Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in
Biomedical Engineering

Beyond static models: Mechanically dynamic matrices reveal new insights into cancer and fibrosis progression

M. Walker[1,2], D. Gourdon[1,2] and M. Cantini[1,2]

The dynamic mechanical nature of extracellular matrices (ECMs) is crucial for the mechanosensitive regulation of cell fate. This is evident in pathological conditions such as cancer and fibrosis, which are characterised by highly fibrotic tissue developing over time. This fibrotic progression not only alters tissue mechanics, but also coincides with the reprogramming of resident cells, promoting their differentiation into aberrant phenotypes and increasing drug resistance. Hydrogels, with their tuneable mechanical and biochemical properties, emerge as powerful ECM mimetics to model and study these abnormal, mechanically-driven cell differentiation phenomena. In this review, after establishing how conventional, mechanically static hydrogels contribute to our understanding of the role of altered mechanosensing in cell differentiation during cancer and fibrosis, we explore the research opportunities given by advanced dynamic matrices. Models employing hydrogels that are fast relaxing, plastic or even with temporally switchable mechanics reveal the otherwise hidden role of time-dependent phenomena during disease development.

Addresses
[1] Centre for the Cellular Microenvironment, Advanced Research Centre, University of Glasgow, UK
[2] Division of Biomedical Engineering, James Watt School of Engineering, University of Glasgow, UK

Corresponding author: Cantini, M (marco.cantini@glasgow.ac.uk)

Current Opinion in Biomedical Engineering 2025, **33:**100570

This review comes from a themed issue on **Cellular and Molecular Aspects of Mechanobiology of the Extracellular Matrix**

Edited by **Delphine Gourdon**

Received 23 September 2024, revised 22 November 2024, accepted 25 November 2024

Available online xxx

https://doi.org/10.1016/j.cobme.2024.100570

Keywords
Extracellular matrix, Cancer, Fibrosis, Mechanosensing, Cell differentiation.

Introduction

ECM remodelling

Abnormal fibrotic wound healing is a highly dynamic, multistage response characterised by inflammation, proliferation, and remodelling [1]; this draws many parallels to the events that occur in the developing tumour stroma [2]. Indeed, tumours are often described as “wounds that do not heal” [3]. The normal wound healing response begins by clotting the injury site and forming a provisional matrix composed of a fibrin-rich polymer interspersed with crosslinked plasma-derived fibronectin (FN) [4]. This matrix serves as a temporary scaffold and mechanical framework to support subsequent cell migration and invasion events; in particular, platelets and immune cells facilitate the recruitment of resident stromal cells by secreting growth factors and cytokines, such as transforming growth factor-beta 1 (TGF-β1) [4]. These recruited cells are key players that carry out significant extracellular matrix (ECM) remodelling by secreting proteases, such as matrix metalloproteinases (MMPs), to replace the provisional matrix with granulation tissue composed of FN, collagen, and various proteoglycans, such as hyaluronic acid (HA) [4]. FN is a key component of this tissue that is crucially involved in regulating cell adhesion dynamics via specific bioactive motifs, such as integrin binding domain arginylglycylaspartic acid (RGD) and provides a template for subsequent collagen deposition [5]. For recruited cells to construct this matrix, they must become activated and differentiate into myofibroblasts. These cells form highly organized cytoskeletons containing α-smooth muscle actin (α-SMA) stress fibres that enable contraction and wound closure. In a healthy microenvironment, activated myofibroblasts undergo apoptosis after a wound healing response; however, in cancer and fibrosis, their persistent activation leads to continued ECM deposition, tissue stiffening, and ultimately, loss of organ function [6].

Cell differentiation in cancer and fibrosis

In the tumour stroma, cancer-associated fibroblasts (CAFs) are one of the most abundant cell populations, derived from the differentiation of numerous cell types that reside in and around the local microenvironment [7]. One of the leading environmental cues that triggers CAF differentiation is mechanics [7]. CAFs contribute to the formation of fibrous desmoplasia and tissue stiffening by excessive secretion of various ECM components, such as collagens and MMPs. Different CAF subsets have also

---

Given the role as Guest Editor, Delphine Gourdon had no involvement in the peer review of the article and has no access to information regarding its peer-review. Full responsibility for the editorial process of this article was delegated to George Alexander Truskey, PhD.

been shown to secrete specific collagens, MMPs, and other ECM molecules, which suggests that heterogeneity occurs within CAF populations and highlights their complex role in the tumour stroma [7]. Various cell types can differentiate into CAFs and have been implicated in cancer and fibrosis, including mesenchymal stem cells (MSCs), endothelial cells, and pericytes [7]. However, most studies using hydrogel models to study abnormal cancer- and fibrosis-related differentiation events focus on epithelial-to-mesenchymal transition (EMT), fibroblast-to-myofibroblast transition (FMT), and stellate cell activation; thus, these are the main events that are introduced and discussed in this review.

EMT is a transient, reversible event in which epithelial cells lose their cell polarity and cell–cell adhesion properties and develop migratory and invasive traits that resemble a mesenchymal phenotype. This involves changes in expression of key protein markers, such as shifting from E-cadherin to N-cadherin, increased nuclear activation of proteins like β-catenin, and increased expression of FN and vimentin [8]. While EMT is important in physiological processes such as tissue morphogenesis and wound healing, during tumour progression, it is thought that abnormal differentiation can form intermediate quasi-mesenchymal states that serve as cancer stem cells (CSCs) [9]. CSCs drive tumour growth and heterogeneity through their self-renewal ability and multi-lineage differentiation capacity. Additionally, EMT has been implicated in various types of fibrotic disease including lung, cardiac, and liver fibrosis, as well as renal fibrosis, where it is believed that over a third of all disease-related fibroblasts are derived from tubular epithelia at the injury site [10]. Within the stromal tumour microenvironment, CAFs have been shown to be the main constituents that express EMT-related genes in various tumour types; however, CAFs have been identified to arise from many cell sources [11,12].

As mentioned in the previous section, under healthy homeostatic conditions, resident stromal fibroblasts orchestrate ECM maintenance, regulate epithelial cell behaviour, and undergo FMT by responding to altered environmental cues during the wound healing response and expressing signature markers such as α-SMA [6]. Fibroblast behaviour is however hijacked during cancer: CAFs are formed from abnormally activated myofibroblasts characterised by a dysregulated wound healing response and excessive ECM production [7]. Additionally, abnormal FMT is also a hallmark of fibrotic disease and occurs in response to dysregulated stromal remodelling as seen in cancer progression [6].

Another class of cells involved in regulating tissue homeostasis and wound healing response are stellate cells of the liver and pancreas, known as hepatic stellate cells (HSCs) and pancreatic stellate cells (PSCs) respectively [13,14]. Both HSCs and PSCs are characterised by the presence of vitamin A-containing lipid droplets in a quiescent state before losing this phenotype and acquiring an activated, myofibroblast morphology following prolonged culture and spreading on tissue culture plastic [13,14]. Abnormal activation of HSCs and PSCs in response to disrupted stromal homeostasis has been correlated with fibrosis and cancer in the liver and pancreas [13,14].

Modelling the tumour and fibrotic microenvironment

The fibrotic and tumour microenvironments are complex and dynamic; their interplay with resident cells regulates cell fate and disease progression. In a bid to further our understanding of these phenomena, researchers have been developing *in vitro* models that recapitulate the biophysical and biochemical cues that cells sense and respond to *in vivo*. These models can be particularly advantageous for recreating the dynamic changes in tissue properties which are seen naturally during fibrotic and cancer disease progression. For example, using tissue engineering approaches, many ECM models have been fabricated with controlled tuneable properties, such as stiffness and viscoelasticity [15,16]. Within this respect, hydrogels are highly desirable because they can be engineered to mimic the structural, mechanical, and biochemical features of native ECMs. Crucially, they can recapitulate the mechanobiological changes observed between healthy and diseased tissues, and they also allow encapsulation of cells within a three-dimensional (3D) environment, recreating the dimensionality that cells encounter *in vivo* [17–19].

Using hydrogel-based models for cancer and fibrosis, researchers have been able to decipher the role of various mechanosensitive signalling players in the differentiation of cells towards pathological phenotypes. These include YAP/TAZ (yes-associated protein 1/transcriptional coactivator with PDZ-binding motif), Rho/ROCK (Rho family of GTPases and Rho-associated kinases), TWIST1 (twist-related protein 1), Piezo1, focal adhesion kinase (FAK), integrin β1 and integrin-linked kinase (ILK). YAP is perhaps the most widely investigated mechanosensitive protein and has been identified as a potent oncogene that is amplified in various cancers and fibrosis [20,21]. Ras homolog family member A (RhoA), known to control cytoskeletal dynamics, has been implicated in cancer and fibrosis through its regulation of many cell processes, including cell cycle maintenance [22,23]. Inhibitors of ROCK, a key cytoskeletal regulator that influences cell morphology and migration, are often employed to understand the role of cytoskeletal-mediated cell events in disease progression, and are potentially interesting for therapeutic targeting [24]. TWIST1 is a key mechanosensitive protein whose nuclear localisation in response to increasing matrix stiffness has been found to promote EMT, migration, and invasion in cancer and fibrosis

[25,26]. Piezo1, a mechanosensitive ion channel that is stretch-activated upon changes in mechanical perception, regulates Ca^{2+} signalling and is essential for key cellular processes, has been implicated in cancer and fibrosis when dysfunctional [27,28]. Various actors involved in cell—matrix interactions have been also implicated in cancer and fibrosis: FAK and its phosphorylated form pFAK, which are essential proteins for the formation of focal adhesions [29,30]; integrin β1, a critical component of the cell-ECM interactive machinery whose dysregulation is associated with cancer and fibrosis [31,32]; and ILK, a multifunctional molecular actor in cell—matrix interactions, cell adhesion, and anchorage-dependent cell growth [33].

Hence, in this review, we discuss how recent advances in hydrogel-based models have progressed our understanding of how mechanosensing and key related signalling pathways are altered during cancer and fibrosis progression, leading to abnormal cell differentiation. After an overview of insights gained using "conventional" mechanobiology approaches that employ mechanically static hydrogels, we then introduce advanced mechanically dynamic models. These include hydrogels with controlled relaxation and plastic deformation, which allow us to investigate the dynamic and dissipative properties of diseased ECMs, and hydrogels with switchable mechanics where cell mechanosensing can be probed in response to temporal changes in matrix mechanics, as seen during disease progression (Figure 1).

Mechanically static hydrogels

Conventional mechanobiological studies consider hydrogels as mechanically static materials, which can be characterised only via their elastic modulus or stiffness. However, hydrogels are inherently viscoelastic and display some viscous, stress relaxing behaviour. Most works disregard this dissipative contribution and make no deliberate effort to either control or alter it. Within this respect, hydrogels are considered elastic, or in any case slow relaxing, and the observed cell responses are ascribed to changes in substrate stiffness alone.

Stiffness relates to the extent an object resists deformation in response to an applied force; this is highly variable across different biological tissues. As highlighted in the introduction, one of the hallmarks of fibrotic and cancerous progression is tissue stiffening accompanied by significant stromal reorganisation and differentiation of multiple cell types into CAFs and fibrotic phenotypes [7]. Hence, changes in stiffness have been modelled using hydrogels of varied elastic modulus to investigate how mechanosensing is consequently altered and contributes to cell differentiation in cancer and fibrosis; examples of these models are summarised in Table 1. Results demonstrate that elastic stiffness is a major player in driving cell differentiation through EMT, FMT or stellate cell activation via altering of mechanosensitive pathways. For example, Lopez-Cavestany et al. showed that EMT of prostate cancer epithelial cells increased on stiffer matrices in a Piezo1-dependent manner (Figure 2a) [34]; similar responses have been observed for other types of cancers, including pancreatic and breast [35—37]. In FMT, Walker et al. showed that stiffer matrices coincide with a cardiac myofibroblast phenotype which was associated with activated YAP expression (Figure 2b) [38]; similar responses have also been observed in other cardiac fibrosis studies [39] and in the context of lung fibrosis [40]. In stellate cell activation, Budde et al. showed that PSCs become activated on stiffer substrates, with increased durotaxis which was shown to be a Piezo1-dependent mechanism (Figure 2c) [41]; similar observations have been found in the context of liver fibrosis [42].

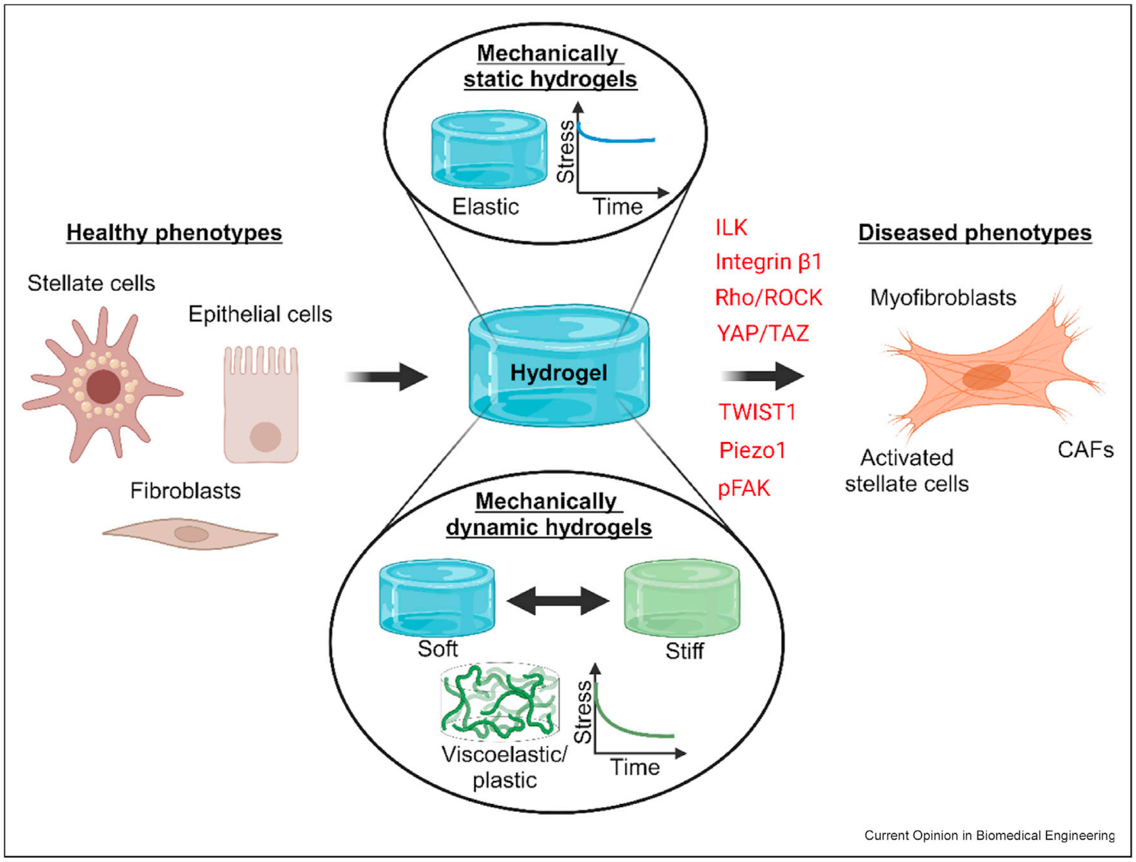
Mechanically dynamic hydrogels

Viscoelastic and plastic hydrogels

Mechanobiological studies in cancer and fibrosis using conventional mechanically static hydrogels have revealed the crucial role of stiffness in driving mechanotransductive changes that promote the differentiation of resident cells into diseased phenotypes, contributing also to drug resistance. However, these matrices do not fully recapitulate the mechanical properties of native tissues, which are instead highly dynamic and dissipative environments [43]. Indeed, most biological tissues exhibit viscoelasticity as they present both elastic and viscous properties, demonstrated for example by a time-dependent strain when a stress is applied. Recent studies have moreover confirmed that tumour microenvironments not only undergo changes in their stiffness, but also in their viscoelastic properties, likely as a result of abnormal matrix crosslinking [44]. Hence, generating hydrogels with controllable viscoelastic properties has great potential in improving our understanding of cancerous and fibrotic disease progression; these dynamic models could help identify mechanosensitive pathways as potential therapeutic targets. Indeed, the role of environmental viscoelasticity in pathological differentiation, such as EMT, has been discussed and speculated recently [45].

Fabricating viscoelastic hydrogels is a novel area of research which has seen various strategies employed to harness the viscoelastic potential of different systems. Examples of strategies include steric spacing of crosslinking points and incorporation of viscous, linear polymers; these approaches successfully modulate hydrogel viscoelasticity and have a significant influence on key cell behavioural aspects, such as adhesion and differentiation [46,47]. It has also been shown that the interplay between matrix stiffness, viscoelasticity, and ligand density influences cell behaviour and should be considered when developing viscoelastic materials to

Figure 1



Hydrogel-based models to study mechanobiology of cell differentiation in cancer and fibrosis. Cancer and fibrosis microenvironments support the differentiation of healthy cell phenotypes into diseased phenotypes that sustain disease progression; the changes in microenvironmental mechanical properties observed *in vivo* can be modelled *in vitro* using conventional mechanically 'static' (elastic) hydrogels, or more advanced 'dynamic' matrices. These models can help to decipher changes in key mechanosensitive signalling pathways and players, including YAP/TAZ, Rho/ROCK, TWIST1, Piezo1, pFAK, Integrin β1 and ILK. Figure prepared in Biorender.

understand disease progression [48]. Hence, researchers have been recently addressing cell behaviour during cancer and fibrosis in response to dynamic and dissipative properties of the microenvironment, revealing a link between viscoelasticity and cancer/fibrotic progression.

In the context of hepatocellular carcinoma, Fan et al. showed that disease progression was found to be accompanied by changes in collagen architecture that enhance ECM viscoelasticity, with greater viscous dissipation and faster stress relaxation, but no changes in stiffness. Mechanistically, viscoelasticity was demonstrated to promote proliferation and invasion via an integrin-β1—tensin-1-Rho/ROCK—YAP mechanotransductive pathway (Figure 3a) [49]. EMT in breast cancer has also been linked to altered ECM viscoelasticity; Sacco et al. showed that overexpression of ILK increases cell spreading, promotes TGFβ1-induced EMT and abrogates TGFβ1-induced apoptosis in cells cultured on viscoelastic polyacrylamide gels with a storage modulus of 2 kPa [50].

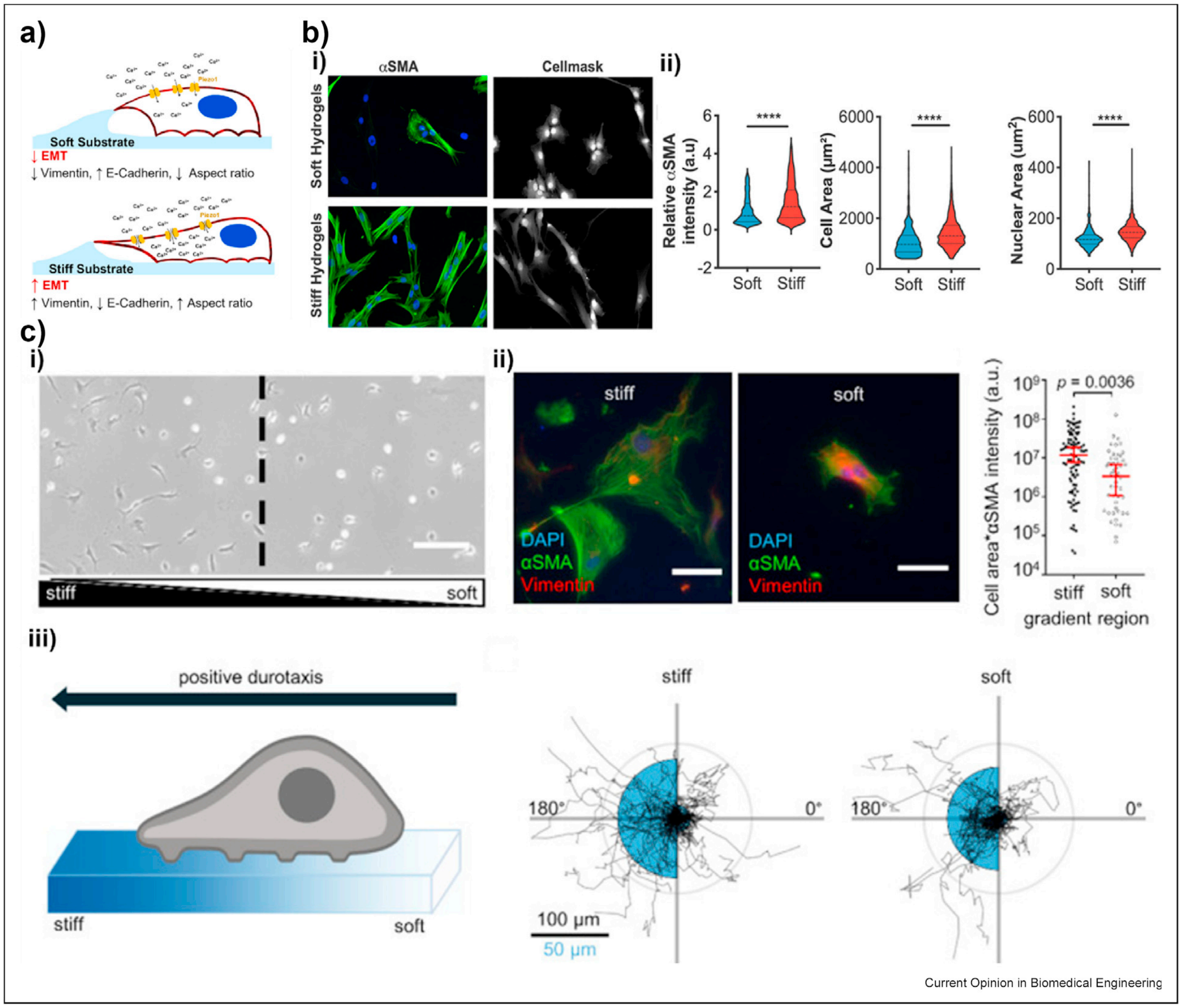
Besides viscoelasticity, a known dissipative feature of ECMs is their plasticity, which relates to permanent microstructural rearrangements in the matrix. Wisdom et al. developed hydrogels with tuneable plasticity using soft (∼2 kPa) alginate gels with varied polymer molecular weight and crosslinking ratios [51]. Interestingly, in gels with high plasticity, various breast cancer cell lines showed increased invasive and migratory behaviour by opening channels in pores through progressively widening and lengthening protrusions, which suggests that more aggressive cell behaviour is promoted in this setting. Additionally, this study showed that the observed cell behaviour was independent of protease-mediated matrix remodelling, which is known to promote cancer and fibrosis, suggesting that the stress-relaxing properties of soft tissue could be crucial as well in driving cancer progression (Figure 3b).

Table 1

Mechanically static hydrogel-based models reveal that stiffness-induced mechanobiological changes regulate differentiation towards cell phenotypes implicated in cancer and fibrosis.

Differentiation	Disease	Cell/tissue type	Material	Ligand	Dimensionality	Stiffness	Cell response	Signalling	Ref
EMT	Pancreatic ductal adenocarcinoma	Primary human PDAC cells	Self-assembled peptide based		3D	1, 10 kPa	In stiffer hydrogels: - Increased EMT marker - Increased ECM protein expression - Increased resistance to gemcitabine and paclitaxel	In stiffer hydrogels: - Increased nuclear YAP/TAZ	[35]
	Breast cancer	DU4457	Fibrin		3D	0.17, 0.52 kPa	In stiffer hydrogels: - Increased EMT marker expression	In stiffer hydrogels: - RhoA-dependent response	[36]
		Patient-derived tumour xenografts	Collagen type I		3D	140 Pa, 1.2–2 kPa	In stiffer matrices: - Increased EMT expression - More metastatic lesion formation	In stiffer matrices: - Upregulation of adhesion markers integrin β1 and p-FAK - Increased YAP activation	[37]
	Prostate cancer	PC3, DU145	Polyacrylamide	Collagen type I	2D	5, 60 kPa	On stiffer hydrogels: - Increased EMT marker expression	On stiffer hydrogels: - Increased intracellular Ca^{2+} - Piezo1 agonist Yoda1 enhances EMT - Piezo1 antagonist GsMTx-4 inhibits EMT	[34]
FMT	Cardiac fibrosis	Primary adult rat ventricular fibroblasts	Polyethylene glycol-norbornene	RGD	2D	6, 41 kPa	On stiffer hydrogels: - Increased expression of myofibroblast markers		[39]
		Primary porcine valve interstitial cells	Polyethylene glycol acrylate	RGD	2D	5, 13 kPa	On stiffer hydrogels: - Increased expression of myofibroblast markers - Chromatin remodelling	On stiffer hydrogels: - Increased YAP activation - YAP inhibition with verteporfin reverses myofibroblast activation	[38]
	Lung fibrosis	Primary human lung fibroblasts	Gelatin methacrylate		2D	5, 15 kPa	On stiffer hydrogels: - Increased myofibroblast activation	On stiffer hydrogels: - Increased YAP activation	[40]
Stellate cell activation	Hepatocellular carcinoma	Human primary HSCs	Polyacrylamide	Collagen type I	2D	1, 32 kPa	On stiffer hydrogels: - Myofibroblast activation of HSCs - Increased carcinoma growth and metastasis	On stiffer hydrogels: - Response mediated by E2F transcription factor 3	[42]
	Pancreatic adenocarcinoma	Murine primary PSCs	Polyacrylamide	ECM mixture	2D	0.75, 5, 13.5 kPa	On stiffer hydrogels: - Myofibroblast-like phenotype and marker expression - Increased durotaxis towards stiffer regions	On stiffer hydrogels: - Pharmacological alteration of Piezo1 activity abolishes durotaxis - Mechanosensitive ion channels TRPV4 and TRPC1 also involved	[41]

Figure 2



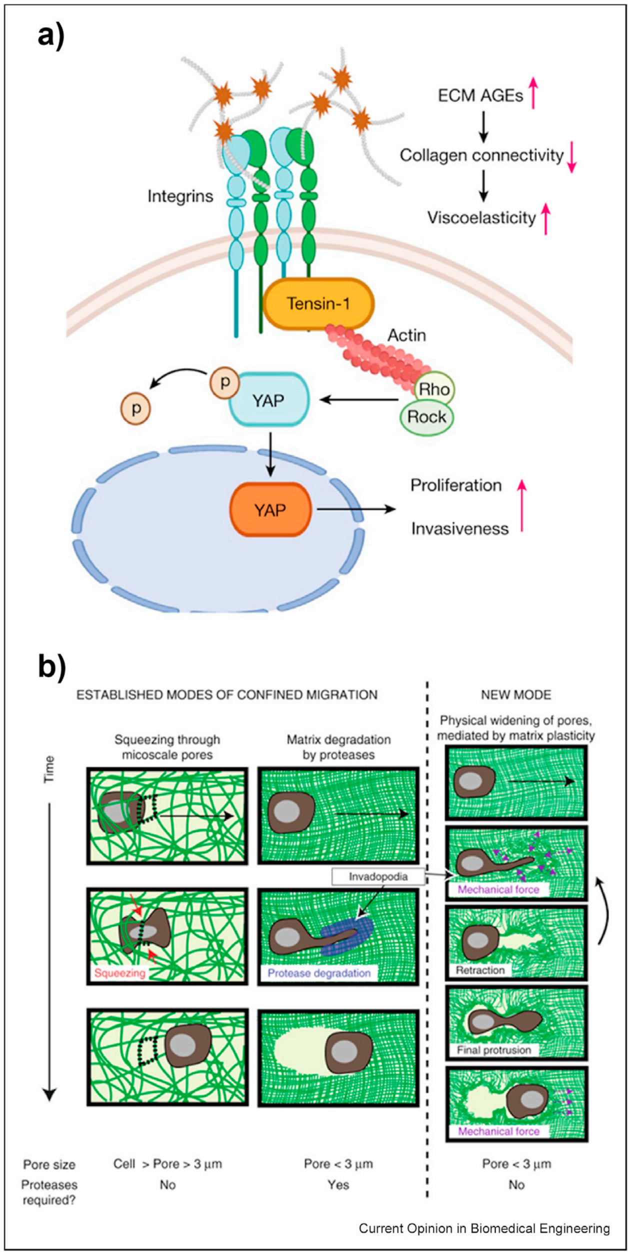
Current Opinion in Biomedical Engineering

Examples of using mechanically static hydrogels to investigate differentiation towards disease phenotypes via EMT (a), FMT (b) and stellate cell activation (c): a) Diagram showing the influence of Piezo1-mediated Ca^{2+} signalling during mechanosensitive EMT on soft and stiff polyacrylamide substrates, reproduced with permission [34]. **b)** Representative images of male valve interstitial cells (VICs) cultured on stiff or soft polyethylene glycol acrylate hydrogels when immunostained for α-SMA (green) and DAPI (blue), cell mask, scale bar = 100 um, (**i**) Quantification of myofibroblast-like phenotypes using α-SMA relative fluorescence intensity, cell area, and nuclear area for VICs on soft or stiff hydrogels. n > 592 cells (**ii**), reproduced with permission [38]. **c)**, Representative phase-contrast image of PSCs on an ECM-coated gradient polyacrylamide hydrogel. The dashed line indicates the centre of the gradient separating “stiff” and “soft” gradient regions. Scale bar = 250 µm, (**i**), Representative immunofluorescence images of the myofibroblastic PSC marker α-SMA (green), vimentin (red) and DAPI (blue) of PSCs seeded on stiff (left) and soft (right) regions, respectively, from N = 4 experiments. Scale bar = 150 µm, (**ii**), Scatter plot of total PSC α-SMA fluorescence assessed by multiplying cell area with α-SMA fluorescence intensity. n cells measured/N experiments ≥51/4, (**iii**) Schematic depiction of “positive” durotaxis. The cells move towards the stiffer side of the gels (left) and durotaxis polar plots depict individual PSC trajectories over 24 h (black lines). The radius of the blue half circles is proportional to the mean cellular displacement into the directions 0° and 180°, respectively. Radial lines indicate 0°, 90°, 180°, 270°. The scale bar corresponds to two sizes: 100 µm for the trajectories and 50 µm for the half circles. Radius of the concentric circle is a visual aid for the scale bar (right), reproduced with permission [41]. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

Switchable mechanics

As mentioned in the introduction, desmoplasia in cancer and fibrosis is characterised by stromal reorganisation over time. To model this, recent studies have developed hydrogel-based systems that can undergo switchable, on-demand changes in mechanics. While dynamically stiffening microenvironments can model disease progression within the stroma, softening hydrogels have also demonstrated to be useful platforms, for example to study fibrotic regression.

Figure 3



Current Opinion in Biomedical Engineering

Examples of using fast-relaxing hydrogels to investigate pathological cell responses in cancer and fibrosis: a) Schematic of the role of tensin 1, which functions as a key component of the ECM mechanosensor complex by binding to integrin β1 in the high-viscoelasticity ECM which arises in liver cancer through increased production of advanced glycation end-products (AGEs) and disruption of collagen architecture. The diagram was created using BioRender. Reproduced with permission [49]. **b)** Known modes and newly discovered mode of confined migration in cancer. For pores sizes smaller than a cell but larger than ~3 μm, it is thought that cells can squeeze through pores to migrate, without requiring proteases. It is thought that for pores smaller than ~3 μm, cells are considered confined to such a degree that they require proteases to migrate. The authors report a migration mode that is plasticity-mediated and protease-independent: if pores are smaller than ~3 μm and the matrix is sufficiently plastic, then cells can use progressively widening and lengthening protrusions to physically open up a channel in the surrounding matrix and enable cell migration. Reproduced with permission [51].

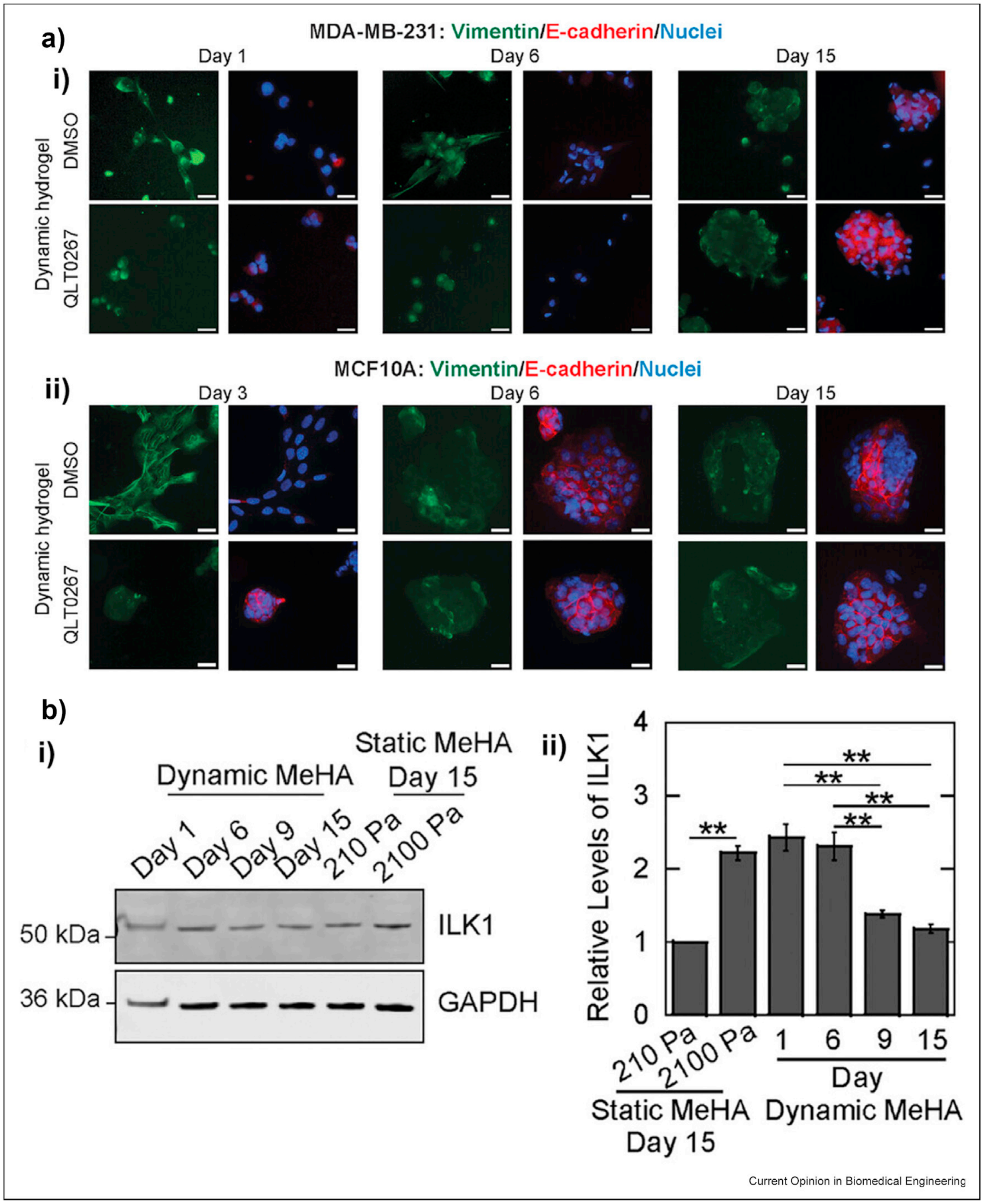
Stiffening

In the context of cancer progression, Joyce et al. used an alginate-Matrigel platform capable of undergoing dynamic ECM stiffness modulation via Ca^{2+} ion release under near infra-red light to mimic the increase in tissue stiffness seen in breast cancer. They observed that dynamic stiffening of MDA-MB-231 breast cancer cell-laden gels from 0.2 to 1.6 kPa or 2–3 kPa resulted in significantly increased EMT and greater chemotherapeutic resistance than their static gel counterparts. This suggests that dynamic stiffening hydrogels may serve as more effective models for drug studies compared to static ones [52]. Shou et al. also showed that dynamic stiffening is important for EMT in breast cancer; MCF-7 cells encapsulated in 3D gelatin–hyaluronic acid–magnetic microparticle (GHAM) hydrogels that were dynamically stiffened using magnetic actuation from 0.5 to 2.5 kPa showed upregulated EMT marker expression, increased drug resistance and elevated mechanotransduction of YAP and TWIST1 [53]. Also in the context of breast cancer, Ondeck et al. developed a methacrylated hyaluronic acid (MeHA) hydrogel that can undergo dynamic stiffening from 0.15 to 3 kPa via photopolymerisation to mimic mechanical changes from healthy to malignant breast cancer tissue. They demonstrated that dynamic stiffening supported EMT of mammary epithelial cells via a mechanism mediated through mechanosensitive transcription factors TWIST1 and YAP [26]. Nguyen et al. instead developed a highly flexible dynamic hydrogel using an adapted gelatin-norbornene system that can independently increase not only elasticity, but also stress relaxation; in this study, dynamic increases in elasticity from 2 to 6 kPa, rather than stress relaxation, enhanced EMT of COLO-357 cells in the context of pancreatic cancer [54]. Finally, Caliari et al. developed MeHA hydrogels that undergo dynamic stiffening from 1.75 to 33 kPa to mimic mechanical changes in liver fibrosis. In this study, they showed that dynamic stiffening induced myofibroblast activation of primary rat HSCs through upregulation of myofibroblast marker α-SMA and nuclear activation of mechanosensitive transcription factors YAP/TAZ [55].

Softening

Using magnetically softening GHAM hydrogels, Shou et al. showed that softening of the ECM from 2.5 to 0.5 kPa reverts MCF-7 breast cancer cells to a more epithelial phenotype by reversing EMT responses through reduced mechanotransduction of YAP and TWIST1, improving drug efficacy [53]. Sankhe et al. also showed that dynamic softening from 2.1 to 0.2 kPa results in a reversion of the EMT phenotype of MDA-MB-231, MCF7 and MCF10A breast cancer cells using MeHA gels that soften upon pentaerythritol tetrakis (3-mercaptopropionate) hydrolysis; this response was further linked to an attenuation of ILK (Figure 4) [57]. These studies could hence provide platforms to investigate not only the role of substrate mechanics in EMT

Figure 4



Current Opinion in Biomedical Engineering

Example of dynamically softening hydrogels to investigate pathological cell responses in cancer and fibrosis: a) Immunofluorescence staining for vimentin (green) and E-cadherin (red) showing changes in vimentin and cadherin expression across time for **i)** MDA-MB-231 breast cancer cells and **ii)** MCF10A mammary epithelial cells cultured on dynamic hydrogels and treated with ILK inhibitor QLT0267 or vehicle control. Scale bars: 25 μm. **b) i)** Western blotting for ILK1 in MDA-MB-231 breast cancer cells seeded on static and dynamic MeHA-based hydrogels for various time points. **ii)** Densitometric analysis of ILK1 from blot shown in panel a, showing that ILK levels decrease as a function of time as the matrix softens. Data normalized with respect to static MeHA 210 Pa day 15 sample. Data indicate mean ± sem for n = 3 trials. $^{**}p < 0.01$. Reproduced with permission [57]. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

plasticity, but also matrix softening strategies as anti-cancer treatments. In the context of fibrosis, Caliari et al. developed MeHA hydrogels with hydrolytically degradable crosslinks which could gradually soften from ∼15–20 to ∼1–3 kPa over 14 days to mimic mechanical changes during liver fibrosis regression. Here, they mechanically primed rat HSCs on stiff substrates to induce a myofibroblast phenotype and showed that, in response to hydrogel softening, they reverted to an intermediate phenotype, akin to that reported *in vivo*, with downregulation of myofibroblast marker α-SMA and mechanosensitive rheostats YAP/TAZ [56]. This study demonstrates that dynamically softening hydrogels represent an optimal tool to mimic fibrosis regression.

Outlook

Hydrogels, thanks to their tuneability as ECM mimetics, provide effective mechanobiological models to study the mechanosensitive processes that regulate pathological cell differentiation in cancer and fibrosis. Conventional mechanically static hydrogels have demonstrated that the stiffer environments encountered during cancer and fibrosis progression coincide with the activation of key signalling molecules, such as the mechanical rheostat YAP/TAZ, which facilitate differentiation into diseased phenotypes through EMT, FMT or stellate cell activation. These models can hence facilitate the identification of potential mechanosensitive therapeutic targets to disrupt abnormal cell differentiation events.

More complex dynamic models that better recapitulate the mechanical properties of the diseased stroma have further revealed that the viscoelastic and plastic properties of the matrix cannot be overlooked, as they are able to reveal cellular behaviours, like modes of migration and invasion, that conventional models cannot predict. Dynamic models have also allowed temporal control over changes in mechanical properties. Besides being able to mimic the timescales associated with disease progression, hence constituting an inherently improved disease model, these platforms have further revealed the limitations of mechanically static hydrogels by showing that drug resistance is influenced by these dynamic changes. Dynamic stiffening and softening hydrogels hence pave the way to more complete mechanobiological models which can improve our understanding of disease dynamics and potentially reveal new treatment options.

Despite the aforementioned advances in hydrogel technologies, there are still limitations of these mechanobiological platforms which future research will need to address. Static systems provide researchers with a high degree of control over the physical properties of the microenvironment, but are not appropriate for predicting real-time changes in cell phenotype during disease progression as a function of dynamically altered mechanics. To predict cell responses that are clinically relevant (e.g., drug resistance) these studies must be conducted in line with timescales appropriate to pathological progression. In this way, changes in cell phenotype can be investigated gradually throughout the transition between healthy and diseased tissue, in response to appropriate time-dependent mechanics that regulate mechanosensitive responses. While dynamic platforms certainly progress towards mimicking these time-dependent mechanical changes, precise control over them to coincide with *in vivo* timescales remains an ongoing challenge. Another challenge in the field of dynamic ECM systems is that biochemical cues also change alongside mechanics during the transition from health to disease. To more accurately recapitulate these complex changes, researchers should endeavour to introduce functionalities that present ECM cues to cells dynamically, in a manner which captures the transition from healthy to pathological tissue. Collaboration between material scientists, bioengineers, biophysicists and cell biologists has been encouraged in previous reviews of static and dynamic systems [58]; this will be critical to develop more accurate disease models that encompass the complex myriad of dynamic changes that the ECM experiences as disease progresses.

Going forward, it is hence crucial that we build upon the mechanobiological knowledge gathered using conventional platforms, progressing towards the development of models with dynamic signals which are reminiscent of those seen during disease progression. This will provide new insights into how cells perceive and respond to changes in mechanical stimuli, better informing cancer and fibrosis researchers and ultimately providing clinically relevant diagnostic and therapeutic information.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

M.C. acknowledges funding from the Medical Research Council (MR/S005412/1), the Royal Society (RGS/R1/231400) and the Carnegie Trust for the Universities of Scotland (RIG013300). D.G. acknowledges the Royal Society under the Wolfson award RSWF/FT/191020.

Data availability

No data was used for the research described in the article.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
* * of outstanding interest

1. Talbott HE, Mascharak S, Griffin M, Wan DC, Longaker MT: **Wound healing, fibroblast heterogeneity, and fibrosis**. *Cell Stem Cell* 2022, **29**:1161–1180.
2. Wu B, Sodji QH, Oyelere AK: **Inflammation, fibrosis and cancer: mechanisms, therapeutic options and challenges**. *Cancers* 2022, **14**:552.
3. Harold F, Dvorak M: **Tumors: wounds that do not heal**. *N Engl J Med* 1986, **315**:1650–1659.
4. Diller RB, Tabor AJ: **The role of the extracellular matrix (ECM) in wound healing: a review**. *Biomimetics* 2022, **7**:87.
5. Sottile J, Hocking DC: **Fibronectin polymerization regulates the composition and stability of extracellular matrix fibrils and cell-matrix adhesions**. *Mol Biol Cell* 2002, **13**:3546–3559.
6. Younesi FS, Miller AE, Barker TH, Rossi FMV, Hinz B: **Fibroblast and myofibroblast activation in normal tissue repair and fibrosis**. *Nat Rev Mol Cell Biol* 2024, **25**:617–638.
7. Lavie D, Ben-Shmuel A, Erez N, Scherz-Shouval R: **Cancer-associated fibroblasts in the single-cell era**. *Nat Can (Ott)* 2022, **3**:793–807.
8. Huang Y, Hong W, Wei X: **The molecular mechanisms and therapeutic strategies of EMT in tumor progression and metastasis**. *J Hematol Oncol* 2022, **15**:129.
9. Orrapin S, Udomruk S, Lapisatepun W, Moonmuang S, Phanphaisarn A, Phinyo P, *et al.*: **Clinical implication of circulating tumor cells expressing epithelial mesenchymal transition (EMT) and cancer stem cell (CSC) markers and their perspective in HCC: a systematic review**. *Cancers* 2022, **14**:3373.
10. Liu L, Sun Q, Davis F, Mao J, Zhao H, Ma D: **Epithelial–mesenchymal transition in organ fibrosis development: current understanding and treatment strategies**. *Burns & Trauma* 2022, **10**.
11. Yang D, Liu J, Qian H, Zhuang Q: **Cancer-associated fibroblasts: from basic science to anticancer therapy**. *Exp Mol Med* 2023, **55**:1322–1332.
12. Szabo PM, Vajdi A, Kumar N, Tolstorukov MY, Chen BJ, Edwards R, *et al.*: **Cancer-associated fibroblasts are the main contributors to epithelial-to-mesenchymal signatures in the tumor microenvironment**. *Sci Rep* 2023, **13**:3051.
13. Cogliati B, Yashaswini CN, Wang S, Sia D, Friedman SL: **Friend or foe? The elusive role of hepatic stellate cells in liver cancer**. *Nat Rev Gastroenterol Hepatol* 2023, **20**:647–661.
14. Menezes S, Okail MH, Jalil SMA, Kocher HM, Cameron AJM: **Cancer-associated fibroblasts in pancreatic cancer: new subtypes, new markers, new targets**. *J Pathol* 2022, **257**:526–544.
15. Long Y, Niu Y, Liang K, Du Y: **Mechanical communication in fibrosis progression**. *Trends Cell Biol* 2022, **32**:70–90.
16. Sievers J, Mahajan V, Welzel PB, Werner C, Taubenberger A: **Precision hydrogels for the study of cancer cell mechanobiology**. *Adv Healthcare Mater* 2023, **12**, 2202514.
17. Walker M, Pringle EW, Ciccone G, Oliver-Cervello L, Tassieri M, Gourdon D, *et al.*: **Mind the viscous modulus: the mechanotransductive response to the viscous nature of isoelastic matrices regulates stem cell chondrogenesis**. *Adv Healthcare Mater* 2023, e2302571.
18. Walker M, Luo J, Pringle EW, Cantini M: **ChondroGELesis: hydrogels to harness the chondrogenic potential of stem cells**. *Mater Sci Eng C* 2021, **121**, 111822.
19. Asadishekari M, Mpoyi EN, Li Y, Eslami J, Walker M, Cantini M, *et al.*: *Three-dimensional tunable fibronectin-collagen platforms for control of cell adhesion and matrix deposition*, vol. 10; 2022.
20. Piccolo S, Panciera T, Contessotto P, Cordenonsi M: **YAP/TAZ as master regulators in cancer: modulation, function and therapeutic approaches**. *Nat Can (Ott)* 2023, **4**:9–26.
21. Mia MM, Singh MK: **New insights into hippo/YAP signaling in fibrotic diseases**. *Cells* 2022, **11**:2065.
22. Schaefer A, Der CJ: **RHOA takes the RHOad less traveled to cancer**. *Trends in Cancer* 2022, **8**:655–669.
23. Zhao Y-Q, Deng X-W, Xu G-Q, Lin J, Lu H-Z, Chen J: **Mechanical homeostasis imbalance in hepatic stellate cells activation and hepatic fibrosis**. *Front Mol Biosci* 2023, **10**.
24. You Y, Zhu K, Wang J, Liang Q, Li W, Wang L, *et al.*: **ROCK inhibitor: focus on recent updates**. *Chin Chem Lett* 2023, **34**, 108336.
25. Mirjat D, Kashif M, Roberts CM: **Shake it up baby now: the changing focus on TWIST1 and epithelial to mesenchymal transition in cancer and other diseases**. *Int J Mol Sci* 2023, **24**, 17539.
26. Ondeck MG, Kumar A, Placone JK, Plunkett CM, Matte BF, Wong KC, *et al.*: **Dynamically stiffened matrix promotes malignant transformation of mammary epithelial cells via collective mechanical signaling** 2019, **116**(9):3502–3507.
27. Xu Y, Huang Y, Cheng X, Hu B, Jiang D, Wu L, *et al.*: **Mechanotransductive receptor Piezo1 as a promising target in the treatment of fibrosis diseases**. *Front Mol Biosci* 2023, **10**.
28. Zhao F, Zhang L, Wei M, Duan W, Wu S, Kasim V: **Mechanosensitive ion channel Piezo1 signaling in the Hall-marks of cancer: structure and functions**. *Cancers* 2022, **14**:4955.
29. Chuang H-H, Zhen Y-Y, Tsai Y-C, Chuang C-H, Hsiao M, Huang M-S, *et al.*: **FAK in cancer: from mechanisms to therapeutic strategies**. *Int J Mol Sci* 2022, **23**:1726.
30. Wen D, Gao Y, Ho C, Yu L, Zhang Y, Lyu G, *et al.*: **Focusing on mechanoregulation Axis in fibrosis: sensing, transduction and effecting**. *Front Mol Biosci* 2022, **9**.
31. Su C, Mo J, Dong S, Liao Z, Zhang B, Zhu P: **Integrinβ-1 in disorders and cancers: molecular mechanisms and therapeutic targets**. *Cell Commun Signal* 2024, **22**:71.
32. Hu Q, Saleem K, Pandey J, Charania AN, Zhou Y, He C: **Cell adhesion molecules in fibrotic diseases**. *Biomedicines* 2023, **11**:1995.
33. Górska A, Mazur AJ: **Integrin-linked kinase (ILK): the known vs. the unknown and perspectives**. *Cell Mol Life Sci* 2022, **79**:100.
34. * Lopez-Cavestany M, Hahn SB, Hope JM, Reckhorn NT, Greenlee JD, Schwager SC, *et al.*: **Matrix stiffness induces epithelial-to-mesenchymal transition via Piezo1-regulated calcium flux in prostate cancer cells**. *iScience* 2023, **26**.

This study expands on the understanding of how EMT is controlled via the mechanosensitive calcium channel Piezo1 in cancerous cells, which senses changes in ECM stiffness. Overall, the authors conclude that Piezo1-regulated calcium flux plays a role in prostate cancer cell metastatic potential by sensing changes in ECM stiffness and modulating EMT markers.

35. Liu Y, Okesola BO, Osuna de la Peña D, Li W, Lin M-L, Trabulo S, *et al.*: **A self-assembled 3D model demonstrates how stiffness educates tumor cell phenotypes and therapy resistance in pancreatic cancer**. *Adv Healthcare Mater* 2024, **13**, 2301941.
36. Heilala M, Lehtonen A, Arasalo O, Peura A, Pokki J, Ikkala O, *et al.*: **Fibrin stiffness regulates phenotypic plasticity of metastatic breast cancer cells**. *Adv Healthcare Mater* 2023, **12**, 2301137.
37. Stashko C, Hayward MK, Northey JJ, Pearson N, Ironside AJ, Lakins JN, *et al.*: **A convolutional neural network STIFMap reveals associations between stromal stiffness and EMT in breast cancer**. *Nat Commun* 2023, **14**:3561.
38. Walker CJ, Batan D, Bishop CT, Ramirez D, Aguado BA, Schroeder ME, *et al.*: **Extracellular matrix stiffness controls cardiac valve myofibroblast activation through epigenetic remodeling**. *Bioengineering & Translational Medicine* 2022, **7**, e10394.

39. Felisbino MB, Rubino M, Travers JG, Schuetze KB, Lemieux ME, Anseth KS, *et al.*: **Substrate stiffness modulates cardiac fibroblast activation, senescence, and proinflammatory secretory phenotype**. *Am J Physiol Heart Circ Physiol* 2024, **326**:H61–H73.

40. Blokland KEC, Nizamoglu M, Habibie H, Borghuis T, Schuliga M, Melgert BN, *et al.*: **Substrate stiffness engineered to replicate disease conditions influence senescence and fibrotic responses in primary lung fibroblasts**. *Front Pharmacol* 2022, **13**.

41. * Budde I, Schlichting A, Ing D, Schimmelpfennig S, Kuntze A, Fels B, *et al.*: **Piezo1-induced durotaxis of pancreatic stellate cells depends on TRPC1 and TRPV4 channels**. *bioRxiv : the preprint server for biology* 2024 Apr 15, 2023.12.22.572956.

The authors, using a two-dimensional stiffness gradient mimicking PDAC, show that PSC activation and migration towards a stiffer substrate is diminished by altering Piezo1 activity. Moreover, disrupting TRPC1 along with TRPV4 abolishes PSC durotaxis even when Piezo1 is functional. The findings suggest that mechanosensitive ion channels, particularly Piezo1, detect the mechanical microenvironment to guide PSC migration.

42. Liu Z, Mo H, Liu R, Niu Y, Chen T, Xu Q, *et al.*: **Matrix stiffness modulates hepatic stellate cell activation into tumor-promoting myofibroblasts via E2F3-dependent signaling and regulates malignant progression**. *Cell Death Dis* 2021, **12**: 1134.

43. Cantini M, Donnelly H, Dalby MJ, Salmeron-Sanchez M: **The plot thickens: the emerging role of matrix viscosity in cell mechanotransduction**. *Adv Healthcare Mater* 2020, **9**, 1901259.

44. Chaudhuri O, Cooper-White J, Janmey PA, Mooney DJ, Shenoy VB: **Effects of extracellular matrix viscoelasticity on cellular behaviour**. *Nature* 2020, **584**:535–546.

45. Barriga EH, Mayor R: **Adjustable viscoelasticity allows for efficient collective cell migration**. *Semin Cell Dev Biol* 2019, **93**: 55–68.

46. Lou J, Mooney DJ: **Chemical strategies to engineer hydrogels for cell culture**. *Nat Rev Chem* 2022, **6**:726–744.

47. Charrier EE, Pogoda K, Li R, Park CY, Fredberg JJ, Janmey PA: **A novel method to make viscoelastic polyacrylamide gels for cell culture and traction force microscopy**. *APL Bioeng* 2020, **4**.

48. Gong Z, Szczesny SE, Caliari SR, Charrier EE, Chaudhuri O, Cao X, *et al.*: **Matching material and cellular timescales maximizes cell spreading on viscoelastic substrates**. *Proc Natl Acad Sci USA* 2018, **115**:E2686–E2695.

49. ** Fan W, Adebowale K, Vancza L, Li Y, Rabbi MF, Kunimoto K, *et al.*: **Matrix viscoelasticity promotes liver cancer progression in the pre-cirrhotic liver**. *Nature* 2024, **626**:635–642.

Enhanced viscoelasticity promotes hepatocellular carcinoma cell proliferation and invasion through an integrin-β1–tensin-1–YAP mechanotransductive pathway in animal studies and 3D cell cultures. The authors reveal that structural changes enhance ECM viscoelasticity, and that viscoelasticity can promote cancer progression *in vivo*, independent of stiffness.

50. Sacco JL, Vaneman ZT, Gomez EW: **Extracellular matrix viscoelasticity regulates TGFbeta1-induced epithelial-mesenchymal transition and apoptosis via integrin linked kinase**. *J Cell Physiol* 2024, **239**, e31165.

51. Wisdom KM, Adebowale K, Chang J, Lee JY, Nam S, Desai R, *et al.*: **Matrix mechanical plasticity regulates cancer cell migration through confining microenvironments**. *Nat Commun* 2018, **9**:4144.

52. Joyce MH, Lu C, James ER, Hegab R, Allen SC, Suggs LJ, *et al.*: **Phenotypic basis for matrix stiffness-dependent chemoresistance of breast cancer cells to doxorubicin**. *Front Oncol* 2018, **8**:337.

53. * Shou Y, Teo XY, Li X, Zhicheng L, Liu L, Sun X, *et al.*: **Dynamic magneto-softening of 3D hydrogel Reverses malignant transformation of cancer cells and enhances drug efficacy**. *ACS Nano* 2023, **17**:2851–2867.

Using a novel 3D dynamic hydrogel whose stiffness can be reversibly changed using an external magnet, the authors demonstrate that malignant transformations (e.g., denser cell organization, EMT and hypoxia) induced by increased stiffness can be halted or reversed via matrix softening. This 'mechanical rescue' potentiates drug efficacy thanks to reduced solid stress from the matrix and downregulation of cell mechano-transductors including YAP1. This platform can be used to deepen our understanding of the impact of matrix softening on cancer biology, an important but rarely studied phenomenon.

54. Nguyen HD, Lin C-C: **Viscoelastic stiffening of gelatin hydrogels for dynamic culture of pancreatic cancer spheroids**. *Acta Biomater* 2024, **177**:203–215.

55. Caliari SR, Perepelyuk M, Cosgrove BD, Tsai SJ, Lee GY, Mauck RL, *et al.*: **Stiffening hydrogels for investigating the dynamics of hepatic stellate cell mechanotransduction during myofibroblast activation**. *Sci Rep* 2016, **6**, 21387.

56. Caliari SR, Perepelyuk M, Soulas EM, Lee GY, Wells RG, Burdick JA: **Gradually softening hydrogels for modeling hepatic stellate cell behavior during fibrosis regression**. *Integr Biol* 2016, **8**:720–728.

57. * Sankhe CS, Sacco JL, Lawton J, Fair RA, Soares DVR, Aldahdooh MKR, *et al.*: **Breast cancer cells exhibit mesenchymal-epithelial plasticity following dynamic modulation of matrix stiffness**. *Advanced biology* 2024, e2400087.

Using HA-based hydrogels with dynamic and tuneable stiffness that mimic normal and tumorigenic mammary tissues, the authors reveal intermediate epithelial/mesenchymal states as cells move along a matrix stiffness-mediated differentiation path, suggesting an important role for matrix mechanics in regulating mesenchymal-epithelial plasticity. This is related to the expression of ILK, with its inhibition impacting proliferation, apoptosis, and gene expression in breast cancer cells cultured on stiff and dynamic hydrogels.

58. Burdick JA, Murphy WL: **Moving from static to dynamic complexity in hydrogel design**. *Nat Commun* 2012, **3**:1269.