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REVIEW

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Designing hydrogel dimensionality to investigate mechanobiology

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Hydrogels are indispensable tools for mechanobiology, providing tunable platforms that mimic the complex extracellular matrix and facilitate the study of cell–microenvironment interactions. This review highlights recent advances in the design of hydrogel systems with dimensionality ranging from 2D to 3D, including innovative 2.5D and sandwich configurations, to dissect the role of biophysical cues in cellular behavior and phenotype regulation. Special attention is given to alginate and gelatin methacrylamide (GelMA) hydrogels, which offer unique mechanical and biochemical properties tailored for diverse applications in 3D cell culture. Cutting-edge strategies to dynamically modulate hydrogel stiffness, viscoelasticity, and spatial confinement are discussed, showcasing their impact on cancer progression, stem cell differentiation, and collective cell migration. By integrating advanced hydrogel fabrication methods, including photopolymerization, dual cross-linking, and microfabrication techniques, this review underscores the transformative potential of hydrogels for unraveling the complexities of cellular mechanotransduction in evolving environments. We also explore the clinical potential of engineered hydrogels across applications including tissue regeneration, disease modeling, and controlled drug delivery. Finally, we discussed key challenges in replicating the dynamic mechanical complexity of living tissues and highlight emerging opportunities in the development of smart and adaptive hydrogel systems. Together, these innovations are paving the way toward next-generation biomimetic platforms that bridge fundamental research and translational applications in mechanobiology.

1. Introduction

In the human body, cells reside within a complex tissue microenvironment, primarily composed of water, proteoglycans (glycosaminoglycans), and structural proteins such as collagen, elastin, fibronectin, and laminin. This non-cellular component, known as the extracellular matrix (ECM), plays a central role in regulating cellular behavior, tissue development, and homeostasis. The ECM exhibits significant variability between tissues due to differences in composition, architecture, and biochemical properties—each influencing specific cellular function.

To replicate these native cues, the design of biomimetic scaffolds has become a major focus in tissue engineering and mechanobiology. These scaffolds aim to recreate both the biochemical and biophysical signals of the ECM, including mechanical properties such as stiffness, porosity, and topography, as well as the presence of adhesion ligands, growth factors, and dynamic signaling molecules. A broad range of biomaterials have been developed to mimic ECM characteristics such as fibrillar architecture, viscoelasticity, enzyme responsiveness, and the spatiotemporal presentation of bioactive factors. Among these, hydrogels have emerged as particularly versatile platforms for engineering physiologically relevant *in vitro* environments. These soft, water-swollen polymer networks permit nutrient diffusion while offering tunable mechanical properties, making them ideal substrates for applications in tissue engineering, drug delivery, and advanced cell culture systems.

2. Hydrogels as customizable substrates

Hydrogels are especially attractive for *in vitro* and *in vivo* applications due to their high-water content, and ability to be engineered with precise control over structural and mechanical properties. Their three-dimensional architecture and permeability allow for nutrients exchange, while their physical properties can be fine-tuned to mimic the diverse range of tissue mechanics found in the body. The field of hydrogel-based biomaterials continues to evolve, offering increasingly sophisticated strategies to recreate the mechanical, compositional, and structural complexity of the native ECM. Hydrogels can be derived from natural polymers (*e.g.*, collagen, fibrin, alginate),

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synthetic polymers (*e.g.*, polyacrylamide (PAAm), polyethylene glycol (PEG)), or hybrid combinations. The design of hydrogels for mechanobiology applications typically involves selecting an appropriate polymer backbone and crosslinking and crosslinking mechanism, which determines the network architecture and its mechanical properties[1,2] (Fig. 1A).

Physically crosslinked hydrogels (or supramolecular hydrogels) are formed through non-covalent interactions such as ionic bonding, hydrogen bonding, hydrophobic interactions, π–π stacking, or protein–protein interactions (*e.g.*, antibody–antigen). These hydrogels tend to be softer and more dynamic, often exhibiting shear-thinning and stress relaxation properties that more closely resemble native ECM behavior. However, the reversibility of these interactions can introduce mechanical instability under physiological conditions. For instance, the stiffness of alginate-based hydrogels can be tuned by varying the concentration of divalent calcium ions, but fluctuations in Ca^{2+} levels can affect cell viability and signaling, especially in calcium-sensitive cells.[3,4]

In contrast, chemically crosslinked hydrogels are stabilized by covalent bonds, resulting in more stable and tunable mechanical properties. Covalent crosslinking can be achieved through a wide array of chemistries, including radical polymerization (*e.g.*, UV-initiated polymerization with Irgacure 2959 or lithium phenyl-2,4,6-trimethylbenzoylphosphinate LAP), amine-carboxylic acid coupling, thiol–ene reactions, copper-catalyzed azide–alkyne cycloaddition, and enzyme-mediated crosslinking (*e.g.*, transglutaminases). These hydrogels offer a broader range of mechanical properties, improved reproducibility, and better structural stability for long-term studies.[1] However, the permanent nature of covalent bonds mean that these systems often lack key dynamic features of native tissues, such as stress relaxation, which has emerged as a critical factor *n* regulating cell–matrix interactions.[5]

While elasticity has long been recognized as a key regulator of cell behavior, recent studies have emphasized the importance of viscoelasticity, mechanical plasticity, and nonlinear elasticity in recapitulating the mechanical environment of living tissues. Natural hydrogels often exhibit low stiffness (on the order of a few Pascals), whereas synthetic systems allow for a broader range—from a few Pascals to hundreds of kilo-Pascals—enabling investigation of diverse cell types and mechanosensitive behaviors.

Viscoelasticity, in particular, is increasingly appreciated as a defining feature of ECM mechanics,[5,6] as well as mechanical plasticity[7] and nonlinear elasticity. Viscoelasticity arises from

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Sylvain Gabriele

Sylvain Gabriele is a tenured Associate Professor at the University of Mons (Belgium), where he leads the Mechanobiology & Biomaterials Lab. He obtained his MS degree in Physical Chemistry from the University of Pierre & Marie Curie (France) and completed his PhD in Polymer Physics at the University of Mons (Belgium) in 2006. He then held a CNRS postdoctoral position at Aix-Marseille University (France), where he developed microfluidic devices to investigate the confined migration of leukocytes through narrow capillaries. In 2008, he joined Harvard University (USA) as a postdoctoral researcher in the Disease and Biophysics Group, studying the propagation of mechanical signals within neuronal networks. He was subsequently appointed Chargé de Recherche at the FNRS and became an Assistant Professor at the University of Mons. Recognized as an Emerging Investigator by Soft Matter in 2013, he has also served as President of the Research Institute for Biosciences at the University of Mons since 2016. He was a Visiting Professor at Stanford University (USA) in 2017 and at the Mechanobiology Institute in Singapore in 2023. His contributions to physico-chemistry for life sciences were honored with the Louis Melsens Prize for the period 2016–2020, awarded by the Royal Academy of Science, Letters, and Fine Arts of Belgium in 2021.

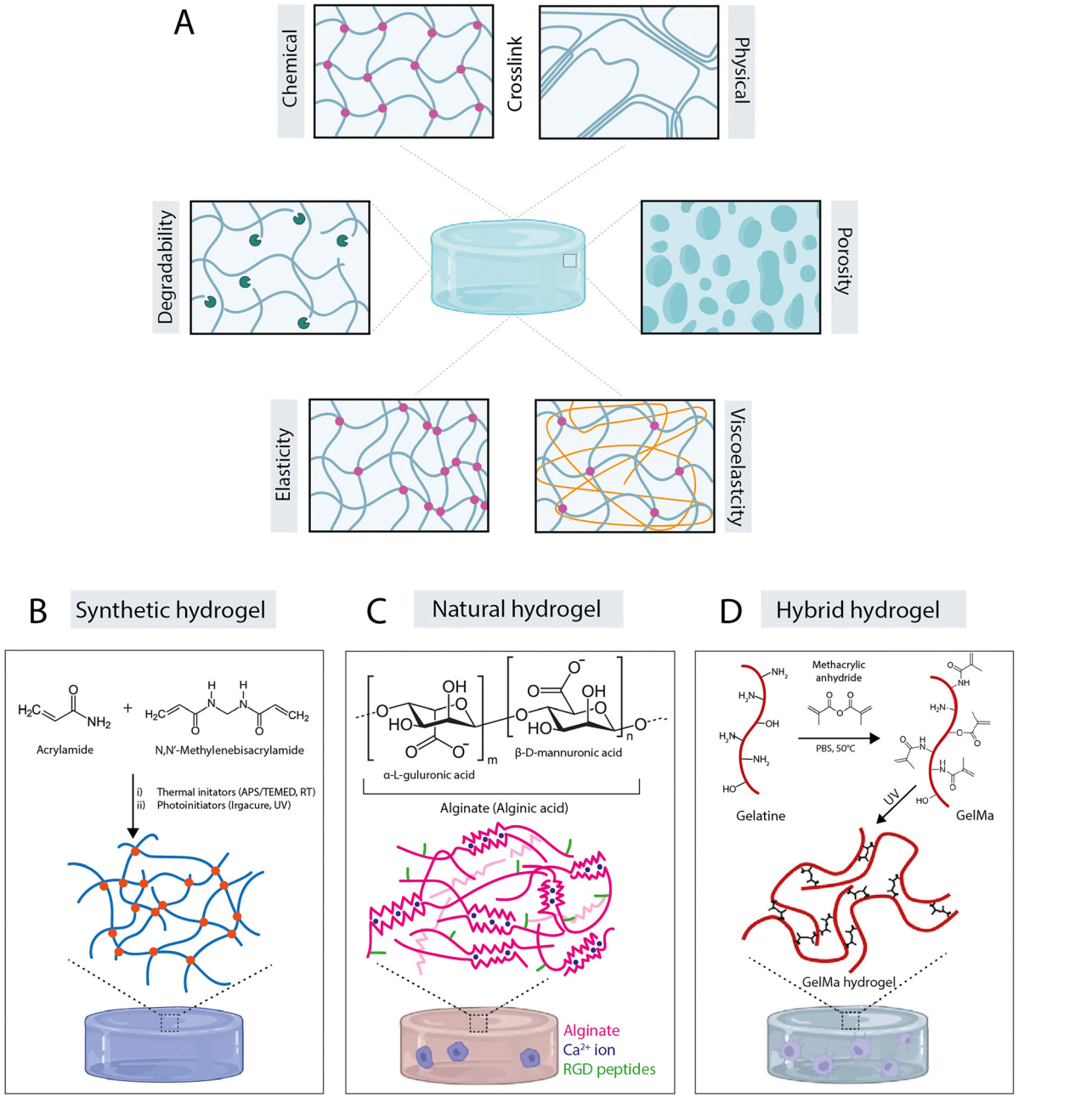

Fig. 1 Overview of different types of hydrogels. (A) Hydrogels can be designed using either physical or chemical cross-linking strategies. Their degradability depends on the nature of polymer network. Some natural hydrogels (*e.g.* collagen, matrigel, and fibrin) are proteolytically degraded by cell-secreted enzymes such as matrix metalloproteinases (MMPs). Most synthetic hydrogels are inherently non-degradable, but they can be engineered to become susceptible to enzymatic or hydrolytic degradation. Pore size is influenced by the cross-linking density, which, along with polymer concentration, also governs the mechanical properties of the hydrogel. Time-dependent mechanical behviors, such as viscoelasticity, can be introdiced by incorporating linear polymer chains. Hydrogels can be derived from natural, synthetic or hybrid materials that contain both material types. (B) (top) Chemical structures of acrylamide and *N*,*N*′-methylenebisacrylamide used to form polyacrylamide (PAAm) hydrogel *via* thermal or photo-initiated polymerization. (bottom) Schematic representation of PAAm network (blue) cross-linked by bisacrylamide monomers (orange). (C) (top) Chemical structures of α-L-guluronic acid and β-(1–4)-D-mannuronic acid (M-blocks) found in alginate. (bottom) Schematic representation of chemically modified 3D alginate hydrogel (pink), cross-linked (blue) with calcium ions (Ca^{2+}) and functionalized with arginine–glycine–aspartic acid (RGD) peptides (green). (D) Schematic of the chemical reaction used to modify gelatin with methacrylic anhydride, yielding gelatin methacrylamide (GelMa), followed by photopolymerization to form a GelMa hydrogel.

time-dependent molecular rearrangements such as reversible bond rupture, polymer chain entanglement, and protein unfolding. Hydrogels engineered to exhibit both energy storage (elastic) and dissipation (viscous) behaviors can better mimic how cells experience and respond to dynamic mechanical cues. For instance, recent work with viscoelastic polyacrylamide

hydrogels has shown that incorporating stress-relaxing behavior modulates epithelial cell spreading, focal adhesion maturation, and migration in a stiffness-dependent manner.[8] This highlights how the same base material, when altered in its dynamic mechanical properties, can elicit profoundly different cellular responses.

These findings collectively highlight that mechanical design parameters—including crosslinking type, network architecture, and viscoelasticity—are central to the development of hydrogels for mechanobiological research. These parameters not only define the physical characteristics of the material but also fundamentally influence cell behavior, governing processes such as adhesion, migration, proliferation, and differentiation. As the field progresses, an increasingly nuanced understanding of these material properties will be essential for building predictive and physiologically relevant *in vitro* models.

In the following section, we highlight three commonly used hydrogel systems that illustrate the breadth of available material platforms: a fully synthetic hydrogel (polyacrylamide), a naturally derived hydrogel (alginate), and a hybrid hydrogel (gelatin methacrylamide), which combines synthetic tunability with biological functionality.

2.1. Synthetic materials: polyacrylamide

Polyacrylamide (PAAm) hydrogels are widely used synthetic materials, particularly in 2D mechanobiology studies, due to their excellent mechanical tunability (Fig. 1B). PAAm networks are formed by free-radical polymerization of acrylamide monomers in the presence of a cross-linker such as *N*,*N*′-methylenebisacrylamide. Polymerization can be initiated either chemically, using ammonium persulfate (APS) and tetramethylethylenediamine (TEMED), or photochemically, using photoinitiators such as Irgacure 2959.[9,10] One of PAAm's major advantages is the precise and independent control over stiffness, which can be tuned from a few hundred Pascals to several hundred kiloPascals, without altering other properties such as porosity. This has made PAAm an indispensable tool for dissecting cell responses to substrate mechanics. However, PAAm is intrinsically non-adhesive to cells, necessitating surface functionalization to permit cell adhesion. To overcome this, various strategies have been employed to introduce bioactivity, including surface modification using sulfo-SANPAH, hydrazine, or periodate oxidation,[11] UV activation to create reactive groups,[12] and incorporation of NHS-esters during polymerization.[13] More recently, hydroxy-PAAm variants have been developed by incorporating hydroxyethyl acrylamide monomers, providing enhanced biofunctionality without increased cost or complexity.[14] Despite its utility in 2D, PAAm is generally unsuitable for 3D cell encapsulation due to the toxicity of unreacted monomers and the absence of degradable, cell-instructive features. Nonetheless, PAAm remains one of the most precisely tunable and reproducible platforms for studying mechanotransduction in controlled settings.

2.2. Natural materials: alginate

Alginate is a naturally derived polysaccharide extracted from brown algae. It consists of alternating blocks of β-D-mannuronic acid (M) and α-L-guluronic acid (G) units (Fig. 1C). The proportion and sequence of M and G residues influence the mechanical and structural properties of the resulting hydrogel.[15,16] Gelation occurs through ionic crosslinking, whereby divalent cations (*e.g.*, Ca^{2+}, Ba^{2+}, Mg^{2+}) create interchain bridges between G-blocks of adjacent chains, forming reversible interchain bridges that result in a physically crosslinked 3D network.[17] Due to its gentle gelation conditions and excellent biocompatibility, alginate is widely used for 3D cell encapsulation and bioprinting. However, native alginate lacks intrinsic integrin-binding sites and exhibits minimal protein adsorption, limiting cell–matrix interactions. To overcome this, alginate is often chemically modified with adhesive peptides such as RGD through covalent coupling to its carboxylic acid groups (Fig. 1C). One of the key advantages of alginate hydrogels is their reversibility: they can be depolymerized using chelating agents (*e.g.*, EDTA), allowing efficient recovery of viable cells from 3D matrices, which is particularly useful in studies focused on dynamic cell–matrix interactions in 3D. A major limitation of unmodified alginate is its resistance to enzymatic degradation, particularly by matrix metalloproteinases (MMPs), preventing cell-driven matrix remodeling. This limitation has spurred efforts to engineer MMP-degradable alginate variants by introducing cleavable peptide linkers or incorporating degradable moieties. For example, a recent study demonstrated the successful synthesis of MMP-sensitive alginate hydrogels using thiol–ene chemistry to introduce degradable crosslinks, promoting cell spreading and viability within the matrix.[18]

2.3. Hybrid materials: gelatin methacrylamide

Gelatin methacrylamide (GelMA) is a hybrid hydrogel system that combines the biological activity of gelatin with the spatial control offered by photopolymerizable chemistries (Fig. 1D). Gelatin, a denatured form of collagen obtained through collagen hydrolysis, loses the native triple-helix structure but retains similar chemical and biological properties, albeit without the fibrillar organization. GelMA is typically synthesized by reacting gelatin with methacrylic anhydride (MAA), introducing methacrylamide and methacrylate groups that enable covalent crosslinking upon UV exposure in the presence of a photoinitiator.[19,20] The degree of methacrylation can be precisely controlled by adjusting the gelatin-to-MAA ratio, allowing fine-tuning of hydrogel stiffness, degradation kinetics, and network density. This modified gelatin retains key bioactive motifs, such as RGD sequences and MMP-sensitive sites, supporting cell adhesion and enzymatic remodeling. As a result, GelMA hydrogels are well-suited for both 2D and 3D applications in mechanobiology, offering excellent biocompatibility, mechanical tunability, and compatibility with various biofabrication techniques. Its compatibility with advanced fabrication methods, such as photopatterning and 3D bioprinting, further enhances its appeal for tissue engineering and organ-on-chip applications.

Thanks to their versatility and tunability, hydrogels have become essential tools in mechanobiology, enabling the

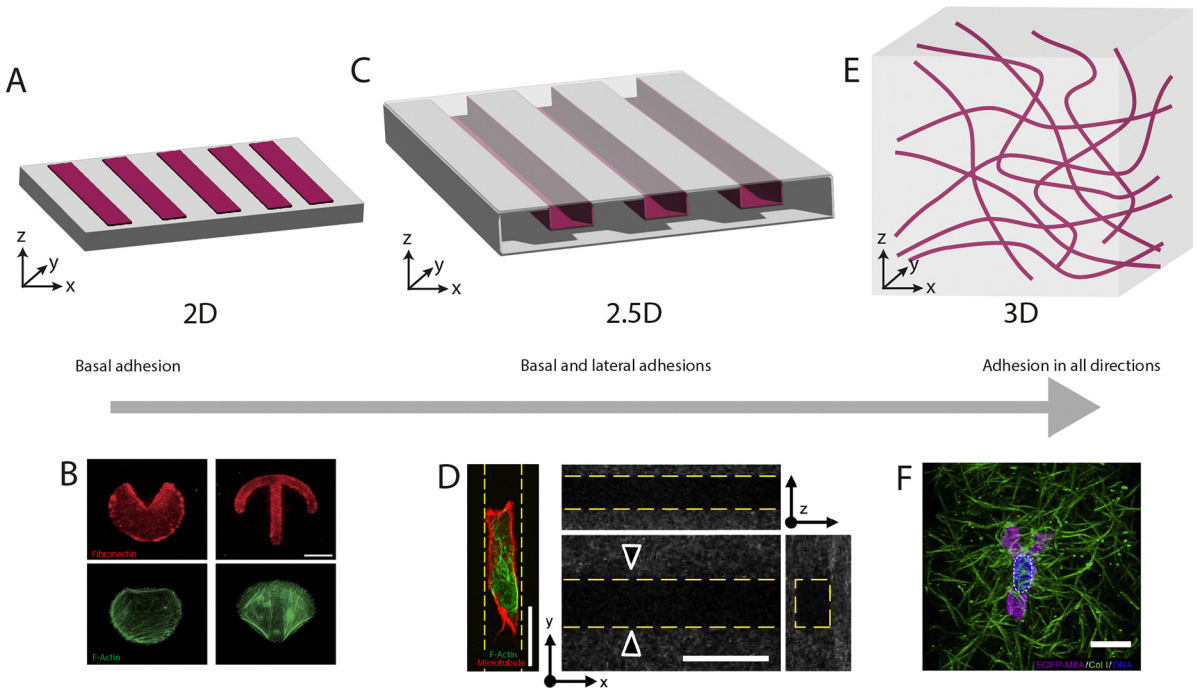

Fig. 2 Dimensionality modulation of hydrogels as a versatile tool for studying mechanobiology. (A) Schematic representation a micropatterned soft two-dimensional (2D) hydrogel coated with proteins (purple). (B) Cells plated on Pacman (left) and crossbow (right) fibronectin micropatterns (red). Cells are stained with phalloidin to visualize F-actin filaments (green). Scale bar: 10 μm. Reproduced from ref. 12 with permission from The Royal Society of Chemistry, copyright 2011. (C) Schematic representation of an intermediate-dimensional (2.5D) hydrogel system. (D) Confocal fluorescent image of a cell within a collagen microtrack, labelled for F-actin filaments (green) and microtubules (red). Orthogonal views depict the structure of the microtrack with yellow dashed lines outlining its boundaries. Scale bars: 25 μm. Reproduced from ref. 21 with permission from The American Physiological Society copyright 2015. (E) Representation of a three-dimensional (3D) matrix hydrogel. (F) Maximum intensity projection of a fibroblast expressing TagGFP2-LifeAct (magenta) spreading in collagen (green). Scale bar: 20 μm. Reproduced from ref. 22 with permission from Elsevier, copyright 2021.

exploration of how cells respond to the mechanical and structural properties of their microenvironment. Continued innovation in hydrogel design—including dimensionality (from 2D to 3D and intermediate 2.5D configurations), degradability, viscoelastic tuning, and biofunctionalization—is deepening our understanding of how cells interpret and respond to mechanical cues. Novel platforms such as sandwich gels, curved hydrogels, and gradient systems (Fig. 2) are helping to reveal how cells process spatial and temporal information in complex environments. These technological advances not only offer insight into fundamental cell–matrix interactions but also pave the way for the development of next-generation biomimetic scaffolds tailored to specific physiological and therapeutic goals.

3. Tailoring the hydrogel dimensionality

While the role of biochemical cues in directing cell fate has been extensively studied, there is a growing body of evidence that biophysical cues are equally critical in regulating cell mechanobiology and guiding complex cellular processes, such as differentiation.[23] Most research to date has focused on two-dimensional (2D) hydrogels with homogeneously coated or microprinted proteins (Fig. 2A and B). In one influential study, mesenchymal stem cells (MSCs) were cultured on PAAm substrates with varying stiffness to explore how matrix mechanics influence stem cell differentiation. It was found that MSCs differentiated into neurocytes, skeletal muscle cells, and osteoblasts on soft (0.1–1 kPa), intermediate (8–17 kPa), and stiff (>34 kPa) hydrogels, respectively, demonstrating the matrix's pivotal role in cell lineage specification.[23] However, traditional 2D planar culture substrates lack the ability to modulate key parameters such as cell spreading and confinement, which are characteristic of dense epithelial monolayers. Additionally, they fail to replicate the complexity of the native 3D microenvironment. While substrate stiffness plays a central role in influencing cell morphology in 2D culture systems—an essential factor in guiding mesenchymal stem cell fate along stiffness gradients[24]—this relationship changes in 3D environments. For instance, MSC morphology is significantly influenced by stiffness in 2D cultures,[25,26] yet during osteogenic differentiation within 3D alginate hydrogels,[6,27] cell morphology becomes largely independent of matrix stiffness. These observations underscore the need for intermediate hydrogel platforms that bridge the gap between 2D planar cultures and fully 3D systems. Such platforms are crucial for isolating and studying the distinct effects of biophysical cues, including cell shape and spatial confinement.[28,29]

To address this, intermediate hydrogel systems, such as 2.5D platforms, have emerged as a crucial tool for dissecting the individual effects of biophysical cues like cell shape, spatial confinement, and stiffness (Fig. 2C and D).[21] These systems provide the precision necessary to better understand cellular responses to their microenvironment while bridging the gap between traditional 2D and complete 3D culture systems. In contrast, fully 3D systems with encapsulated cells provide a more physiologically relevant environment by replicating the complex spatial and mechanical properties of native tissues, enabling more accurate studies of cell behavior under *in vivo*-like conditions (Fig. 2E and F). This article will present significant results in mechanobiology achieved using hydrogel-based platforms with multiscale dimensions, transitioning from 2D to 3D through innovative 2.5D and sandwich strategies.

3.1. 2D microprinted hydrogels

Considerable effort has been directed toward developing synthetic hydrogels that mimic the natural ECM. However, culturing cells on 2D hydrogels alone falls short of isolating specific mechanical cues or fully elucidating how these cues drive cellular phenotypic and functional responses. To address this, micropatterning techniques, originally designed for rigid substrates,[30,31] were adapted to hydrogels of varying stiffnesses. Early techniques for controlling cell adhesion patterns emerged in the 1970s,[32,33] and by the 2000s, microcontact printing had become the preferred approach (Fig. 3).[34,35] This soft lithography technique facilitates the creation of protein adhesive islands, providing precise control over cell and small cell cluster dimensions[36] (Fig. 3). Micropatterning approaches, offering a powerful tool for examining specific mechanical cues

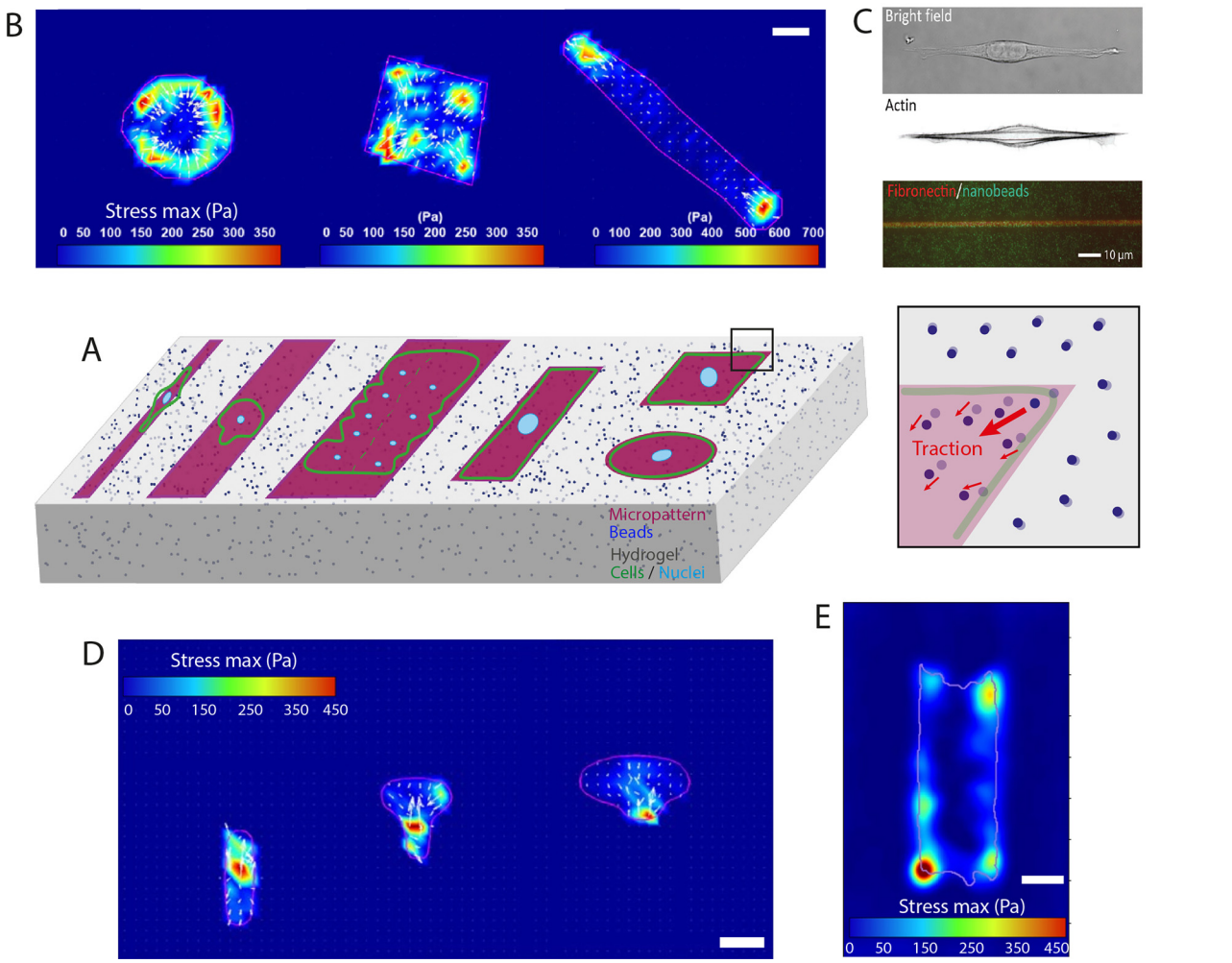

Fig. 3 Integrating hydrogel micropatterning with traction force microscopy (TFM) to study cell mechanics and confined migration. (A) Schematic representation of a 2D hydrogel with various microprinted geometries (purple). Fluorescent beads embedded within the hydrogel enable simultaneous micropatterning and force measurements using TFM. Typical traction force maps obtained *via* TFM for (B) different myoblast morphologies. Reproduced from ref. 43 with permission from Springer Nature, copyright 2019. (C) From top to bottom: Bright-field image, actin cytoskeleton visualization, and fluorescent bead (green) imaging of cell adhered to a 2 μm-wide micropatterned fibronectin line (red) with nanobeads (green) to determine traction forces. Adapted from ref. 51. Typical traction force maps obtained *via* TFM for (D) confined and unconfined migrating cell morphologies and (E) during the migration of an epithelial cluster. (D) Reproduced from ref. 52 with permission from Springer Nature, copyright 2019. (E) Reproduced from ref. 53 with permission from Springer Nature, copyright 2024. Scale bars are: (B) 20 μm, (D) 15 μm and (E) 50 μm.

in cellular environments, were naturally extended to hydrogels with ECM-like properties, enhancing the ability to control cell adhesion area and morphology. For example, before microcontact printing on hydrogels was developed, early studies created protein islands on PAAm hydrogels by incubating a collagen solution on a flexible, perforated PDMS membrane over a hydrogel. These studies revealed that cells reorient their actin stress fibers and focal adhesions according to the imposed geometry, especially in square and rectangular corners.[37] PAAm hydrogels with elastic properties allowed for the observation of traction forces exerted by contractile stress fibers concentrated in these corners, correlating with focal adhesion formation and high lamellipodial activity.[37] However, conventional PAAm hydrogel functionalization, which typically uses UV-activated sulfo-SANPAH, often suffers from variability due to the compound's instability in aqueous solutions,[38,39] highlighting the need for more stable functionalization methods. To address this issue, new strategies, such as the functionalization of PAAm hydrogels with hydroxyl groups,[14,40] aldehyde,[38] or photocleavable comonomers containing a caged amine,[41] have advanced the application of direct microcontact printing on hydrogels by enabling the binding of a broad range of ECM proteins. Applying protein micropatterns with different shapes and stiffnesses on hydrogels has shed light on how matrix stiffness modulates growth dynamics, synaptic density, and electrophysiological activity in cortical neuronal networks.[42] The development of microprinting techniques on hydrogels has made it possible not only to control cell spreading on islands of defined sizes and geometries while regulating the stiffness of the cellular matrix within physiologically relevant ranges but also to combine micropatterns with force measurements using traction force microscopy (TFM) (Fig. 3A). Indeed, many synthetic hydrogels, such as PAAm, exhibit linear elastic properties that allow the generation of a map of contractile forces exerted on the substrate based on the deformation map obtained by observing the displacement of fluorescent beads ($\sim$250 nm in diameter) embedded in the hydrogel through microscopy (Fig. 3). The integration of protein micropatterns with traction force microscopy (TFM) has revealed that cell elongation enhances actomyosin contractility in myoblasts (Fig. 3B). This increased contractility influences actin network organization, nuclear orientation, and cytoplasmic localization of the yes-associated protein (YAP), highlighting YAP's central role in mechanotransduction in myoblasts.[43] Indeed, YAP is a mechanotransducer in the Hippo signaling pathway that translates mechanical cues from the cellular environment, such as stiffness or tension, into biochemical signals to regulate gene expression, cell proliferation, and differentiation. Similarly, studies manipulating endothelial cell elongation on rectangular micropatterns have demonstrated that contractile stress fiber organization not only governs nuclear orientation and deformation but also affects chromatin condensation.[44,45] Using micropatterned PAAm hydrogels in TFM has further shown that elevated actin filament tension induces nuclear indentations, with LINC complex accumulation at these sites, emphasizing the role of cellular tension in shaping nuclear structure and chromatin organization.[46] Advancements in micropatterning techniques, such as adhesive micropattern creation through deep UV photoillumination with a photomask,[12] have enabled more versatile approaches for micropatterning on PAAm hydrogels. These innovations expand the potential for precise control of cellular environments and mechanobiological investigations by showing for instance that cells integrate ECM geometry at the whole-cell level by reorganizing the actin network[47] and that the nucleus is essential for proper mechanical responses, though it may be dispensable for polarization and migration in 2D environments.[48] The functionalization of hydrogels with protein micropatterns has not only enabled the identification of organizational rules for cytoskeletal components and organelle arrangement in response to changes in cell geometry,[36] biochemical composition,[49] or substrate stiffness[50] but has also transformed our approach to studying cell migration dynamics. By using protein lines ranging from just a few microns to several tens of microns in width, researchers have been able to mimic the morphologies of cells migrating within 3D environments (Fig. 3C).[51] The integration of TFM with protein lines of varying widths has provided valuable insights into how spatial confinement influences the distribution of contractile forces exerted by migrating cells. This approach has revealed the critical role of stick-slip mechanisms in driving symmetry breaking[51] (Fig. 3C), and has shown how the modulation of spatial confinement can modulate cell migration speed through modification of actin treadmilling activity within the lamellipodium (Fig. 3D).[52] Recently, the advantages of micropatterned soft hydrogels, which enable spatial confinement and measurement of substrate contraction forces, have demonstrated that the migration efficiency of cell clusters is strongly influenced by contact geometry and the orientation of cell–cell junctions relative to the migration direction (Fig. 3E).[53] This approach highlights how geometric constraints and intercellular organization play crucial roles in modulating collective cell migration dynamics.

3.2. 2.5D hydrogel platforms

Expanding micropatterning techniques to create pseudo-3D structures within hydrogels has unlocked new opportunities for investigating cellular behavior in constrained environments. This approach has led to the development of 2.5D hydrogel platforms—systems that bridge 2D and 3D environments—including microtracks, microwells, pillars, and fibers (Fig. 2C, D and 4). Using embossing techniques, open or closed microtracks with a hydrogel cap were engineered within collagen matrices to replicate the size and topography of tracks generated by proteolytically active cancer cells. The level of spatial confinement can vary in these 2.5D microtracks (open configuration) to a pseudo-3D configuration (close configuration) depending on the width and depth of the microtrack. These innovative microtracks have revealed that cells can migrate through a 3D ECM without the need for matrix metalloproteinases (MMPs), a surprising finding that suggests alternative pathways for cell movement.[54] Notably, while

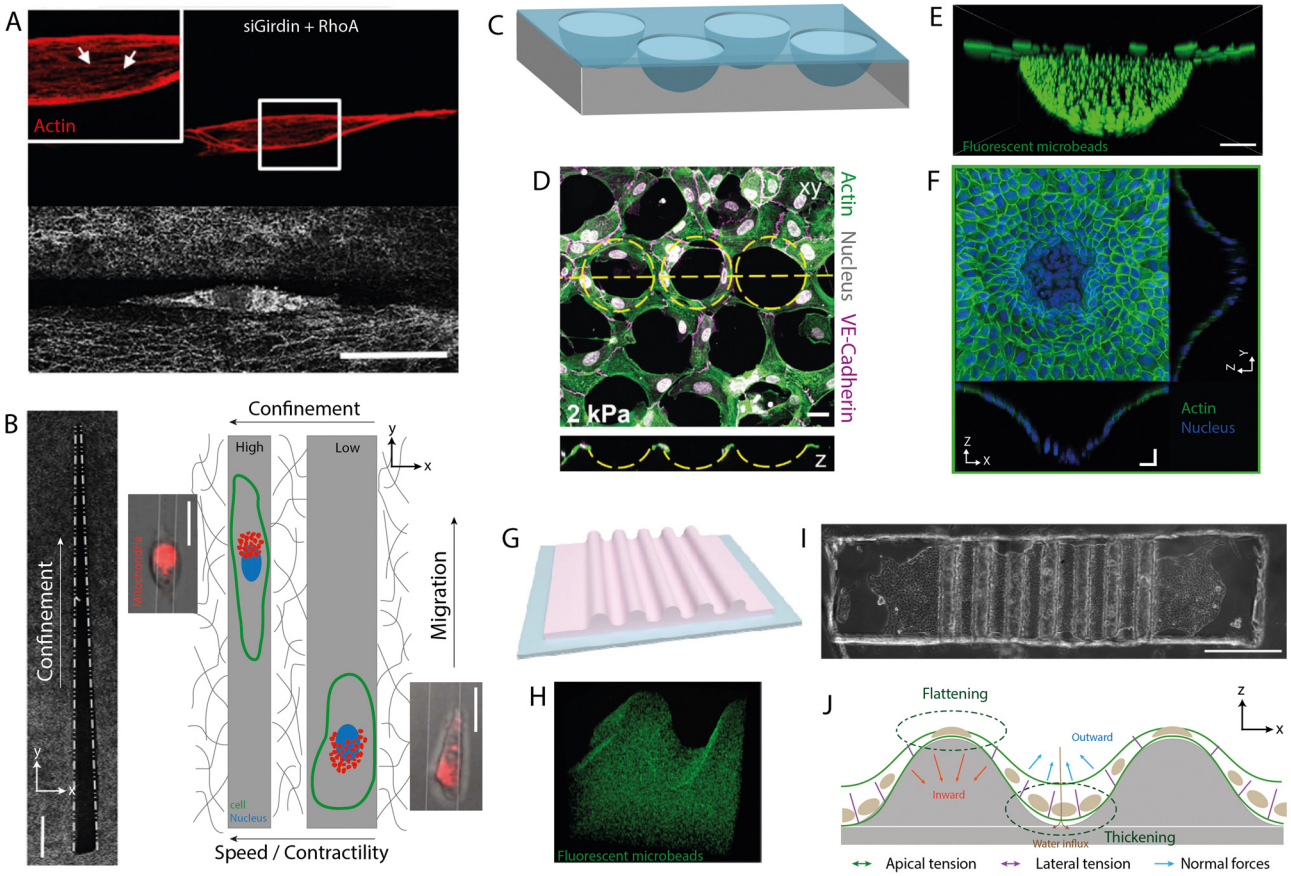

Fig. 4 Cellular organization and mechanobiology in various 2.5D hydrogel systems. (A) Confocal immunofluorescence (top) and reflectance (bottom) images showing actin filament organization (red) in RhoA activator treated girdin knockdown MDA-MB-231 cells during 3D microtrack migration. Insets (2× magnification) highlight actin stress fibers (white arrows). Scale bar: 25 μm. Reproduced from ref. 57 with permission from Springer Nature, copyright 2018. (B) (left) Confocal reflectance image of a collagen microtrack with a tapering width (20 to 5 μm). Scale bar: 25 μm. Schematic and representative images of cells in fully confined and partially confined zone, with labelled mitochondria. Scale bar: 20 μm. Reproduced from ref. 58 with permission from Cell Press, copyright 2019. Reproduced from ref. 59 with permission from Royal Society of Chemistry, copyright 2024. (C) Schematic representation of a multiple microwells system. (D) Confocal images of cells stained for actin (green), nucleus (gray), and VE-cadherin (magenta) in hemispherical microwells. Top (*xy*) and side (*xz*) views are shown, with yellow dashed lines indicating microwell positions and axes for side views. Scale bar: 25 μm. Reproduced from ref. 60 with permission from American Association for the Advancement of Science, copyright 2022. (E) Confocal side-view (*xz*) image of a single 3D microwell. Scale bar: 30 μm. Reproduced from ref. 10 with permission from Wiley, copyright 2024. (F) Confocal images showing top (*xy*) and side (*xz*; *yz*) views of 3D microwells covered by a confluent epithelial monolayer. Scale bar: 20 μm. (G) Schematic representation of a corrugated hydrogel. Reproduced from ref. 10 with permission from Wiley, copyright 2024. (H) 3D visualization of fluorescent bead distribution within a polyacrylamide hydrogel. (I) Bright field image of a cell monolayer grown on a corrugated substrate. Scale: 500 μm. Reproduced from ref. 61 with permission from eLife Sciences Publications Ltd, copyright 2024. (J) Schematic of an epithelial monolayer grown on a corrugated hydrogel, where the substrate profile alternates between convex and concave curvature. Apical tension (green) and lateral tension (purple) modulate monolayer thickness, leading to flattening on crests and thickening in valleys. Convex regions enhance adhesion and reduce apoptosis, while concave regions promote cell extrusion. Reproduced from ref. 9 with permission from Nature Portfolio, copyright 2024.

cell–matrix mechanocoupling is critical for migration through unstructured 3D environments, it is not necessary for microtrack migration. Instead, migration within these tracks is driven by cytoskeletal dynamics—including actin polymerization, cortical tension, and microtubule organization[21] – in coordination with the focal adhesion protein vinculin.[55] Further studies using this platform have shown that girdin, a pro-metastatic protein essential for cell polarity, is a key factor in 3D collagen microtrack migration (Fig. 3A). Knockdown of girdin reduces migration speed, disrupts cell morphology and orientation, and leads to a loss of directed movement. Interestingly, girdin depletion also impairs actin organization and stress fiber formation, defects that can be reversed by upregulating the GTPase RhoA (Fig. 4A, inset), which is involved in the regulation of actin cytoskeleton dynamics and plays a key role in cellular contractility and mechanotransduction.[56] This suggests that RhoA plays an important role in restoring cytoskeletal structure and migration capacity when girdin is absent.[57] More recently, the collagen microtrack platform has been used to explore how confinement primes epithelial cancer cells for rapid migration. Studies have shown that migration through confined tracks enhances cell speed and causes migratory machinery (Fig. 4B)—including actin and metabolically active mitochondria—to accumulate at the leading edge of breast

cancer cells (Fig. 4B). This suggests that active mitochondrial localization within confined spaces may facilitate even faster migration once the cells exit the confined environment.[58,59] Together, these findings from collagen microtrack studies provide new insights into how confinement conditions prime cancer cells for enhanced future migration. They also point to potential therapeutic targets for disrupting confinement-induced adaptations to inhibit breast cancer metastasis.

Other 2.5D structures in hydrogels such as microwell arrays have proven highly effective as high-throughput platforms for cell culture and imaging (Fig. 4C–F). It was shown that microwell arrays in polyethylene glycol (PEG) allow single-cell spatial confinement within microfabricated cavities, facilitating continuous, long-term observation of individual cells and their progeny.[62,63] Hydrogel microwell arrays have also enabled the production of uniformly sized multicellular tumor spheroids, which are valuable for mechanobiology studies and advanced drug screening. These tumor spheroids are structurally robust and can be easily transferred to standard 2D culture substrates without disrupting the engineered 3D multicellular configuration.[64] Additionally, variations in the aspect ratio of soft hydrogel microwells have revealed new modes of endothelial cell self-organization. Independent of protein or chemical patterning, these geometrical and mechanical cues alone have been shown to guide cell behavior (Fig. 4D). For example, endothelial cells in soft (2 kPa) gelatin microwells are approximately 30 times more likely to migrate to the edges and organize into ring-like structures compared to cells in stiffer (35 kPa) microwells, demonstrating the impact of combined stiffness and geometry cues on cell arrangement.[60]

Recently, a photopolymerization technique using an optical photomask has been developed to fabricate bowl-shaped microwells and wavy corrugations in PAAm hydrogels (Fig. 4E and F). Microwells can replicate the curvature and dimensions of lobular tissue structures, enabling investigations into how Gaussian curvature at the microwell entrance affects epithelial tissue mechanobiology.[10] Notably, cells cultured in these microwells form supracellular contractile actin cables along the edge of the microwell (Fig. 4F), which drive the vertical orientation of nuclei toward the microwell bottom. Other forms of curved hydrogels such as corrugated hydrogels (Fig. 4G–J) have allowed to investigate how epithelial cell monolayers sense and adapt to concave and convex structures by adjusting their thickness and encoding these thickness variations through changes in nuclear deformation, positioning, and function.[9] Furthermore, convex surfaces on elastomeric substrates were shown to drive cellular forces that counteracted osmotic gradients, enhancing cell adhesion and reducing apoptosis (Fig. 4I and J).[61] Conversely, concave regions diminished these forces, collectively creating a curvature-dependent spatial pattern in cell extrusion (Fig. 4I and J). The role of basal hydraulic stress in regulating cell extrusion is further highlighted by the reduced extrusion rates observed in monolayers grown on wavy hydrogel substrates. Unlike elastomeric materials, hydrogels allow both water and solutes to permeate, mitigating increases in solute concentration caused by apical-to-basal ion transport, thereby influencing the overall extrusion dynamics.[61] These findings from 2.5D hydrogel platforms highlight how microenvironmental curvature influences cytoskeletal organization and nuclear positioning, offering valuable models for studying curvature-induced mechanotransduction in epithelial cells.[65]

Alternatively, various hydrogel microstructures have been developed to mimic cellular interactions with ECM microstructures or to replicate the specific cellular arrangements found in native tissues. For example, hydrogel micropillars created through casting techniques allow for passive longitudinal tension generation,[66] as myoblasts exert contractile forces on the pillars. This configuration restricts cell compaction along the longitudinal axis, promoting alignment and maturation similar to native muscle tissue. Myoblasts cultured on these micropillars show significant alignment within two days, progressing to a dense cellular construct by day five, and ultimately forming compact muscle bundles by day twelve, closely resembling the microarchitecture of skeletal muscle.[67] While these microstructured hydrogels provide powerful platforms for studying the fundamental mechanisms of cellular adaptation to various ECM cues and for creating organized tissue structures, they often lack the 3D signals needed to fully replicate a physiological-like microenvironment. To address this limitation, hydrogel microfibers—produced *via* techniques like electrospinning, extrusion, or microfluidic spinning—offer an ideal scaffold to promote cell elongation and alignment along biomimetic cylindrical shapes, like natural collagen fibrils.[68] Hydrogel microfibers are increasingly gaining attention as advanced constructs for skeletal muscle tissue engineering, due to their ability to direct cellular organization and mimic the natural fiber-like structure of muscle.[69]

3.3. Sandwiched and 3D hydrogel platforms

Advanced strategies have recently been developed to create 3D cell cultures from hydrogels with controlled physico-chemical properties, producing 3D platforms that closely mimic physiological conditions (Fig. 2E and F). Notably, an alternative to embedding cells within hydrogels has emerged: a ''sandwich culture'' method, where cells are cultured between two hydrogel layers (Fig. 5A). This approach offers several advantages, including the ability to precisely adjust the mechanical properties of each layer and improved accessibility for optical microscopy. For instance, this technique has been used to investigate the effects of hydrogel stiffness on mechanotransduction in mouse induced pluripotent stem cells (iPSC)-derived embryoid bodies (iPSC-EBs). When iPSC-EBs are seeded between PAA hydrogels with varying stiffness levels (E' = 54.3 ± 7.1 kPa for hard, 28.1 ± 2.3 kPa for moderate, and 5.1 ± 0.1 kPa for soft) and cultured for two days, stiffness cues activate the YAP mechanotransducer and trigger actin cytoskeleton reorganization in a stiffness-dependent manner.[70] Additionally, cell culture on hydrogels of moderate stiffnesses specifically upregulates the protein expression of ectoderm and mesoderm lineage markers in iPSC-EBs, driven by YAP-mediated mechanotransduction. Advanced sandwiched platforms have recently been developed

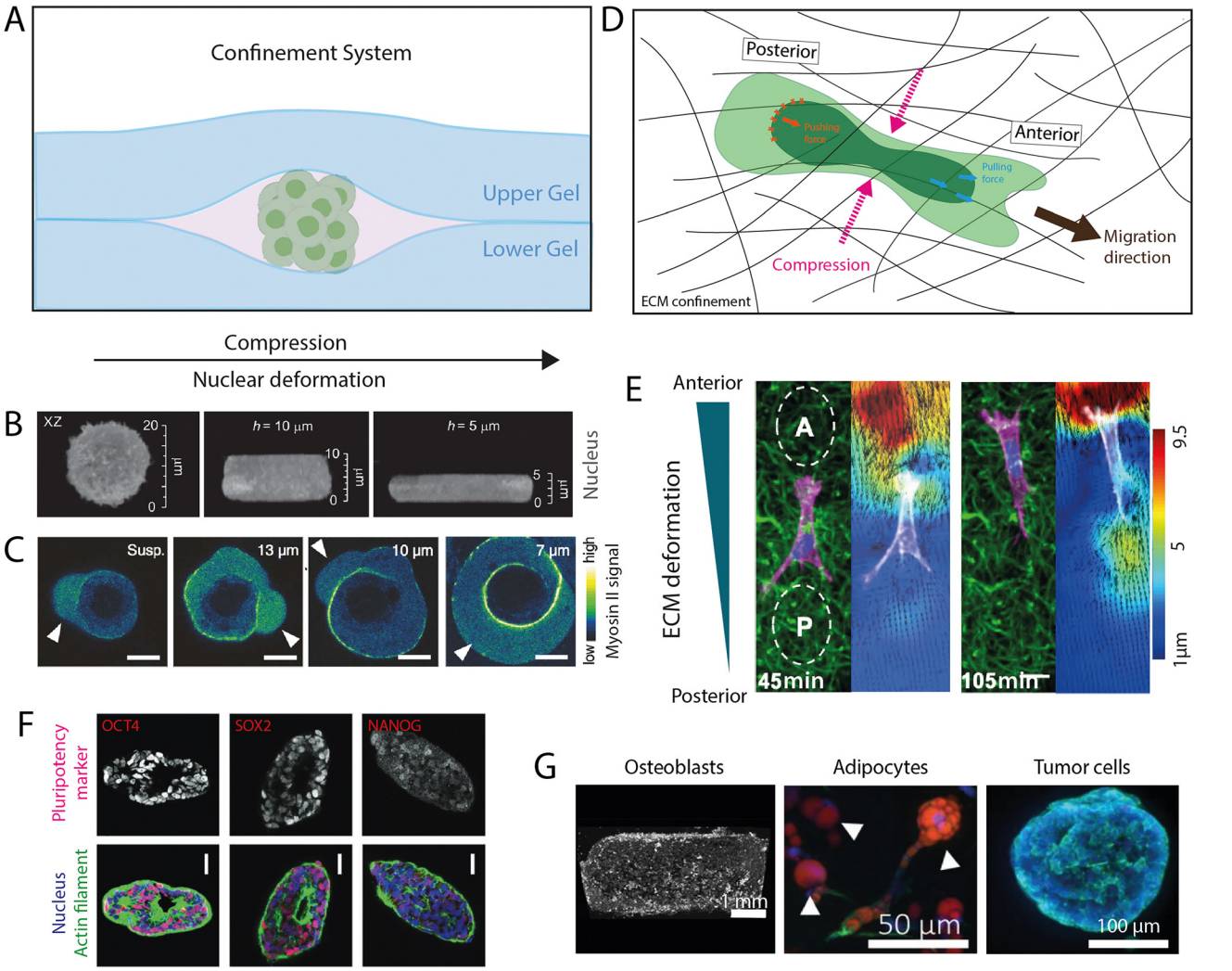

Fig. 5 Advanced 3D hydrogel systems for cell confinement, migration, and differentiation studies. (A) Schematic representation of a "sandwich culture" method, where cells (green) are cultured between two hydrogel layers (blue). (B) 3D images (*xz* views) of the same cell at different heights within a hydrogel. Reproduced from ref. 73 with permission from American Association for the Advancement of Science, copyright 2020. (C) Confocal fluorescence images of control progenitor stem cells in suspension (Susp.) and under varying confinement heights, expressing Myl12.1-eGFP (myosin II). Scale bars: 10 μm. Reproduced from ref. 72 with permission from American Association for the Advancement of Science, copyright 2020. (D) Schematic representation of a cell embedded in a 3D collagen matrix and undergoing compression during a confining migration event. (E) Time-lapse images of human fibroblasts expressing TagGFP2-LifeAct (magenta) embedded in a 3D collagen matrix (green), migrating directionally. "A" (anterior) and "P" (posterior) ovals represent deformations associated with cell migration. Color-coded bar indicates ECM deformation. Reproduced from ref. 22 with permission from Cell Press, copyright 2021. (F) Representative immunohistochemical stains of pluripotency markers OCT4, SOX2, and NANOG for cells encapsulated in RGD alginate hydrogels. The scale bar: 25 μm. Reproduced from ref. 76 with permission from Wiley, copyright 2021. (G) Left top to right: Micro-computed tomography (μCT) image showing osteoblast mineralization (minerals in white) in GelMA hydrogel, immunofluorescence image of lipid droplets formation in cell, and immunofluorescence image of spheroids in GelMa hydrogel. Reproduced from ref. 77 with permission from Wiley, copyright 2021.

to mimic the spatial confinement experienced by cells while independently tuning the microenvironment's stiffness within the physiological range. These systems provide valuable tools for studying cellular behavior under conditions that closely resemble the tumor microenvironment. For instance, a confinement device based on molded agarose pads was used to investigate how colorectal cancer cells adapt to prolonged confined environments. The study revealed, for the first time, a mechano-adaptive response during mitosis, characterized by a decrease in nuclear size.[71] This adaptation relies on the nuclear tension sensor cPLA—an enzyme that acts as a sensor of intracellular calcium levels, playing a critical role in releasing arachidonic acid—and the cellular contractility machinery (Fig. 5B and C),[72,73] suggesting a mechanism that may be crucial for cellular resilience to mechanical stresses in the tumor microenvironment. Similarly, a hydrogel-based microchannel platform—which serves as an intermediate model between 2D microtracks and fully 3D cultures—with independently tunable channel stiffness and width demonstrated that cancer cell migration speed is influenced by the synergistic

effects of these parameters.[74] Furthermore, the mesenchymal-to-amoeboid transition—a key feature of cancer cell plasticity—was found to strongly correlate with channel stiffness, underscoring the role of microenvironmental mechanics in regulating migration modes. While sandwiched hydrogels are invaluable for studying processes such as apico-basal competition in response to physicochemical changes within the cellular microenvironment, they fall short of replicating the complexity of true 3D matrices, which are designed to more accurately mimic the anatomical structures and functions of human tissues (Fig. 5D). For instance fibroblasts and MSCs migrate in 3D environments by generating and maintaining matrix prestrain through myosin IIA-dependent anterior contractions, enabling directional migration along ECM stiffness gradients[22] (Fig. 5E). In contrast, glioblastoma (GBM) cells exhibit both mesenchymal and amoeboid migration modes, with the transition to amoeboid behavior driven by hyaluronic acid (HA) content in 3D collagen matrices. This HA-induced shift involves ROCK-dependent mechanisms, highlighting the plasticity of 3D migration strategies in different cellular contexts.[75]

To emulate the specific physicochemical properties of tissues, including matrix composition and stiffness, a diverse array of hydrogel systems and fabrication strategies has been developed. These systems employ different materials, such as silk, hyaluronic acid, peptide-based and PEG-based hydrogels, alginate, GelMA and various synthesis methods, including ionic or covalent cross-linking, phase transition, cell-mediated cross-linking, free-radical polymerization, photopolymerization, and click chemistry.[78,79] In the following section, we will focus specifically on alginate and GelMA hydrogels, which represent a significant portion of recent efforts aimed at studying 3D mechanobiology using hydrogels.

3.3.1. Alginate hydrogels. They are widely used due to their excellent biocompatibility, making them particularly suitable for applications such as vascular, cartilage, and bone tissue engineering. However, these scaffolds face challenges such as limited cellular adhesion, slow degradation rates, and inadequate mechanical strength, particularly when aiming to replicate the properties of bone tissue. To overcome these limitations, alginate can be chemically modified or cross-linked using advanced strategies to enhance its functional properties. For example, the incorporation of arginine–glycine–aspartic acid (RGD) peptides (Fig. 1B) has been shown to improve cell adhesion and facilitate the development of functional cardiac muscle tissue.[80] Interestingly, the inclusion of RGD peptides not only increased cellular adhesion and accelerated cell proliferation but also revealed that the size of the spheroids formed was governed solely by the stiffness of the hydrogel, rather than the presence of adhesion motifs.[81] Alginate hydrogels with tunable mechanical properties have been utilized to explore the emergence of drug resistance in breast cancer cells, providing a novel approach to mimic the evolving tumor microenvironment. A dynamic alginate–matrigel system was developed in which stiffness was precisely modulated by activating calcium-loaded liposomes embedded within the hydrogel through near-infrared light exposure.[82] As the elastic modulus increased to mimic the early stages of breast tumor progression, tumoral breast epithelial cells (MDA-MB-231) displayed reduced sensitivity to the chemotherapeutic agent doxorubicin. Notably, the stiffened microenvironment also induced the expression of mesenchymal phenotype markers, highlighting a mechanotransduction-driven link between matrix stiffness, phenotypic plasticity, and chemoresistance.

Recently, various strategies have been developed using alginate hydrogels to create robust platforms that effectively mimic ECM viscoelasticity in 3D cell culture studies.[83] It was shown that human-induced pluripotent stem cells (hiPSCs) exhibit extended maintenance of pluripotency within alginate hydrogels compared to the shorter durations reported in reconstituted basement membrane matrices (Fig. 5F).[76]

Lumen formation within these hydrogels is governed by actomyosin contractility and is associated with the cytoplasmic translocation of the mechanotransduction regulator YAP from the nucleus. One straightforward and cost-effective method involves reducing the molecular weight of alginate in a highly controlled manner through serial autoclaving.[84] With each autoclave cycle, intrinsic viscosity, hydrodynamic radius, and molecular weight are proportionally reduced, while the polymer's chemical composition remains unchanged. This technique offers a simple yet effective way to tailor alginate properties for specific applications. Another approach utilizes light-triggered tethering of poly(ethylene glycol) (PEG) to alginate to modulate hydrogel stress relaxation rates temporally or spatially.[85] This method allows fine-tuning of the stress relaxation rate without affecting the elastic modulus of the hydrogel, providing precise control over viscoelastic properties. Notably, dual-crosslinking strategies leverage the unique degradability of each hydrogel: ionically crosslinked alginate permits stress relaxation due to its reversible ionic bonds, while radical-mediated photocrosslinking of GelMA provides structural stability and controlled degradation. This combination has been shown to be cytocompatible, maintaining the viability of mouse bone marrow stromal cells and offering a versatile platform for 3D cell culture studies.[86]

3.3.2. GelMA hydrogels. Gelatin methacrylamide (GelMA) is a widely utilized biomaterial for hydrogel fabrication due to its biocompatibility, tunable properties, and enzymatically degradable structure, which supports dynamic cellular remodeling (Fig. 1D). Hydrogel stiffness plays a pivotal role in regulating cellular behavior and phenotype, particularly in the context of breast cancer research. GelMA hydrogels have emerged as a transformative material in 3D tumor modeling due to its unique physicochemical properties, including tunable stiffness, biocompatibility, and degradability *via* matrix metalloproteinases (MMPs).[87] This combination of features makes GelMA an ideal platform for studying tumor microenvironments (TMEs). Innovative techniques have further expanded the utility of GelMA in modeling TMEs. A two-step photolithography approach has been employed with GelMA hydrogels to construct a model of the breast TME. This strategy revealed that tumor cells located in the periphery exhibited faster and more directed migration compared to cells within the tumor core,

highlighting spatial differences in cellular behavior within the TME.[88] Similarly, recent works explored the versatility of GelMA hydrogels for engineering mineralized, adipose, and tumor microtissues derived from human cells (Fig. 5G). By replicating the local microenvironment of cancer cells invading the bone marrow, it was demonstrated that the presence of human adipocytes significantly promoted tumor growth within the bone marrow niche.[77]

A study investigating the effects of a clinically relevant range of microenvironmental stiffness on breast cancer cells over a 21-day culture period used GelMA hydrogels to uncover distinct cellular responses.[89] Tumoral breast epithelial cells (MCF7) cultured in high-stiffness hydrogels (10 wt%; 28 kPa) exhibited downregulation of the epithelial marker E-cadherin and upregulation of mesenchymal markers N-cadherin and vimentin, indicating epithelial-to-mesenchymal transition (EMT). In contrast, highly aggressive tumoral breast epithelial cells (MDA-MB-231), when cultured in hydrogels of similar stiffness (10 wt%; 33 kPa), did not show changes in EMT markers.[90] When both cell lines were cultured in softer hydrogels (5 wt%; 11 kPa), their original phenotypes were maintained over the same period. These findings underscore the importance of precisely controlling hydrogel mechanical properties when studying breast cancer cell behavior and phenotype transitions. GelMA hydrogels have also been employed to explore cardiac cell mechanobiology. H9C2 cardiac-derived myoblasts were encapsulated as individual cells and as cell spheroids within stiffness-gradient GelMA hydrogels to examine their individual and collective mechanosensation in 3D.[91] Across a stiffness range of 3.7–17.5 kPa, individual H9C2 cells demonstrated a limited ability to adapt their volume to increasing substrate stiffness, as evidenced by minimal changes in cell volume and shape. Morphological observations were correlated with the expression of mechanosensitive markers YAP, myocardin-related transcription factor A (MRTF-A), and lamin-A, which were more strongly associated with cell and nuclear volume than with substrate stiffness. When cultured as spheroids, H9C2 cells displayed distinct morphologies, including smaller nuclei compared to individually cultured cells, while maintaining overall cell volume. Spheroids were sensitive to stiffness cues, as indicated by decreasing nuclear localization of YAP and MRTF-A, increasing lamin-A expression, and elevated vinculin expression with rising substrate stiffness.

These findings suggest that cell–cell and cell matrix interactions within spheroids enhance cardiac cell volume adaptation and mechanosensitivity, illustrating the complex interplay between mechanical environment and multicellular organization in 3D systems.

4. Cell–matrix and cell–cell interactions within hydrogel systems

Cellular interactions within hydrogels are dynamic and multifaceted, encompassing both cell–matrix adhesion and cell–cell communication. These interactions play crucial roles in physiological processes such as tissue development and remodeling, as well as in pathological contexts like cancer invasion or fibrosis. Cells sense the mechanical properties of their surrounding environment through integrin-based focal adhesions,[92] which activate mechanotransduction pathways regulating cytoskeletal organization, nuclear mechanics,[93] and intercellular signaling.[56] Cells receive cues by sensing the mechanical properties of the substrate, but they are also able to generate traction forces that induce deformation and structural changes in the hydrogel. The magnitude of these forces depends on hydrogel stiffness, network structure, and the density and affinity of adhesion ligands. Studies have shown that matrix stiffness can significantly influence cell morphology and migration.[94] On soft substrates, cell–ECM adhesion weakens, and cells tend to cluster due to stronger cell–cell interactions, a phenomenon observed in compaction behavior.[95]

To better understand these behaviors, physical and computational models have been developed to describe cell–matrix adhesion dynamics. For example, a stochastic model of integrin–ligand binding revealed that these interactions are highly cooperative, leading to integrin clustering under favorable conditions such as high stiffness and ligand density.[96] The ability of integrins to form strong and robust adhesion complexes is promoted by stiff gels and high ligand density, while it may be impaired under less favorable conditions, leading to unstable adhesions and reduced force transmission.

Simultaneously, cadherin-mediated cell–cell adhesions contribute to tissue cohesion and collective behaviors. As tissues mature, these junctions strengthen and become mechanically integrated with the cytoskeleton. For instance, tissue aging has been associated with cortical recruitment of cadherins and maturation of cell–cell junctions, alongside increasing cell–substrate forces.[97,98] These processes are tightly coupled with ECM mechanics, underscoring the need for hydrogel systems that accurately recapitulate the dynamic evolution of tissue structure and function.

To better mimic the native ECM, synthetic fibrous hydrogels based on polyisocyanide (PIC) polymers have been engineered with RGD peptides to support integrin-mediated adhesion.[99] These matrices recapitulate key aspects of fibrillar ECM architecture and porosity, enabling robust cell spreading, dynamic matrix remodeling, and the study of processes such as collective migration and tissue morphogenesis. Beyond stiffness, hydrogel viscoelasticity and plasticity have emerged as key determinants of cell behavior. Viscoelastic hydrogels enable time-dependent stress relaxation, while plasticity describes the permanent deformation of the matrix under contractile cellular forces.[7] These properties profoundly affect how cells migrate, remodel their environment, and maintain tissue integrity. However, the molecular pathways by which cells sense and transduce matrix plasticity remain poorly defined.

Recent studies have started to address this gap. For example, collagen–hyaluronic acid hydrogels with tunable plasticity were used to study endothelial cell outgrowth.[100] In highly plastic hydrogels, integrin clustering and focal adhesion formation

increased, while VE-cadherin expression at cell–cell junctions decreased, resulting in reduced adherens junction integrity. To explain these observations, a motor–clutch computational model was employed to simulate how endothelials cells respond to plastic matrix deformation. The model confirmed that increased matrix plasticity results in enhanced cell contractility and perturbed cell–cell adhesion, offering a predictive framework to guide future hydrogel designs for collective tissue applications.

These findings underscore the importance of not only tuning mechanical properties to influence individual cell behavior, but also engineering hydrogels to support coordinated multicellular processes such as epithelial sheet formation, vasculature development, and collective migration. Combining experimental and computational approaches is therefore essential for validating hydrogel designs in complex biological contexts.

5. Modeling cell behavior in hydrogel systems

Hydrogels offer a versatile platform for constructing *in vitro* models that replicate the dimensionality, mechanical complexity, and biochemical cues of the ECM. To advance our understanding of how cells sense and respond to such environments, mechanobiology is increasingly supported by physical and computational modeling. These models allow for the decoupling of mechanical parameters such as stiffness, viscoelasticity, and spatial confinement, providing mechanistic insight into cellular processes including mechanotransduction, migration, and matrix remodeling. Physical models, often adapted from soft matter physics and mechanical engineering, are now widely employed to capture cell behaviors at multiple scales—from subcellular focal adhesion dynamics to tissue-level coordination.

For instance, simulations have been instrumental in elucidating how individual cell motility integrates into collective migration behavior.[53,98,101,102] Recently, a highly efficient discrete vertex model was introduced to investigate the spatiotemporal dynamics of epithelial sheet expansion. Their study revealed that mechanical waves can spontaneously emerge during tissue expansion, with wave frequency governed by a power-law relationship involving local cell density and cortical elasticity.[103] In pathological contexts such as atherosclerosis, understanding the viscoelastic properties of soft tissues like atherosclerotic plaques at rupture-prone scales is essential for assessing their mechanical vulnerability. To address this challenge, a hybrid hierarchical theory–microrheology (HHM) approach has been developed.[104] Enabling precise characterization of atherosclerotic plaque components and their mechanical vulnerability through a two-stage power-law model.

More broadly, translational mechanobiology now plays a pivotal role in guiding the design of synthetic hydrogel matrices to develop more predictive *in vitro* models. For instance, it was demonstrated that tailoring the mechanical and biochemical properties of hydrogels enhances the ability to direct cell fate decisions, offering better platforms for regenerative medicine and cell-based therapies.[105]

6. Clinical applications of hydrogels

Hydrogels hold immense potential for applications in regenerative medicine, cell transplantation, disease modeling, and targeted drug delivery. According to the ClinicalTrials.gov database (**https://clinicaltrials.gov/**), over 350 clinical studies are currently investigating hydrogel-based systems across diverse medical fields, including ophthalmology, cardiology, dermatology, and gynecology. Despite this progress, relatively few hydrogel-based products have advanced beyond early-stage trials, and only a small subset has received regulatory approval for clinical use. This translational gap is largely attributed to challenges in reproducibility, limited long-term *in vivo* performance data, regulatory complexity, and issues related to manufacturing scalability. Moreover, achieving consistent mechanical properties, predictable degradation rates, and immunocompatibility in diverse physiological environments remains a technically demanding task.

A compelling recent advancement is the development of a stimuli-degradable hydrogel implant designed for the reversible occlusion of fallopian tubes[106] (Fig. 6A and B). This system offers a non-hormonal contraceptive approach and may mitigate endometriosis by preventing retrograde menstruation—a mechanism implicated in the disease's spread. Endometriosis is a chronic and often overlooked disease that affects roughly 10% of women and is characterized by uterine-like tissue growing outside the uterus. There is growing evidence that fallopian tubes facilitate the spread of endometriotic tissue by acting as conduits for proinflammatory mediators during retrograde menstruation. These hydrogels are also being investigated for the prevention of intrauterine adhesions, notably in Asherman's syndrome. The hydrogel systems are composed of two different acrylamide-based polymers crosslinked with the disulfide crosslinker *N*,*N*′-bis(acryloyl)cystamine (BAC) (Fig. 6A). The system consists of a proprietary transcervical catheter and two teaspoons of liquid precursors that fill the uterine cavity and to form a soft hydrogel that separates the uterine walls during the healing process (Fig. 6B). This hydrogel has shown biocompatibility in porcine models and effective implantation under ultrasound guidance in a human-scale uterus model.[107] It completely blocks the passage of both sperm and endometrial cells through the fallopian tubes (Fig. 6C–F). Nevertheless, translation into human trials will require robust safety and efficacy data, particularly given the complex hormonal and immunological context of the female reproductive system. The Juveena Hydrogel System, currently under evaluation [NCT06634719], is designed to reduce post-surgical scarring and to control heavy menstrual bleeding. Building on a decade of success with hyaluronic acid-based hydrogels, it delivers therapeutic agents directly to target tissues and has demonstrated efficacy in adhesion prevention and endometrial repair.[108–110]

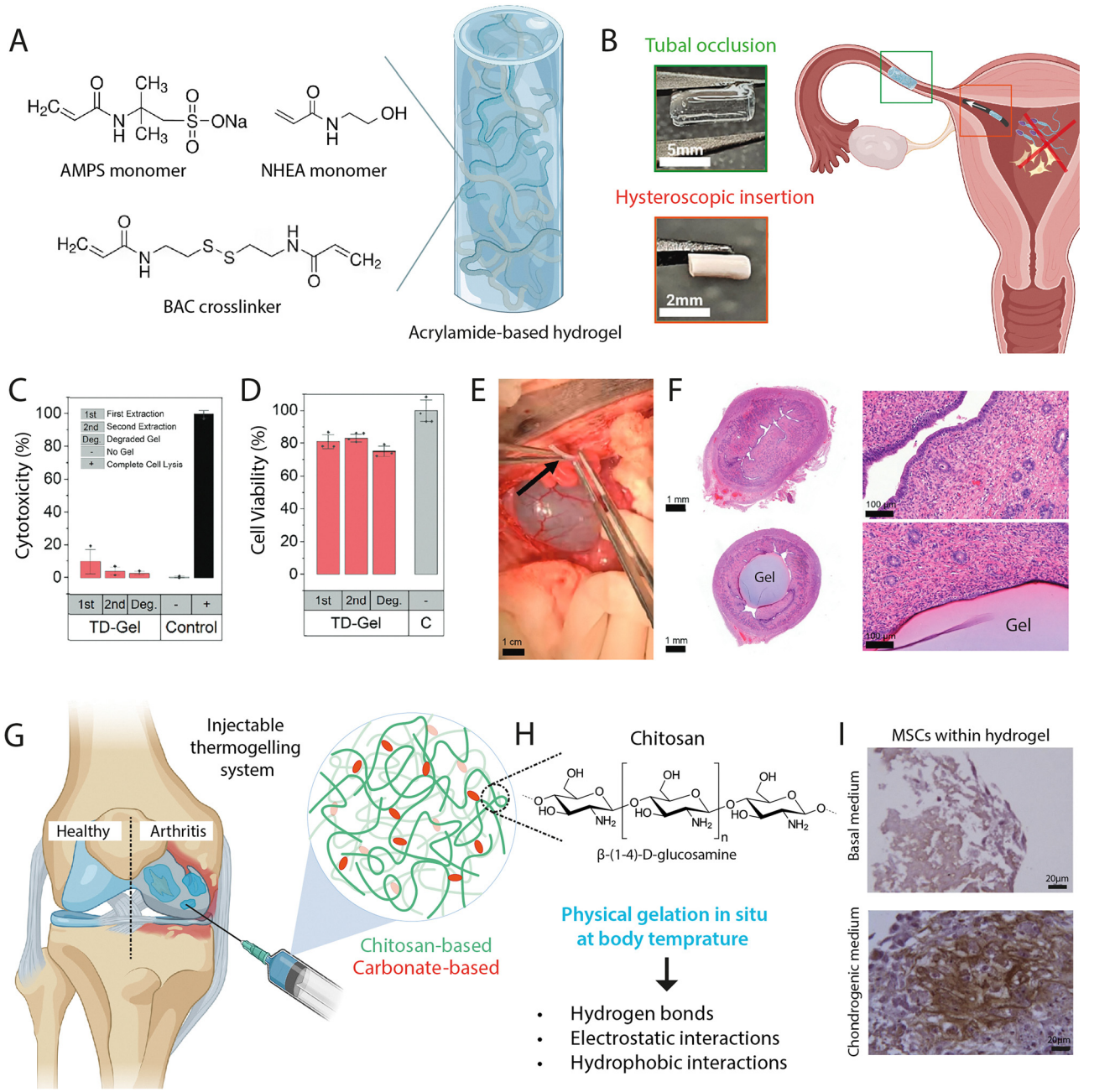

Fig. 6 Clinical applications of hydrogels. (A) Chemical structures of AMPS (2-acrylamido-2-methyl-1-propanesulfonic acid), NHEA (*N*-(2-hydroxyethyl) acrylamide) monomers and BAC (*N*,*N*′-bis(acryloyl)cystamine), the disulfide-based crosslinker used to synthesize the thiol-degradable (TD) hydrogel. (B) Schematic representation of the stimuli-removable hydrogel platform during the placement using a hysteroscope (orange) and following tubal occlusion (green). (C) Cytotoxicity assay based on lactate dehydrogenase (LDH) release in human fibroblasts cultured in medium conditioned by various TD hydrogel states. Reproduced from ref. 106 with permission from Wiley, copyright 2024. (D) Viability of human fibroblasts after 24 hours of incubation with hydrogel-conditioned media. Reproduced from ref. 106 with permission from Wiley, copyright 2024. (E) *In vivo* surgical placement of the hydrogel into porcine fallopian tubes to assess long-term biocompatibility. Reproduced from ref. 106 with permission from Wiley, copyright 2024. (F) Hematoxylin and eosin staining of porcine oviduct sections (isthmic region): top, unblocked control; bottom, TD-gel-blocked oviduct after three weeks *in vivo*. Reproduced from ref. 106 with permission from Wiley, copyright 2024. (G) Schematic of a healthy *versus* osteoarthritic knee, showing the application of the injectable thermogelling system JointRep™. This implant, composed of chitosan and glucosamine carbonate, is designed to fill cartilage defects and promote hyaline cartilage regeneration. (H) Chemical structure of chitosan, a linear polysaccharide composed of randomly distributed β-(1 → 4)-linked D-glucosamine (deacetylated units) and *N*-acetyl-D-glucosamine (acetylated units). (I) Immunostaining for human type II collagen (a marker of chondroblasts) showing negative control samples (top) and positive staining in the extracellular matrix of treated samples (bottom). Reproduced from (111) with permission from Elsevier, copyright 2019.

Hydrogels have already reached clinical maturity in several domains. To date, more than 100 hydrogel-based products have received approval from the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA). These include natural, synthetic, and hybrid hydrogels used in wound care, ophthalmic procedures, dermal fillers, contact lenses, and as drug delivery systems.

6.1. Chitosan-based injectable hydrogel for cartilage repair

Cartilage defects, often resulting from trauma or degenerative conditions like osteoarthritis, pose a clinical challenge due to the tissue's limited self-healing capacity (Fig. 6G). This highlights the need for innovative therapies capable of supporting tissue repair and regeneration. Among promising solutions, injectable hydrogel systems offer a biomimetic environment that closely resembles the native ECM, thereby facilitating cell adhesion, proliferation, and matrix deposition (Fig. 6G and H). A clinically advanced example is JointRep™, a CE-marked chitosan-based hydrogel developed for the minimally invasive treatment of cartilage lesions (Fig. 6H). This formulation remains liquid at room temperature and rapidly solidifies at body temperature, forming a scaffold that promotes cellular ingrowth and adheres to cartilage lesions through the cationic nature of chitosan.[111] Clinical outcomes are encouraging with WOMAC scores (a standardized index for osteoarthritis pain and function) decreasing by 88% at 6 months and 93% at 12 months. Histological analysis confirmed the formation of type II collagen and hyaline-like cartilage in the repaired tissue[112] (Fig. 6I). A new clinical trial is underway to further validate these outcomes and long-term efficacy [NCT04840147].

6.2. PEG-based hydrogel for ophthalmic surgery

Ophthalmic procedures, particularly those involving the cornea, demand precise wound closure to preserve intraocular pressure and prevent complications such as leakage or infection. Traditional suturing techniques often cause patient discomfort, prolonged healing, and increased risk of inflammation. As a result, there is a growing need for advanced biomaterials that can provide effective, biocompatible, and minimally invasive alternatives for ocular wound sealing. In this context, ReSure®—an FDA-approved PEG-based hydrogel—has emerged as an effective alternative. It is applied as a liquid sealant that polymerizes *in situ* to form a soft, adherent barrier, sealing clear corneal incisions after cataract surgery.[113] Compared to sutures, ReSure® has been shown to reduce the incidence of wound leaks and infections, while improving patient comfort and recovery outcomes.

While these clinical examples demonstrate the versatility and promise of hydrogel technologies, their successful translation into therapeutic applications depends on more than functional performance alone. Critical design considerations such as biodegradability, immune compatibility, and *in vivo* stability must be carefully addressed to ensure safety, efficacy, and long-term integration within physiological environments.

6.3. Biodegradability, immune response, and *in vivo* stability of hydrogels in clinical applications

The clinical translation of hydrogel-based systems necessitates careful consideration of important physico-chemical properties, such as their biodegradability, immune response, and *in vivo* stability to ensure safety and efficacy.

Biodegradability is essential for hydrogels used in regenerative medicine, as it allows the scaffold to be gradually replaced by native tissue. The degradation rate must align with tissue healing processes, providing support during initial stages and degrading as new tissue forms. The degradability of hydrogels mainly relies on their sensitivity to enzymatic degradation driven by matrix metalloproteinases (MMPs) secreted by cells. Some natural hydrogels are like GelMA are enzymatically degradable and have been shown to support tissue regeneration effectively. Synthetic hydrogels, such as those based on poly(ethylene glycol) (PEG), can be engineered with hydrolytically or enzymatically cleavable linkages to achieve controlled degradation profiles.[114,115]

Immune response is a critical factor influencing the biocompatibility of hydrogels. While PEG has been widely used due to its perceived inertness, recent studies have highlighted the potential for anti-PEG immune reactions. For instance, research indicates that pre-existing anti-PEG antibodies can influence the efficacy of PEG-based hydrogel implants and tissue engineering applications. Additionally, studies have shown that lipid nanoparticles containing PEG can induce anti-PEG antibodies, leading to accelerated blood clearance upon repeated administration.[114] These findings underscore the importance of assessing and mitigating immune responses when designing PEG-based hydrogels for clinical use.[115]

In vivo stability ensures that hydrogels maintain their structural and functional integrity under physiological conditions. Factors such as mechanical stress, enzymatic activity, and fluid dynamics can affect the longevity and performance of hydrogels. For example, injectable GelMA hydrogels have been developed to deliver therapeutic agents and have demonstrated stability and efficacy *in vivo*. Similarly, studies on *in situ*-forming PEG-collagen hydrogels have shown promising biocompatibility and stability when applied to corneal defects in animal models.[116,117]

Addressing these parameters is central to designing clinically viable hydrogels. Advances in smart and responsive materials, informed by recent preclinical and clinical studies, continue to enhance the optimization of hydrogel systems for therapeutic applications.

7. Challenges and opportunities in hydrogel design for mechanobiology

Hydrogels have emerged as powerful tools in mechanobiology due to their tunable mechanical properties, biocompatibility, high water content, active exchange capabilities, and structural similarity to native ECMs. Nevertheless, significant challenges remain in designing hydrogel systems that can faithfully

recapitulate the dynamic, heterogeneous nature of living tissues. Replicating the multifaceted mechanical environment of the ECM—characterized not only by stiffness but also by viscoelasticity, plasticity, and non-linear elasticity—remains a complex task. Many conventional hydrogels offer static mechanical properties and lack the ability to undergo time-dependent deformations, limiting their effectiveness in mimicking the adaptive behaviors of biological matrices under physiological conditions.

To address these limitations, recent technological advances have enabled the development of more sophisticated and responsive hydrogel systems. For example, 3D printing technologies now offer unprecedented control over the spatial architecture of hydrogels, allowing the fabrication of intricate, tissue-like structures with defined mechanical and compositional heterogeneity.[118,119] To address these limitations, recent technological advances have enabled the development of more sophisticated and responsive hydrogel systems. For example, 3D printing technologies now offer unprecedented control over the spatial architecture of hydrogels, allowing the fabrication of intricate, tissue-like structures with defined mechanical and compositional heterogeneity.[120] At the same time, nanotechnology is opening new avenues for engineering multifunctional hydrogels. The incorporation of nanomaterials—such as graphene oxide,[121] gold nanoparticles,[122] or silica nanostructures[123,124]—can enhance mechanical strength, introduce electrical conductivity, or confer responsiveness to external stimuli like light, pH, or magnetic fields. These functionalized hydrogels represent promising platforms for studying dynamic cell–matrix interactions and for delivering spatially and temporally controlled therapeutic cues.

An important and rapidly advancing category within hydrogel design for mechanobiology is that of dynamically tunable hydrogels, which enable real-time control of biomechanical properties through external physical or chemical stimuli. These materials represent a significant step toward precision biomimetic environments, allowing spatiotemporal modulation of stiffness, viscoelasticity, or ligand presentation in response to triggers such as light, temperature, or magnetic fields. Such systems address the growing need for culture platforms that mimic not only the structural and mechanical complexity of native tissues but also their inherent dynamic behavior. Recent advancements in photoresponsive hydrogels have demonstrated the ability to achieve spatial and temporal control over matrix mechanics using light, enabling researchers to dissect cellular responses to dynamic environments with high precision.[125,126] Similarly, thermoresponsive hydrogels that undergo reversible sol–gel transitions in response to temperature changes offer versatile platforms for modulating cell behavior in a controlled manner.[127] These approaches move beyond static matrices and begin to capture the continuous remodeling that occurs in both the material and cellular components during culture—an essential feature for understanding mechanobiological processes such as sprouting angiogenesis and cancer invasion. For example, spheroids growing within confined hydrogels generate compressive stresses that alter matrix architecture and cell behavior, depending on the matrix's viscoelastic and degradable properties.[128] Precision hydrogel systems that incorporate dynamic mechanical tuning are particularly promising for recapitulating the tumor microenvironment (TME), where tissue remodeling and mechanical stress evolve together during disease progression. Engineering hydrogels with independent and reversible control over structural and biochemical features enables the construction of 3D environments that guide cellular processes such as invasion, morphogenesis, and differentiation. Overall, these innovations are opening new possibilities in mechanobiology, providing experimental systems with unprecedented control over biophysical signals and their timing. The integration of such dynamic hydrogel systems is expected to significantly advance our understanding of cell–matrix interactions in both cancer and regenerative contexts.[129,130]

One particularly promising application of next-generation hydrogel systems is the development of cancer organoid models to advance the clinical translation of novel therapies.[131,132] Organoids are three-dimensional, self-organizing structures that capture key features of native tissues, including the architecture, cell diversity, and matrix interactions. Hydrogels designed with tunable stiffness, degradability, and bioactive motifs have enabled the generation of tumor organoids that replicate *in vivo* mechanical and biochemical conditions. For instance, recent studies have leveraged engineered hydrogels to model tumor growth and invasion dynamics, providing insights into how mechanical cues regulate collective cell migration and metastatic potential.[131] These organoid systems are increasingly used in drug screening and personalized medicine, underscoring the clinical relevance of precision-engineered hydrogel environments.

Emerging analytical technologies hold significant promise for advancing our understanding of how individual cells, particularly within complex multicellular systems like organoids, perceive and respond to mechanical cues in hydrogel-based environments. High-resolution methods such as spatial transcriptomics and mass spectrometry imaging could be transformative in mapping cell-specific responses to mechanical stress, yet their application to hydrogel systems remains limited. Integrating molecular sensors directly into hydrogel networks—capable of reporting on forces or mechanical deformation in real time—would allow for more refined, spatiotemporal analysis of cell–matrix interactions than current techniques like traction force microscopy. It would also be highly valuable to enhance the optical properties of hydrogels in order to improve the quality of microscopic observations,[133] which are currently severely limited by their low refractive index, close to that of water. Such advances could significantly enhance the resolution at which we study cell behavior in 3D microenvironments and contribute to the rational design of next-generation hydrogels for mechanobiology.

Looking ahead, the intersection of materials science, engineering, and biology is expected to drive major breakthroughs in hydrogel technologies. Designing hydrogels that are not only structurally and mechanically accurate, but also dynamic and

interactive, remains a key goal for the field. Integrating advanced fabrication techniques, nanotechnology, and computational modeling will be crucial for realizing hydrogel systems that can replicate the full complexity of tissue mechanics and guide multicellular processes such as morphogenesis, repair, and disease progression. As our understanding of mechanobiology deepens, these engineered matrices will play an increasingly central role in both fundamental research and translational applications.

8. Conclusion

Hydrogels have become essential tools in mechanobiology, enabling the precise exploration of cell–ECM interactions across a spectrum of dimensional and mechanical complexities. Advances in hydrogel design, spanning from 2D to 3D systems, including intermediate 2.5D and sandwich configurations, have enabled researchers to explore the complex interplay of biophysical cues, such as stiffness, viscoelasticity, and spatial confinement, in regulating cellular behavior and phenotype. Alginate-based and GelMA-based hydrogels, with their tunable mechanical properties, biocompatibility, and biochemical modifiability, have emerged as pivotal materials for advancing 3D cell culture systems. These systems have illuminated mechanisms underlying critical processes, such as cancer progression, stem cell differentiation, and collective migration. The integration of cutting-edge fabrication techniques, such as micropatterning, micromolding and photopolymerization, continues to expand the potential of hydrogel-based platforms. To further enhance the biomimicry of dynamic cellular microenvironments, hydrogels with temporally tunable stiffness are increasingly being developed. These approaches employ various innovative strategies temporally modulate hydrogel mechanical properties. For instance, the light-triggered release of calcium or a chelator from liposomes has been used to adjust the stiffness of alginate hydrogels dynamically.[134] An other method involves the light-triggered tethering of PEG to the hydrogel network, enabling precise control of hydrogel stress relaxation rates in both time or space.[85]

Additionally, a dual-crosslinking strategy has been employed to create biocompatible 3D viscoelastic interpenetrating network (IPN) hydrogels based on GelMA-alginate, which can progressively stiffen over time. Incorporating degradable structural components or reversible dynamic crosslinks into hydrogels allows for efficient cellular adaptation to the matrix, supporting critical functions such as migration, proliferation, and differentiation. For instance, dynamic bioengineered hydrogels have been successfully utilized as scaffolds for advanced stem cell and tissue engineering applications, highlighting the role of tunable matrix properties in promoting desired cellular outcomes.[135] Recent innovations have advanced the integration of multiple biologically relevant dynamic processes into fully synthetic materials, overcoming the typical limitation of addressing only a single dynamic process.[136] This innovation is driven by the molecular dynamics of supramolecular polymers, which dictate interactions with covalent polymer networks and ultimately define the mechanical, structural, and dynamic properties of hybrid hydrogel networks. Cell growth studies have demonstrated the biological significance of these engineered timescales, with molecular dynamics influencing cell spreading by modulation of bulk and nonlinear stiffness, as well as dynamic ligand presentation. Looking ahead, future research should focus on tailoring bioactive cues to match specific cellular response timescales. These dynamic properties are particularly valuable for investigating transient cellular processes and mechanotransduction within evolving microenvironments. Moving forward, integrating such adaptive hydrogel systems with real-time monitoring techniques and multi-parameter control will pave the way for deeper insights into complex biological phenomena and the development of innovative therapeutic and tissue engineering strategies.[137]

Author contributions

S. G. and M. L. conceived the project, written and corrected the manuscript. The figures were created by M. L.

Data availability

No primary research results, software or code have been included and no new data were generated or analyzed as part of this review.

Conflicts of interest

The authors declare no competing interests.

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