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Tuning the matrix: Recent advances in mechanobiology unveiled through polyacrylamide hydrogels

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Over the past 30 years, polyacrylamide (PAAm) hydrogels have become essential tools to mimic the mechanical properties, chemical composition, and dimensionality of the extracellular matrix (ECM) in in vitro mechanobiology studies. This brief review highlights recent developments that have transformed PAAm hydrogels from simple 2D static elastic hydrogels to complex ECM-mimicking systems involving protein micropatterning, mechanical patterning, stretching, DNA tension probes, viscoelasticity, and the microfabrication of 3D systems. We focus on novel mechanobiological questions that have been elucidated using these platforms and give a perspective on the future of PAAm hydrogels for mechanobiology research.

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Introduction

Cellular mechanobiology studies how mechanical forces regulate cell behavior. Specifically, one key aspect of this field is to understand how extracellular matrix (ECM) physicochemical properties—which dynamically evolve during development and disease—are sensed and transduced into mechanotransduction pathways, from integrins and focal adhesions through the cytoskeleton down to the nucleus [1].

The ECM has complex physicochemical properties, including confined geometries [2], heterogeneous protein composition [3], mechanical gradients [4], viscoelasticity [5], and 3D curvature [6]. The mechanistic understanding of the effects of these properties on cell behavior requires the development of well-controlled systems where each variable can be experimentally decoupled from the others.

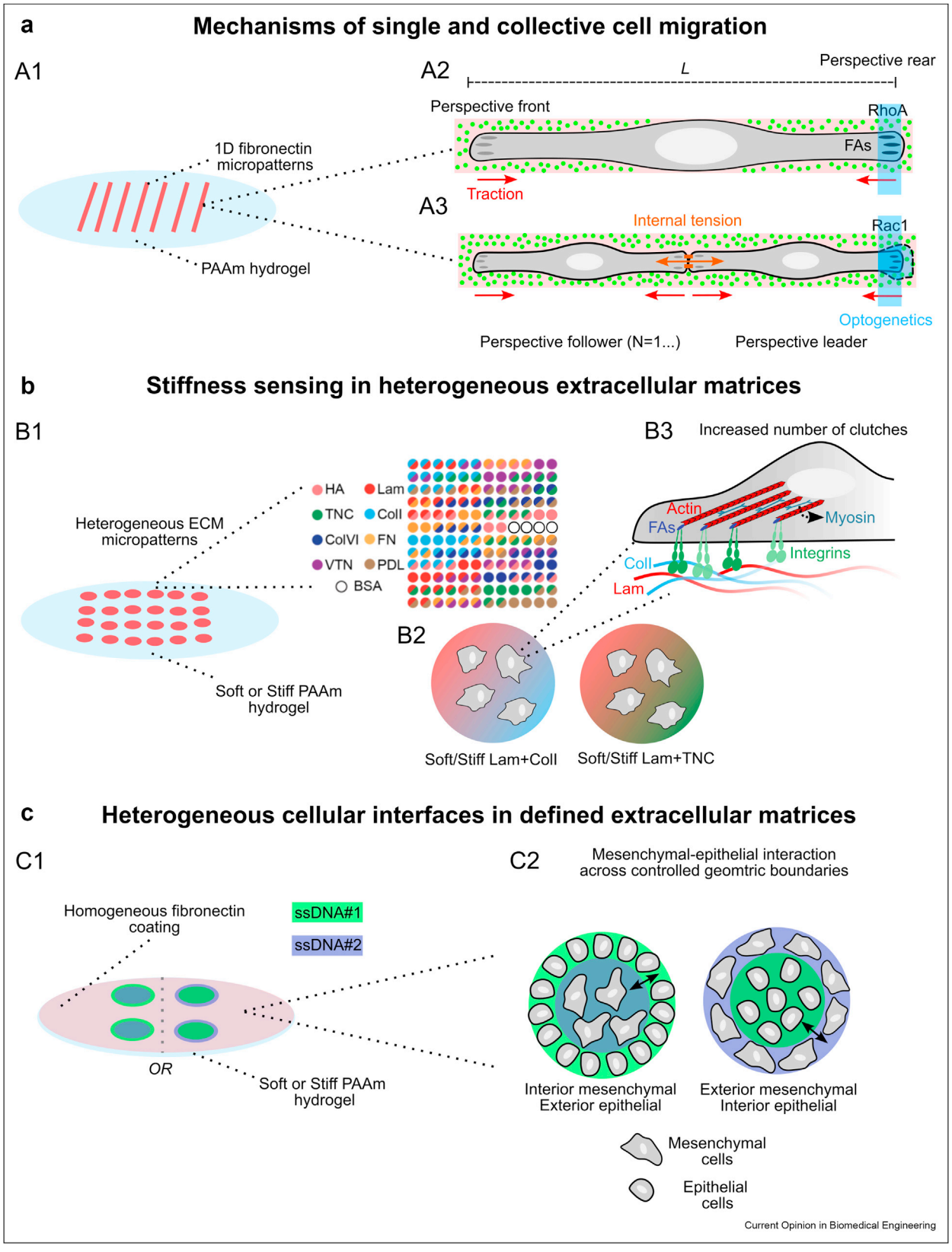
In this regard, polyacrylamide (PAAm) hydrogels have been instrumental in advancing the field of cellular mechanobiology [7]. Being easily synthesized from commercially available reagents following standardized protocols, PAAm hydrogels are the substrate of choice for most mechanobiology laboratories due to their widely tunable physicochemical properties while retaining compatibility with high-resolution optical microscopy.

The creative use of this simple material system has allowed researchers to reliably mimic the complex properties of the in vivo ECM on a coverslip. This brief review explores novel and recent implementations of PAAm hydrogels that have helped to elucidate increasingly complex questions regarding the role of mechanical forces on cellular behavior. This work complements recent reports focusing on the material science of PAAm hydrogels [8] and strategies for their bioligand functionalization [9].

Micropatterned polyacrylamide hydrogels: controlling cell migration, extracellular matrix protein composition, and cellular interfaces

PAAm hydrogels are chemically inert, which means they require chemical functionalization with adhesive sequences or ECM proteins to support cell adhesion [9]. This offers a breadth of possibilities on how to conjugate and present ECM proteins on the surface of PAAm hydrogels. This, together with their tunable Young's modulus (E), recapitulating that of soft biological tissues (from 100s of Pa to 10s of kPa), provides a flexible and robust system for decoupling mechanics and ligand-driven effects on cell behavior. In recent years, soft lithography techniques have been developed to introduce micrometric protein patterns (or micropatterns) on PAAm hydrogels [10–12]. Recent implementations of one-dimensional (1D) micropatterns on PAAm hydrogels (Figure 1 A1), combined with live traction force

Figure 1


a
Mechanisms of single and collective cell migration
A1
1D fibronectin micropatterns
PAAm hydrogel
A2
L
Perspective rear
Perspective front
RhoA
FAs
Traction
A3
Internal tension
Rac1
Optogenetics
Perspective follower (N=1...)
Perspective leader
b
Stiffness sensing in heterogeneous extracellular matrices
B1
Heterogeneous ECM micropatterns
HA
Lam
TNC
Coll
ColVI
FN
VTN
PDL
BSA
Soft or Stiff PAAm hydrogel
B3
Increased number of clutches
Actin
FAs
Myosin
Integrins
Coll
Lam
B2
Soft/Stiff Lam+Coll
Soft/Stiff Lam+TNC
c
Heterogeneous cellular interfaces in defined extracellular matrices
C1
Homogeneous fibronectin coating
ssDNA#1
ssDNA#2
OR
Soft or Stiff PAAm hydrogel
C2
Mesenchymal-epithelial interaction across controlled geomtric boundaries
Interior mesenchymal
Exterior epithelial
Exterior mesenchymal
Interior epithelial
Mesenchymal cells
Epithelial cells
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microscopy (TFM) measurements enabled by embedding fluorescent microbeads within the hydrogels and optogenetic activation of cytoskeletal contractility, have been used to reveal novel insights into mechanisms of single and collective cell migration in different cell lines [13,14]. Hennig and colleagues micropatterned fibronectin (FN) lines on PAAm hydrogels to investigate whether symmetry breaking, the first step required to initiate cell migration, can occur in the absence of any pre-established polarity cues [13]. Using both NIH-3T3 fibroblasts, a mesenchymal cell line, and RPE1 cells, an epithelial cell line, and focusing on the (a)symmetry of tractions stresses in the front and rear of 1D cells, as well as cell morphological parameters and adhesion dynamics, the authors established a stochastic mechanism of symmetry breaking based on symmetric accumulation of traction forces and adhesion strength, which eventually leads to the stochastic disassembly of adhesions at one end, becoming the rear, while the opposite end becomes the front. This was framed within a physical model based on stick-slip dynamics, as the cell alternates between symmetric (stick) and asymmetric (slip) phases. Combining this with further experiments involving local optogenetic activation of RhoA, a master regulator of rear retraction and cell contractility, the authors controlled cell migration on demand, confirming the proposed stick-slip model (Figure 1 A2). Overall, the novel assay based on 1D micropatterning of PAAm gels, live force measurements, and optogenetics established a general framework of cell migration based on stick-slip dynamics, relating cellular morphology, adhesion strength, and contractility, initiated without a priori existence of polarization.

Similarly, the combination of 1D FN micropatterns on the surface of PAAm hydrogels with TFM and optogenetically controllable Madin–Darby Canine Kidney (MDCK) epithelial cellular trains has challenged the conventional view of the roles of leader and follower cells in collective cell migration [14]. Specifically, in this work, generating leader cells on demand via local optogenetic activation of Rac1, which causes lamellipodium and focal adhesion formation, it was shown that leaders can only guide one—but not more—follower cells (Figure 1 A3). For coherent motion to occur in larger cell collectives, global supracellular group traction force and tension asymmetry are a necessary requirement, as demonstrated experimentally and predicted by theoretical modeling.

In addition to models for studying cell migration, soft and stiff PAAm gels have been combined with high-throughput microcontact printing to study cell mechanoresponse of different cell lines, including human dermal telomerase-immortalized fibroblast (TIFs, a mesenchymal cell line), human epithelial osteosarcoma

A1) 1D fibronectin (FN) micropatterns (i.e. thin lines) are generated on the surface of polyacrylamide (PAAm) hydrogels of defined stiffness using different techniques, such as microcontact printing or deep UV patterning through a photomask. When adhering to these micropatterns, cells adopt 1D spindle-like morphology and are forced to migrate back and forth in one direction, facilitating the study of cell migration. **A2)** Schematic representation of the experimental system devised in Ref. [13]. NIH-3T3 fibroblasts expressing the optogenetic construct Cry2-CIBN (N-terminal domain of cryptochrome-interacting basic-helix-loop-helix, which, upon blue light exposure, controls the localization of ArhGEF11, an upstream regulator of RhoA) adhere to 2–5 μm FN lines (red shade) on $E \approx 40$ kPa PAAm hydrogels with embedded microspheres for traction force microscopy (TFM) (green), adopting an elongated shape with length L. When the cell is symmetric, traction force magnitudes at either end are also symmetric (red arrows). Eventually, adhesions at one side stochastically collapse, establishing an asymmetric traction profile which allows the cell to migrate in the opposite direction. This behavior is cyclic, and the cell alternates between spreading phases (stick) and migrating phases (slip). By ontogenetically activating RhoA on one side of the cell, adhesions first mature and tractions build up, eventually collapsing and establishing the perspective rear (and consequently the perspective front on the opposite side). FAs = focal adhesions. **A3)** Schematic representation of the experimental system devised in Ref. [14]. OptoMadin-Darby Canine Kidney (MDCK)-Rac1 cells expressing two optogenetic constructs, CIBN-GFP-CAAX and TIAM-CRY2-mCherry (the first is situated at the plasma membrane, whereas the second is cytosolic and carries the catalytic domain of Tiam1, an activator of Rac1. On illumination with blue light, the two constructs bind with high affinity, localizing Tiam1 at the membrane and activating Rac1) adhere to 20 μm FN lines (red shade) on $E \approx$ 18 kPa PAAm hydrogels with embedded microspheres for TFM (green). Cellular trains (i.e. 1D groups of cells) of various cell numbers stochastically form. Here, a train composed of two cells is shown. By shining blue light at the end of one of the cells, Rac1 is activated and a lamellipodium forms (right cell, dashed line). This cell becomes the leader, which can only pull one follower forward (left cell). For larger trains ($2 < N < 3$, N = number of followers), Rac1 activation does not cause collective migration, but rather global traction (red arrows) asymmetry and tension (orange arrows) are required. **B1)** Schematic representation of the experimental system devised in Ref. [15]. Soft ($E \approx 0.5$ kPa) and Stiff ($E \approx 50$ kPa) PAAm hydrogels are micropatterned in a high-throughput fashion with spot arrays (200 μm) made of different combinations of extracellular matrix (EMC) proteins mixed at a 1:1 ratio or alone: Collagen I (ColI), Laminin (Lam), Collagen VI (ColVI), Tenascin C (TNC), Fibronectin (FN), Hyaluronic acid (HA), Vitronectin (VTN), poly-D-lysine (PDL), bovine serum albumin (BSA). Panel containing the protein combinations is taken and edited from Ref. [15] with written permission from the authors. **B2)** ECM mixtures Lam + ColI and Lam + TNC promote equal spreading in soft and stiff hydrogels in human dermal telomerase immortalized fibroblasts (TIFs), whereas human epithelial osteosarcoma cells (U2OS) showed this behavior only on Lam + ColI but not Lam + TNC. **B3).** The stiffness insensitive cell spreading is explained by the molecular clutch model, for which more clutches are engaged due to the presence of distinct ECM proteins. FAs = focal adhesions. **C1)** Schematic representation of PAAm hydrogels ($E \approx 2.4–36$ kPa) for independent cell capture and adhesion developed in Ref. [16]. Different sequences of single-stranded DNA (ssDNA) can be photopatterned onto the hydrogels containing benzophenone-methacrylate, which, upon light activation (250–365 nm), forms a covalent bond with ssDNA oligomers. FN is subsequently conjugated onto the hydrogel via N-hydroxysuccinimide (NHS) groups, introduced on the surface of the hydrogels. Lipid-ssDNA labeled cells are captured by complementary base paring to ssDNA patterns and subsequently adhere to the hydrogels using integrin interactions with FN. **C2)** Lipid-ssDNA labeled MDCK (epithelial cells, labeled with complementary strand to ssDNA 1) and NIH-3T3 fibroblasts (mesenchymal cells, labeled with complementary strand to ssDNA2) were captured over a total circular area of radius $R = 500$ μm and split into two concentric circles patterned with ssDNA#1 and ssDNA#2 and their interaction was studied across two configurations (interior mesenchymal/exterior epithelial and vice versa, each configuration can be patterned on a given gel) on hydrogels with Young's modulus $E \approx 3$ kPa (Soft) or 35 kPa (Stiff). Black arrow shows boundary across which interaction occurs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cells (U2OS), and human epithelial breast adenocarcinoma cells (MDA-MB-231), to ECM stiffness within heterogenous protein environments [15]. The ECM in vivo is in fact composed of a multitude of proteins, yet most studies have investigated cell response to substrate stiffness in the context of single ECM ligands. By micropatterning spot mixtures of different ECM proteins on soft and stiff hydrogels (Figure 1 B1), the authors identified specific ECM combinations promoting equal spreading on soft and stiff hydrogels in both TIFs and U2OS (Figure 1 B2). These results were interpreted in the context of the molecular clutch model, for which the stiffness-insensitive spreading occurs due to a change in the ratio between adhesion (clutches) and contractility (motors) (Figure 1 B3). This study highlights the complex interplay between physical and chemical properties of the ECM in regulating cell behavior and may shine light on how cells in soft tissues function without a rigid ECM.

Further, PAAm hydrogels have been recently combined with single-stranded DNA (ssDNA) micropatterns and cofunctionalized with FN to decouple the processes of cell capturing and cell adhesion [16] (Figure 1C1). Specifically, using lipid-ssDNA labeled cells, different types of cells can be selectively captured on distinct patterns on the same mechanically homogeneous hydrogel, and dynamic tissue interfaces studied in real time. As a proof of concept, the authors generated concentric patterns of two ssDNA sequences, and captured mesenchymal NIH-3T3 fibroblasts and epithelial MDCK cells labeled with complementary sequences to study their interaction (Figure 1C2). Simply swapping the geometrical position of the two cell populations changed extracellular signal-regulated kinase (ERK) signaling activity, an emerging mechanosensor [17], opening new possibilities to study the interaction of distinct cell populations in mechanically and geometrically-defined complex microenvironments.

Overall, the combination of PAAm hydrogels with protein micropatterns and different cell lines is advancing our current knowledge of fundamental cellular processes including cell migration, stiffness sensing, and cell–cell interaction in geometrically and mechanically defined environments.

Mechanically patterned polyacrylamide hydrogels: advances in durotaxis

Along with their versatile chemical modifications, PAAm hydrogels have been instrumental to establish the mechanoresponse of different cell types to ECM mechanical gradients. Mechanical gradients occur in vivo and are essential to steer cell migration during processes such as development, cancer progression, and wound healing [18]. However, methods to robustly generate stiffness gradient hydrogels usually rely on complex methodologies, making them inaccessible to most labs. To overcome this issue, stiffness gradient PAAm hydrogels were recently engineered containing embedded fluorescent beads, whose density positively correlates with substrate stiffness, thus removing the need for specialized atomic force microscopy characterization of the gels (Figure 2 A1) [19]. The robustness of the newly developed PAAm hydrogels was validated through experiments elucidating the mechanosensitivity of fibrillar adhesions in TIFs [19]. Importantly, stiffness-gradient hydrogels allow researchers to significantly improve experimental throughput, enabling the study cell mechanoresponse to a wide range of rigidities on a single coverslip.

In this context, mechanical gradient PAAm hydrogels were first reported more than 20 years ago to describe the process of durotaxis, that is, the migration of cells along stiffness gradients [20]. Recently, elegant work combining the previously described stiffness-gradient hydrogels [19], 1D protein micropatterns, and photopatterned softening of PAAm hydrogels demonstrated that durotaxis can occur against stiffness gradients (i.e. toward softer regions, termed negative durotaxis), challenging the canonical view of cell migration along stiffness gradients (i.e. positive durotaxis). Using tumor cell lines, including U-251 MG human glioblastoma cells and MDA-MB-231 cells, the authors demonstrated that cells can undergo negative durotaxis if the stiffness optimum for maximum traction-force generation is within the gradient, in accordance with the molecular clutch model in the absence of adhesion reinforcement [21]. MDA-MB-231 cells that conventionally undergo positive durotaxis were, in fact, shown to switch to negative durtoaxis when talin-1 and talin-2 expression, the focal adhesions proteins involved in reinforcement, were silenced (Figure 2 A2).

This work, using durotactic PAAm hydrogels in combination with molecular clutch simulations, reframed durotaxis as a dynamic process that can switch direction based on motor-clutch dynamics. Interestingly, seminal work using durotactic PAAm hydrogels and epithelial cell clusters of different types [22] demonstrated that collective durtoaxis (also recently reported in vivo [18]) is far more efficient than single-cell durotaxis, and might be a conserved mechanism of how living systems respond to mechanical gradients. It would therefore next be of interest to understand whether negative durotaxis also occurs in cell collectives.

Stretching polyacrylamide hydrogels and DNA tension probes: cell stiffness sensing

PAAm hydrogels have been instrumental in defining a general framework of ECM stiffness sensing and transduction, based on a mechanical linkage between the ECM and the cytoskeleton via integrins and focal adhesions: the actin-talin-integrin-FN clutch [23]. At the core of this model is the concept of the force loading

rate, that is, the rate at which cells apply force to the underlying ECM (i.e. the product of the deformation speed and the effective substrate stiffness), as the key determinant of clutch engagement and disengagement [24]. Increasing substrate stiffness increases the loading rate indirectly, which generally promotes clutch engagement [24]. However experimental evidence demonstrating the direct involvement of the loading rate in mechanosensing was lacking until recently. By combining Soft ($E \approx 0.6$ kPa) PAAm hydrogels with a microscopy-compatible stretching device to directly control the loading rate via stretch frequency and amplitude in mouse embryonic fibroblasts (MEFs) (Figure 2 B1), the direct involvement of the loading rate in triggering clutch engagement and mechanosensing was demonstrated [25]. Specifically, the authors reported that increasing stretching frequency (i.e. substrate deformation speed) for a given stretching amplitude triggers clutch engagement on soft hydrogels to a level comparable to that observed on a stiffer static gel of approximately equivalent (indirect) loading rate. The authors reported a combinatory biphasic response to stretching frequency and amplitude, explained by a first loading-rate-mediated adhesion reinforcement and a subsequent loading-rate-mediated cytoskeletal softening at higher frequencies, supported by a modified theoretical clutch model [25] (Figure 2 B2). These results were supported by data acquired at the single-adhesion level via optical tweezers, as well as atomic force microscopy experiments performed at the cell or adhesion scale [25].

Overall, these results demonstrated the direct involvement of the loading rate in ECM stiffness sensing, generalizing our understanding of how different physicochemical stimuli (e.g. ligand density, time-dependent mechanics, etc.) may regulate mechanosensing via the modulation of this variable.

Furthering our understanding of how cells sense underlying ECM elasticity, PAAm hydrogels have recently been combined with DNA tension probes to simultaneously measure single adhesion forces as well as conventional traction forces via TFM in different cell types [26] (Figure 2 B3). Strikingly, this study demonstrated that increasing ECM stiffness does not increase the fraction of high load-bearing integrins in MEFs (defined as integrins sustaining more than 50 pN); rather, the proportion of total load-bearing integrins increases (defined as integrins sustaining more than 17 pN). This suggests that cells recruit more integrins, rather than strengthening existing bonds, to sustain the higher loading rates experienced at high substrate stiffness (Figure 2 B4). Further, using locked oligonucleotides to irreversibly bind mechanically stretched DNA probes in both MEFs and U-251 MG cells, the authors were able to measure the binding frequency of integrins to the underlying ECM as a function of substrate elasticity, revealing that sampling frequency presents a biphasic response to ECM stiffness. This means that sampling frequency is more sensitive to ECM stiffness in comparison to the fraction of high load-bearing integrins, which is constant regardless of ECM stiffness.

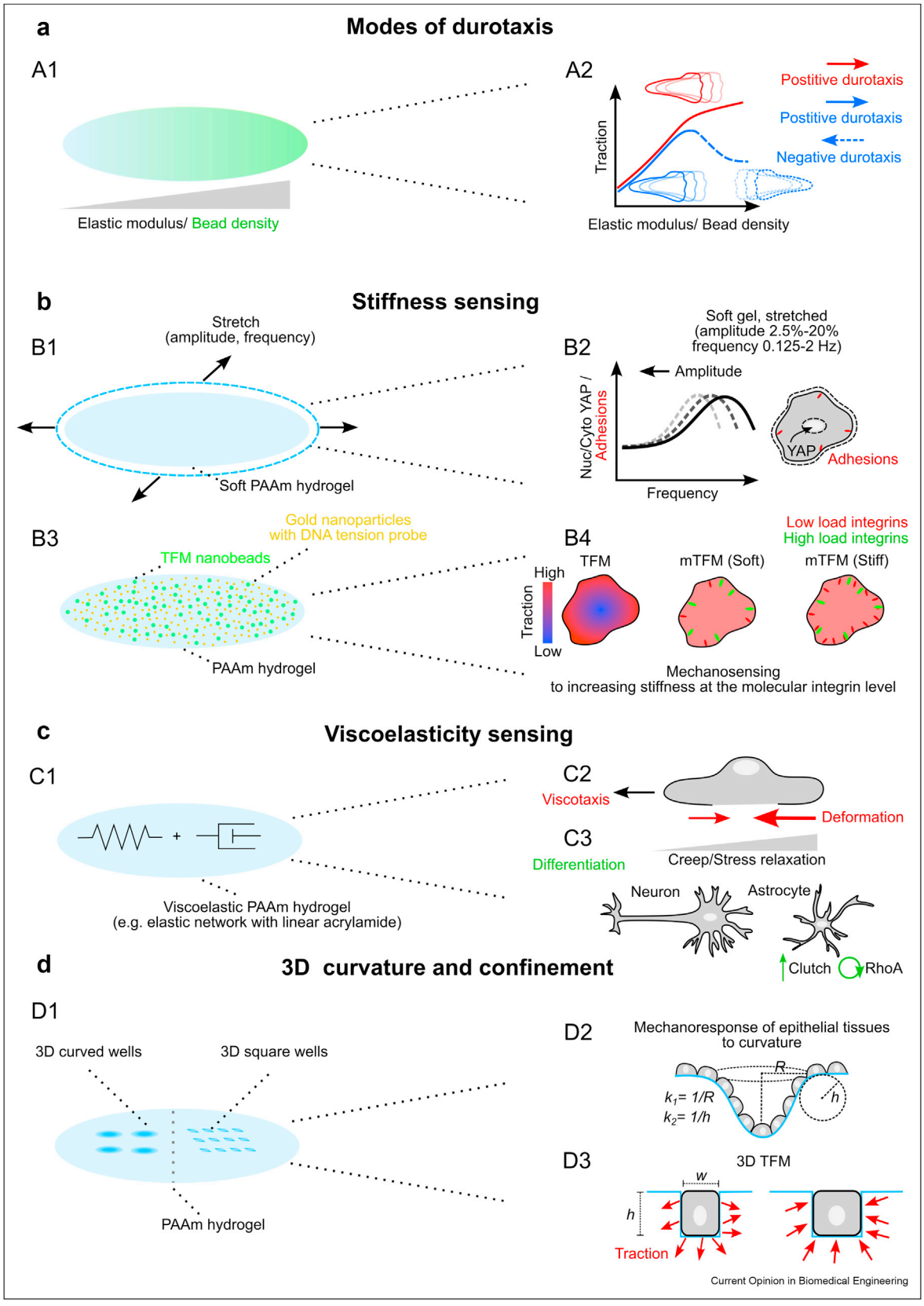
Overall, these recent developments combining PAAm hydrogels with both stretching devices and DNA tension probes [25,26] expand our understanding of stiffness mechanosensing across scales spanning from single adhesions to whole cells, starting to elucidate the timescales of stiffness mechanosensing and mechanotransduction.

Beyond elastic polyacrylamide hydrogels: understanding cell response to substrate viscoelasticity

ECMs in vivo are viscoelastic, meaning that they both store and dissipate energy in response to cell-generated forces and displacements [5]. Importantly, ECM viscoelasticity has recently been shown to be an early indicator of cancer progression in vivo [27]. Yet, commonly used PAAm hydrogels discussed until now show minimal energy dissipation and practically behave as linearly elastic materials under cellular deformations, limiting most of our understanding of cellular mechanosensing and mechanotransduction to the reductive case of linear elastic ECMs. In recent years, the importance of ECM viscoelasticity in regulating cellular mechanobiology is starting to be appreciated [5], and viscoelastic PAAm hydrogels have been developed in this context (Figure 2C1). Different approaches have been devised to independently tune substrate energy dissipation and stiffness in PAAm hydrogels [28–32]. Using these systems, how substrate viscoelasticity affects cell mechanobiological processes is starting to be elucidated. PAAm hydrogel viscoelasticity has been shown to modulate important processes including cell spreading [28,29], differentiation [29,32,33], migration [31,34], adhesion formation and traction force generation [30,33] in a variety of cell types, including human mesenchymal stem cells, NIH-3T3 fibroblasts, and human breast epithelial cells.

Interestingly, using viscoelastic PAAm hydrogels with a loss modulus gradient (produced similarly to durotactic hydrogels reported above), the process of viscotaxis, that is, the migration of cells in response to (against) loss modulus gradients, was reported [31]. Using umbilical cord human mesenchymal stem cells, the authors framed viscotaxis in the context of the creep behavior of the material. Along the gradient of loss modulus, the cell initially applies the same traction force. However, at longer time scales, the substrate deforms more consistently along the gradient. This asymmetric deformation leads to polarization against the gradient, biasing migration toward the side with lower viscoelastic properties (Figure 2C2).

Figure 2


a
Modes of durotaxis
A1
Elastic modulus/ Bead density
A2
Postitive durotaxis
Postitive durotaxis
Negative durotaxis
Traction
Elastic modulus/ Bead density
b
Stiffness sensing
B1
Stretch
(amplitude, frequency)
Soft PAAm hydrogel
B2
Soft gel, stretched
(amplitude 2.5%-20%
frequency 0.125-2 Hz)
Amplitude
Nuc/Cyto YAP /
Adhesions
Frequency
YAP
Adhesions
B3
Gold nanoparticles
with DNA tension probe
TFM nanobeads
PAAm hydrogel
B4
Low load integrins
High load integrins
TFM
mTFM (Soft)
mTFM (Stiff)
High
Traction
Low
Mechanosensing
to increasing stiffness at the molecular integrin level
c
Viscoelasticity sensing
C1
Viscoelastic PAAm hydrogel
(e.g. elastic network with linear acrylamide)
C2
Viscotaxis
Deformation
C3
Differentiation
Creep/Stress relaxation
Neuron
Astrocyte
Clutch
RhoA
d
3D curvature and confinement
D1
3D curved wells
3D square wells
PAAm hydrogel
D2
Mechanoresponse of epithelial tissues
to curvature
R
k_1= 1/R
k_2= 1/h
h
D3
3D TFM
w
h
Traction
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Similarly, recent work employing viscoelastic PAAm hydrogels with mismatched DNA crosslinks and optogenetic modulation of RhoA in adult rat and mouse hippocampal neural stem cells has shown that substrate stress relaxation plays a comparable role in neural stem cell commitment akin to stiffness [32] (Figure 2C3).

These studies underscore the crucial role of ECM viscoelasticity in regulating fundamental cellular processes, such as migration and differentiation, emphasizing the need to incorporate viscoelasticity into cell culture models for mechanobiological research.

Surpassing the second dimension: polyacrylamide hydrogels to understand cell response to three-dimensional (3D) curvature and confinement

Critically, PAAm hydrogel prepolymer solutions are cytotoxic, which prevents encapsulation of cells within them. Conventionally, this limits their applications to two dimensional (2D) cultures. However, cells in the ECM are oftentimes surrounded by a 3D matrix, fundamentally altering how cells sense and transduce surrounding physicochemical properties [35]. The combination of PAAm hydrogels with microfabrication techniques has allowed researchers to generate 3D structures within PAAm hydrogels, on which cells are subsequently seeded, thus widening their application beyond classic 2D substrates (Figure 2 D1).

For example, by developing a novel photopolymerization technique based on selective illumination of the prepolymer solution through a quartz photomask, the effects of 3D curvature on MDCK epithelial tissue mechanobiology were studied [36,37] (Figure 2 D2). Specifically, 3D microwells with physiologically-relevant Gaussian curvature were developed [37]. Higher Gaussian curvature was shown to promote the formation of a supracellular actin cable at the microwell entrance, which, in turn, caused cells to align and migrate into the 3D lobular structure, with effects down to the nucleus in terms of nuclear organization and chromatin compaction.

Other approaches involving the polymerization of PAAm hydrogels against microfabricated structures to generate wells or channels of defined stiffness and geometry are starting to elucidate the roles of 3D confinement on fundamental processes such as traction force generation and confined migration [38,39]. For example, it was

A1) Schematic representation of durotactic polyacrylamide (PAAm) hydrogels devised in Ref. [19]. The substrate's Young's modulus E (ranging from 0.5 kPa to 22 kPa) linearly correlates to the bead density (green shade). **A2)** Using the hydrogels described in A1), durotaxis was described as a bidirectional process. U-251 MG human glioblastoma cells lack adhesion reinforcement and exhibit maximal traction force generation at $E \approx$ 5–10 kPa. As such, when seeded on hydrogels with gradient $E \approx$ 0.5–22kPa, these cells migrated toward their stiffness optimum both from soft tostiff (positive durotaxis, solid half of blue curve and schematic blue cell) and stiff to soft (negative durotaxis, dashed half of blue curve and schematic dashed blue cell), as predicted by the molecular clutch model. On the contrary, MDA-MB-231 human breast adenocarcinoma cells exhibit adhesion reinforcement and therefore show positive durotaxis in the same range (solid red curve and schematic red cell). Upon Talin 1/2 silencing, which reduces adhesion reinforcement, MDA-MB-231 cells show both positive and negative durotaxis, demonstrating the reversibility of durtoaxis, in accordance with the molecular clutch model. **B1)** Schematic representation of Soft ($E \approx$ 0.6 kPa) PAAm hydrogels subject to biaxial stretch using a custom-made microscopy-compatible stretching device to control both stretching frequency and amplitude devised in Ref. [25]. **B2)** Stretching mouse embryonic fibroblasts (MEFs) at increasing frequency (0.125–2 Hz) and different amplitudes (2.5–20%) on these soft PAAm hydrogels causes a biphasic response in YAP nuclear translocation (Nuc/Cyto YAP, Nuc=nuclear, Cyto=cytoplasmic) and adhesion growth. Low frequencies do not cause mechanosensing, because integrins unbind from the soft gel before talin can unfold, described by the initial flat phase of the curve. The increasing phase, where mechanosensing occurs, is comparable to the one that would be observed on progressively stiffer static elastic hydrogels of equivalent loading rate. Increasing the stretching amplitude from 2.5% to 20% causes maximum nuclear YAP and adhesion length to occur at lower frequencies, consistent with how both stretching amplitude and frequency affect the loading rate, as predicted by a modified molecular clutch model. The decreasing phase is caused by cytoskeletal softening, i.e. a partial disruption of the cytoskeleton at high stretching frequencies. **B3)** Schematic representation of modified PAAm hydrogels of tunable Young's modulus ($E \approx$ 1–80 kPa) containing gold nanoparticles with DNA tension probes (yellow) and traction force microscopy (TFM) beads (green) for simultaneous TFM and single-integrin force measurements, developed in Ref. [26]. This innovative method was termed molecular tension fluorescence microscopy (mTFM). **B4)** Using mTFM and MEFs, it was shown that the typical increase in tractionforces with substrate stiffness - measured by standard TFM, here shown as a schematic traction map (leftmost cell) - is explained by recruitment of additional low-load-bearing integrins (red), whereas the number of high-load-bearing integrins (green) is insensitive to substrate stiffness (middle and rightmost cell). **C1)** Schematic representation of PAAm hydrogels with independently tunable elasticity (spring) and viscous dissipation (dashpot) [28–30]. **C2)** Umbilical cord human mesenchymal stem cells migrate against a loss modulus gradient ($G'' \approx$ 1.6–300 Pa) on a PAAm hydrogels with uniform storage modulus ($G' \approx$ 1 kPa). This is explained by the increased deformation (creep, here shown by a red arrow) along the loss modulus gradient at longer time scales. This, together with the higher traction forces against the gradient, causes polarization and migration against the loss modulus gradient, in a process termed viscotaxis [31]. **C3)** Faster stress relaxation in PAAm hydrogels with mismatched DNA crosslinks of uniform shear modulus of 1.5 kPa biases differentiation of adult rat and mouse hippocampal neural stem cells toward astrogenesis and decreases neurogenesis. This is explained by an increased cell-ECM engagement within the molecular clutch model and cyclic oscillatory activation of RhoA (green arrows) [32]. **D1)** Schematic representation of microstructured PAAm hydrogels with 3D features, for example, 3D-curved wells created via photopolymerization of the prepolymer solution against an optical photomask [37], or 3D square wells created via micro molding of the prepolymer solution against polydimethylsiloxane (PDMS) pillars [39]. **D2)** 3D-curved wells of defined radius (25–100 μm) were developed using a photopolymerization technique in order to study the effect of physiologically-relevant Gaussian curvature ($k = k_1 k_2$) on MDCK cell mechanobiology. **D3)** 3D microwells (height h = 11–12 μm and width w = 14–15 μm) were developed in PAAm hydrogels of Young's modulus $E \approx$ 15–150 kPa using a molding technique to study how cell volume regulates traction force generation of human breast epithelial MCF-10A cells in well-defined 3D environments. Increasing microwell volume yields distinct traction force patterns (red arrows), switching from contractile to extensile. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.

recently shown that human breast epithelial MCF-10A cells switch from a contractile to an extensile traction force pattern based on their confining volume [39] (Figure 2 D3), furthering our understanding of how mechanical confinement, an important variable in the in vivo ECM which influences processes such as cancer progression [2], regulates mechanobiological processes.

Conclusions and future perspectives

Over the years, PAAm hydrogels have emerged as extremely versatile systems to mimic the physicochemical properties of the ECM. As such, fundamental concepts that have shaped the field of mechanobiology, including the molecular clutch model describing cell response to substrate stiffness [23,25] and durotaxis [20–22], have been established using this material system.

Using novel chemistries to better control and expand the range of accessible mechanical (including elastic and viscoelastic) properties [32], introducing dynamic changes in mechanical properties that can be controlled on demand via external stimuli such as magnetic actuation of magnetoactive PAAm hydrogels [40] or light-driven softening/stiffening of photosensitive PAAm hydrogels [41] (preprint), and exploiting novel microfabrication techniques to generate complex yet reproducible 3D systems [36–39], is anticipated to open novel avenues to address increasingly complex questions in the field of mechanobiology—both at level of single cells and multicellular tissues.

Author contributions

GC wrote the original manuscript and designed the figures. MSS edited the manuscript and acquired funding.

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.

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Data availability

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

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