
[76]L. Ge, L. Yang, R. Bron, J. K. Burgess, and P. van Rijn, "Topography-mediated fibroblast cell migration is influenced by direction, wavelength, and amplitude," ACS Appl. Bio Mater. **3**, 2104–2116 (2020).
[77]Z. Cao, J. K. Ball, A. H. Lateef, C. P. Virgile, and E. A. Corbin, "Biomimetic substrate to probe dynamic interplay of topography and stiffness on cardiac fibroblast activation," ACS Omega **8**, 5406–5414 (2023).
[78]J. M. Goffin *et al.*, "Focal adhesion size controls tension-dependent recruitment of α-smooth muscle actin to stress fibers," J. Cell Biol. **172**, 259–268 (2006).
[79]M. Bril *et al.*, "Shape-morphing photoresponsive hydrogels reveal dynamic topographical conditioning of fibroblasts," Adv. Sci. **10**, 2303136 (2023).
[80]L. Landolt, G. C. Spagnoli, A. Hertig, I. Brocheriou, and H.-P. Marti, "Fibrosis and cancer: Shared features and mechanisms suggest common targeted therapeutic approaches," Nephrol. Dial. Transplant. **37**, 1024–1032 (2022).
[81]S. Devarasou, M. Kang, T. Y. Kwon, Y. Cho, and J. H. Shin, "Fibrous matrix architecture-dependent activation of fibroblasts with a cancer-associated fibroblast-like phenotype," ACS Biomater. Sci. Eng. **9**, 280–291 (2023).
[82]M. Kalli, P. Papageorgis, V. Gkretsi, and T. Stylianopoulos, "Solid stress facilitates fibroblasts activation to promote pancreatic cancer cell migration," Ann. Biomed. Eng. **46**, 657–669 (2018).
[83]M. Kong *et al.*, "Cardiac fibrotic remodeling on a chip with dynamic mechanical stimulation," Adv. Healthc. Mater. **8**, 1801146 (2019).
[84]F. Alisafaei *et al.*, "Tension anisotropy drives fibroblast phenotypic transition by self-reinforcing cell–extracellular matrix mechanical feedback," Nat. Mater. **24**, 955–911 (2025).
[85]M. D. Mitchell, R. E. Laird, R. D. Brown, and C. S. Long, "IL-1β stimulates rat cardiac fibroblast migration via MAP kinase pathways," Am. J. Physiol. Heart Circ. Physiol. **292**, H1139–H1147 (2007).
[86]C. D. Paul, W.-C. Hung, D. Wirtz, and K. Konstantopoulos, "Engineered models of confined cell migration," Annu. Rev. Biomed. Eng. **18**, 159–180 (2016).
[87]D. J. Tschumperlin, "Fibroblasts and the ground they walk on," Physiology **28**, 380–390 (2013).
[88]M. Miron-Mendoza, X. Lin, L. Ma, P. Ririe, and W. M. Petroll, "Individual versus collective fibroblast spreading and migration: Regulation by matrix composition in 3-D culture," Exp. Eye Res. **99**, 36–44 (2012).
[89]X. Gao *et al.*, "Confined migration drives stem cell differentiation," Adv. Sci. **12**, 2415407 (2025).
[90]Y. Li *et al.*, "Confinement-sensitive volume regulation dynamics via high-speed nuclear morphological measurements," Proc. Natl. Acad. Sci. U. S. A. **121**, e2408595121 (2024).
[91]A. J. Lomakin *et al.*, "The nucleus acts as a ruler tailoring cell responses to spatial constraints," Science **370**, eaba2894 (2020).
[92]S. Cho, J. Irianto, and D. E. Discher, "Mechanosensing by the nucleus: From pathways to scaling relationships," J. Cell Biol. **216**, 305–315 (2017).
[93]K. L. Harper *et al.*, "Virus-modified paraspeckle-like condensates are hubs for viral RNA processing and their formation drives genomic instability," Nat. Commun. **15**, 10240 (2024).
[94]V. Todorovski *et al.*, "Confined environments induce polarized paraspeckle condensates," Commun. Biol. **6**, 145 (2023).
[95]A. Desmoulire and I. Darby, "Apoptosis mediates the decrease cellularity during the transition between granulation tissue scar," Am. J. Pathol. **146**, 56 (1995).
[96]R. Kalluri, "The biology and function of fibroblasts in cancer," Nat. Rev. Cancer **16**, 582–598 (2016).
[97]M. Zhao *et al.*, "Targeting fibrosis: Mechanisms and clinical trials," Signal Transduction Targeted Ther. **7**, 206 (2022).
[98]L. Chadli *et al.*, "Identification of regulators of the myofibroblast phenotype of primary dermal fibroblasts from early diffuse systemic sclerosis patients," Sci. Rep. **9**, 4521 (2019).
[99]A. R. Rigau and C. Dees, "Mechanisms of fibroblast activation during fibrotic tissue remodeling," Fibrosis **2**, 10002 (2024).
[100]R. K. Bhogal, C. M. Stoica, T. L. McGaha, and C. A. Bona, "Molecular aspects of regulation of collagen gene expression in fibrosis," J. Clin. Immunol. **25**, 592–603 (2005).
[101]J. Thorlacius-Ussing *et al.*, "The collagen landscape in cancer: Profiling collagens in tumors and in circulation reveals novel markers of cancer-associated fibroblast subtypes," J. Pathol. **262**, 22–36 (2024).
[102]H. Sato *et al.*, "Multifaced roles of desmoplastic reaction and fibrosis in pancreatic cancer progression: Current understanding and future directions," Cancer Sci. **114**, 3487–3495 (2023).
[103]C. H. Lee *et al.*, "Nanofiber alignment and direction of mechanical strain affect the ECM production of human ACL fibroblast," Biomaterials **26**, 1261–1270 (2005).
[104]I. Druzhkova *et al.*, "Expression of EMT-related genes in hybrid E/M colorectal cancer cells determines fibroblast activation and collagen remodeling," Int. J. Mol. Sci. **21**, 8119 (2020).
[105]M. Tisler *et al.*, "Analysis of fibroblast migration dynamics in idiopathic pulmonary fibrosis using image-based scaffolds of the lung extracellular matrix," Am. J. Physiol. Lung Cell. Mol. Physiol. **318**, L276–L286 (2020).
[106]F. Calvo *et al.*, "Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts," Nat. Cell Biol. **15**, 637–656 (2013).
[107]F. Liu *et al.*, "Mechanosignaling through YAP and TAZ drives fibroblast activation and fibrosis," Am. J. Physiol. Lung Cell. Mol. Physiol. **308**, L344–L357 (2015).
[108]S. Noguchi *et al.*, "TAZ contributes to pulmonary fibrosis by activating profibrotic functions of lung fibroblasts," Sci. Rep. **7**, 42595 (2017).
[109]S. Noguchi, A. Saito, and T. Nagase, "YAP/TAZ signaling as a molecular link between fibrosis and cancer," Int. J. Mol. Sci. **19**, 3674 (2018).
[110]X. He *et al.*, "Myofibroblast YAP/TAZ activation is a key step in organ fibrogenesis," JCI Insight **7**, e146243 (2022).
[111]V. Sharma, J. Letson, and S. Furuta, "Fibrous stroma: Driver and passenger in cancer development," Sci. Signal. **15**, eabg3449 (2022).
[112]M. J. Paszek and V. M. Weaver, "The tension mounts: Mechanics meets morphogenesis and malignancy," J. Mammary Gland Biol. Neoplasia **9**, 325–342 (2004).
[113]Q. Liu, Q. Luo, Y. Ju, and G. Song, "Role of the mechanical microenvironment in cancer development and progression," Cancer Biol. Med. **17**, 282–292 (2020).
[114]A. Nagelkerke, J. Bussink, A. E. Rowan, and P. N. Span, "The mechanical microenvironment in cancer: How physics affects tumours," Semin. Cancer Biol. **35**, 62–70 (2015).
[115]H. T. Nia *et al.*, "Solid stress and elastic energy as measures of tumour mechanopathology," Nat. Biomed. Eng. **1**, 0004 (2016).
[116]M. S. Pepeler *et al.*, "Prognostic impact of bone marrow fibrosis and effects of tyrosine kinase inhibitors on bone marrow fibrosis in chronic myeloid leukemia," Clin. Lymphoma Myeloma Leuk. **24**, e161–e167 (2024).
[117]H. Zhang *et al.*, "Impact of bone marrow fibrosis on outcomes of allogeneic hematopoietic stem cell transplantation in acute myeloid leukemia," Bone Marrow Transplant. **59**, 1654–1666 (2024).
[118]K. R. Levental *et al.*, "Matrix crosslinking forces tumor progression by enhancing integrin signaling," Cell **139**, 891–906 (2009).
[119]A. Sadlonova *et al.*, "Identification of molecular distinctions between normal breast-associated fibroblasts and breast cancer-associated fibroblasts," Cancer Microenviron. **2**, 9 (2009).
[120]R. Nedaeinia *et al.*, "The role of cancer-associated fibroblasts and exosomal miRNAs-mediated intercellular communication in the tumor microenvironment and the biology of carcinogenesis: A systematic review," Cell Death Discovery **10**, 380 (2024).
[121]C. Han, T. Liu, and R. Yin, "Biomarkers for cancer-associated fibroblasts," Biomarker Res. **8**, 64 (2020).
[122]L. Mathieson, L. Koppensteiner, D. A. Dorward, R. A. O'Connor, and A. R. Akram, "Cancer-associated fibroblasts expressing fibroblast activation protein

and podoplanin in non-small cell lung cancer predict poor clinical outcome," Br. J. Cancer **130**, 1758–1769 (2024).
[123]N. Paland *et al.*, "Differential influence of normal and cancer-associated fibroblasts on the growth of human epithelial cells in an in vitro cocultivation model of prostate cancer," Mol. Cancer Res. **7**, 1212–1223 (2009).
[124]G. Klein, "Evolutionary aspects of cancer resistance," Semin. Cancer Biol. **25**, 10–14 (2014).
[125]B. Erdogan *et al.*, "Cancer-associated fibroblasts promote directional cancer cell migration by aligning fibronectin," J. Cell Biol. **216**, 3799 (2017).
[126]Q. Peng *et al.*, "Biological characteristics and genetic heterogeneity between carcinoma-associated fibroblasts and their paired normal fibroblasts in human breast cancer," PLoS One **8**, e60321 (2013).
[127]N. P. Jobe *et al.*, "Simultaneous blocking of IL-6 and IL-8 is sufficient to fully inhibit CAF-induced human melanoma cell invasiveness," Histochem. Cell Biol. **146**, 205–217 (2016).
[128]L. Xiao *et al.*, "TRAP1 suppresses oral squamous cell carcinoma progression by reducing oxidative phosphorylation metabolism of cancer-associated fibroblasts," BMC Cancer **21**, 1329 (2021).
[129]R. Wieder, "Fibroblasts as turned agents in cancer progression," Cancers **15**, 2014 (2023).
[130]Y. Yu *et al.*, "Cancer-associated fibroblasts induce epithelial–mesenchymal transition of breast cancer cells through paracrine TGF-β signalling," Br. J. Cancer **110**, 724 (2014).
[131]B. C. Özdemir *et al.*, "Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival," Cancer Cell **25**, 719–734 (2014).
[132]K. A. Gieniec, L. M. Butler, D. L. Worthley, and S. L. Woods, "Cancer-associated fibroblasts—Heroes or villains?," Br. J. Cancer **121**, 293–302 (2019).
[133]R. Francescone, H. C. Crawford, and D. B. Vendramini-Costa, "Rethinking the roles of cancer-associated fibroblasts in pancreatic cancer," Cell. Mol. Gastroenterol. Hepatol. **17**, 737–743 (2024).
[134]P. B. Rozenchan *et al.*, "Specific upregulation of RHOA and RAC1 in cancer-associated fibroblasts found at primary tumor and lymph node metastatic sites in breast cancer," Tumor Biol. **36**, 9589–9597 (2015).
[135]M. Ao *et al.*, "Stretching fibroblasts remodels fibronectin and alters cancer cell migration," Sci. Rep. **5**, 8334 (2015).
[136]B. G. Kim *et al.*, "Mechanical compression induces VEGFA overexpression in breast cancer via DNMT3A-dependent miR-9 downregulation," Cell Death Dis. **8**, e2646–e2646 (2017).
[137]A. J. Haak, Q. Tan, and D. J. Tschumperlin, "Matrix biomechanics and dynamics in pulmonary fibrosis," Matrix Biol. **73**, 64–76 (2018).
[138]A. L. Olsen *et al.*, "Hepatic stellate cells require a stiff environment for myofibroblastic differentiation," Am. J. Physiol. Gastrointest. Liver Physiol. **301**, G110–G118 (2011).
[139]A. Santos and D. Lagares, "Matrix stiffness: The conductor of organ fibrosis," Curr. Rheumatol. Rep. **20**, 2 (2018).
[140]D. C. Rockey, P. D. Bell, and J. A. Hill, "Fibrosis—A common pathway to organ injury and failure," N. Engl. J. Med. **372**, 1138–1149 (2015).
[141]E. Y. Wang *et al.*, "Biowire model of interstitial and focal cardiac fibrosis," ACS Cent. Sci. **5**, 1146–1158 (2019).
[142]S. Sawamura *et al.*, "Elevated alpha 1(I) to alpha 2(I) collagen ratio in dermal fibroblasts possibly contributes to fibrosis in systemic sclerosis," Int. J. Mol. Sci. **23**, 6811 (2022).
[143]C. Hall *et al.*, "Chronic activation of human cardiac fibroblasts in vitro attenuates the reversibility of the myofibroblast phenotype," Sci. Rep. **13**, 12137 (2023).
[144]Z. Deng *et al.*, "TGF-β signaling in health, disease and therapeutics," Signal Transduction Targeted Ther. **9**, 61 (2024).
[145]S. Mascharak *et al.*, "Modelling and targeting mechanical forces in organ fibrosis," Nat. Rev. Bioeng. **2**, 305–323 (2024).
[146]A. Leask, A. Naik, and R. J. Stratton, "Back to the future: Targeting the extracellular matrix to treat systemic sclerosis," Nat. Rev. Rheumatol. **19**, 713–723 (2023).
[147]M. W. Parker *et al.*, "Fibrotic extracellular matrix activates a profibrotic positive feedback loop," J. Clin. Invest. **124**, 1622–1635 (2014).
[148]H. Saini *et al.*, "The role of tumor-stroma interactions on desmoplasia and tumorigenicity within a microengineered 3D platform," Biomaterials **247**, 119975 (2020).
[149]Y. Han *et al.*, "3D matrix stiffness modulation unveils cardiac fibroblast phenotypic switching," Sci. Rep. **14**, 17015 (2024).
[150]M. Perepelyuk *et al.*, "Normal and fibrotic rat livers demonstrate shear strain softening and compression stiffening: A model for soft tissue mechanics," PLoS One **11**, e0146588 (2016).
[151]G. M. Fomovsky, A. D. Rouillard, and J. W. Holmes, "Regional mechanics determine collagen fiber structure in healing myocardial infarcts," J. Mol. Cell. Cardiol. **52**, 1083–1090 (2012).
[152]X. Huang *et al.*, "Matrix stiffness-induced myofibroblast differentiation is mediated by intrinsic mechanotransduction," Am. J. Respir. Cell Mol. Biol. **47**, 340–348 (2012).
[153]J. He, X. Cheng, B. Fang, S. Shan, and Q. Li, "Mechanical stiffness promotes skin fibrosis via Piezo1-Wnt2/Wnt11-CCL24 positive feedback loop," Cell Death Dis. **15**, 84 (2024).
[154]J. He, B. Fang, S. Shan, and Q. Li, "Mechanical stiffness promotes skin fibrosis through Piezo1-mediated arginine and proline metabolism," Cell Death Discovery **9**, 354 (2023).
[155]D. L. Matera *et al.*, "Microengineered 3D pulmonary interstitial mimetics highlight a critical role for matrix degradation in myofibroblast differentiation," Sci. Adv. **6**, eabb5069 (2020).
[156]D. B. Brückner and C. P. Broedersz, "Learning dynamical models of single and collective cell migration: A review," Rep. Prog. Phys. **87**, 056601 (2024).
[157]A. W. Holle *et al.*, "Cancer cells invade confined microchannels via a self-directed mesenchymal-to-amoeboid transition," Nano Lett. **19**, 2280–2290 (2019).
[158]J. Z. Zhao, J. Xia, and C. P. Brangwynne, "Chromatin compaction during confined cell migration induces and reshapes nuclear condensates," Nat. Commun. **15**, 9964 (2024).
[159]Y. Xu, R. Koya, K. Ask, and R. Zhao, "Engineered microenvironment for the study of myofibroblast mechanobiology," Wound Repair Regener. **29**, 588–596 (2021).
[160]P. Kollmannsberger, C. M. Bidan, J. W. C. Dunlop, P. Fratzl, and V. Vogel, "Tensile forces drive a reversible fibroblast-to-myofibroblast transition during tissue growth in engineered clefts," Sci. Adv. **4**, eaao4881 (2018).
[161]M. Walker, M. Godin, and A. E. Pelling, "Mechanical stretch sustains myofibroblast phenotype and function in microtissues through latent TGF-β1 activation," Integr. Biol. **12**, 199–210 (2020).
[162]Y. Jang *et al.*, "Fibrosis-encapsulated tumoroid, a solid cancer assembloid model for cancer research and drug screening," Adv. Healthc. Mater. **13**, 2402391 (2024).
[163]D. J. Tschumperlin and D. Lagares, "Mechano-therapeutics: Targeting mechanical signaling in fibrosis and tumor stroma," Pharmacol. Ther. **212**, 107575 (2020).
[164]M. Ezzo and B. Hinz, "Novel approaches to target fibroblast mechanotransduction in fibroproliferative diseases," Pharmacol. Ther. **250**, 108528 (2023).
[165]M. S. Chitnis, X. Gao, J. Marlena, and A. W. Holle, "The mechanical journey of primordial germ cells," Am. J. Physiol. Cell Physiol. **327**, C1532–C1545 (2024).
[166]O. Y. Dudaryeva, S. Bernhard, M. W. Tibbitt, and C. Labouesse, "Implications of cellular mechanical memory in bioengineering," ACS Biomater. Sci. Eng. **9**, 5985–5998 (2023).
[167]C. M. Novak, J. S. Wheat, S. N. Ghadiali, and M. N. Ballinger, "Mechanomemory of pulmonary fibroblasts demonstrates reversibility of transcriptomics and contraction phenotypes," Biomaterials **314**, 122830 (2025).
[168]J. W. N. Lee and A. W. Holle, "Engineering approaches for understanding mechanical memory in cancer metastasis," APL Bioeng. **8**, 021503 (2024).