Hydrogels with Tunable Physical Cues and Their Emerging Roles in Studies of Cellular Mechanotransduction

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1. Introduction

Tissue engineering is a broad field that encompasses various aspects of engineering and life sciences, intending to maintain, improve, or restore tissue function.[1] One of the main approaches adopted in tissue engineering is to use a combination of matrices and cells to deliver living elements to specific sites, which then becomes integrated within the body.[1] Tissue engineering can also be used to create in vitro models of organs and disease functions to develop a deeper understanding of the signals involved in vivo, and to use these models as drug testing platforms.[2,3] While the field has progressed considerably, various challenges remain regarding the influence of biophysical cues on how cells sense and transduce mechanical properties (mechanotransduction) that govern the developmental processes and diseases such as cancer.

Cells can be grown on synthetic substrates mimicking the extracellular matrix (ECM) environment. Mimicking the ECM microenvironment is paramount in understanding the signaling behaviors of cells responsible for cellular processes such as differentiation and proliferation. ECM microenvironment can be divided into biophysical (structure, stress, fluid flow, etc.) and biochemical (adhesion ligands, growth factors, etc.) cues. The seminal work conducted by Engler et al.[4] studying 2D cell culture on poly(acrylamide) gels demonstrated the ability to tune the lineage of mesenchymal stem cells (MSC) by altering the matrix stiffness on which the cells were grown. The role of biophysical properties of the ECM on dictating cell behavior has been extensively researched in the past few years.[5–8] There have also been considerable developments with regard to what mechanical properties cells are sensitive to. For instance, the role of dissipative and time-dependent mechanical properties has recently been unveiled as potent regulators of cellular mechanotransduction.[9]

Various material systems in the form of thin films and gels have been explored for imparting biophysical cues to cells.[10–12] For instance, thin films have been investigated to study the potential roles of substrate geometry and protein patterns on cell signaling.[13–15] To mimic the native state of ECM that consists of large amounts of water, hydrogels have been widely used to study cell mechanotransduction. Hydrogels are made of hydrophilic networks of polymers that swell in water. The physical and chemical properties of hydrogels can be tuned to match the viscoelastic properties of ECM.[16] Cells may be grown on the surface of hydrogels (2D) or inside them (3D).[17,18] 3D cell culture naturally mimics the ECM more effectively than 2D cell culture. It is to be noted that there are substantial differences between 2D and 3D matrices. For instance, the cell spreading is dissimilar as the ligand densities presented to the cell are different in 2D and 3D.[19] Moreover, 3D matrices require remodeling properties and the imaging is more arduous in 3D because of the nonplanar...
nature of the 3D matrix.\(^{20}\) 2D cell culture generally allows better control over the spatial and temporal presentation of biophysical and biochemical factors.\(^{21}\) Particularly, 2D culture has been commonly used to study cell adhesion processes and to study cell response to dynamic substrates.\(^{22}\) This review focuses primarily on 2D substrates and for 3D systems the readers are pointed to other excellent reviews in the literature.\(^{23-26}\)

This review covers the design and development of hydrogel-based materials and their influence on cellular mechanotransduction, particularly in regards to the various aspects of mechanical properties. First, Section 2 and 3 go through the components of the cellular microenvironment and signaling cascades involved in mechanotransduction. Various material schemes used for tuning hydrogel viscoelasticity and their associated physicochemical considerations are presented in Section 4 and 5. Then in Section 6, the roles of viscoelasticity in modulating cell behavior is discussed, followed by an overview of some of the recent studies made with cells and substrates focusing on varying mechanical properties. Selected studies investigating the synergistic effects of mechanical properties with various cues such as topography, and patterns on cellular behavior are then presented in Section 7. Finally, a note on prospects with designer hydrogel matrices in terms of studying complex cellular behaviors such as migration and mechanical memory is presented.

### 2. Components of Cellular Microenvironment

Cells sense complex cues and activate signaling cascades in response, affecting processes such as tissue development and disease progression.\(^{27}\) In vivo, cells reside in the ECM. The ECM is a 3D network consisting of many components that contribute to cellular signaling, in addition to providing support to the cells inside it. As the molecules of the ECM interacts with receptors present in the cell membrane, these receptors transmit signals to molecules in the cytoplasm, which, in turn, produces cascades of events travelling into the nucleus affecting the expression of genes, including genes affecting the ECM production or destruction by the cells. These dynamic interactions between cells and the ECM thus lead to the continuous remodeling of the ECM and changes in cellular behavior.

The main components of this dynamic cellular microenvironment can be divided into four main functional elements: the ECM, integrins, cytoskeleton, and the nucleus.

#### 2.1. Extracellular Matrix

The main protein in extracellular matrix is collagen. Collagen is the most abundant protein in the animal kingdom. It is fibrous protein that accounts for \(\approx 30\%\) of the protein mass in our bodies.\(^{28}\) The ECM is also composed of other fibrous proteins, glycosaminoglycans (GAG), proteoglycans, and glycoproteins in addition to abundant amounts of water. GAGs are linear heteropolysaccharide molecules consisting of a disaccharide repeat sequence, typically an amino sugar and uronic acid.\(^{29}\) There are four different families of GAGs: chondroitin/dermatan sulfate, heparan sulfate, keratan sulfate, and hyaluronan.\(^{30}\) GAGs are polar and capable of binding substantial amounts of water, giving resistance to compressive forces.\(^{31}\) GAG chains covalently or noncovalently attached to core proteins form proteoglycans. Both GAGs and proteoglycans play important roles in sequestering growth factors in addition to providing toughness to tissues.\(^{32}\) Elastins and collagens are fibrous proteins in the ECM, which provides the necessary tensile strength.\(^{33,34}\) Collagen exhibits nonlinear mechanical properties, while the elastins, as the name suggests, are highly elastic proteins with high resilience or the ability to recoil when the load is removed.\(^{14}\) Glycoproteins such as fibronectin and laminins are involved in cell surface interactions while simultaneously serving as binding sites for integrins.\(^{35}\) These components of the ECM are shown within Figure 1. It is important to note that the ECM varies considerably between organs and between healthy and disease states. An understanding of the ECM of the target tissue is of relevance when constructing an engineered microenvironment.

#### 2.2. Integrins

Cells interact with the ECM through integrin receptors embedded in the plasma membrane. Integrins are heterodimeric transmembrane receptors consisting of \(\alpha\) and \(\beta\) subunits, and there are 24 identified combinations of heterodimers of integrins in mammalian cells.\(^{36}\) Integrins play an essential role in cell adhesion, motility, and ECM remodeling.\(^{37}\) On the extra-cellular side, integrins can bind to various ligands in the ECM, such as collagen, fibronectin, and laminin.\(^{38}\) At the intracellular side, integrins are connected to the cytoskeleton of the cell via large complexes of proteins, such as vinculin, talin, \(\alpha\)-actinin, and tensin.\(^{39}\) The binding of integrins to ECM ligands on the extra-cellular side supports the adhesion of cells, and they are further involved in force transduction into the cell by recruiting various proteins and triggering signaling cascades, a process referred to as outside-in signaling.\(^{36}\) The cytoplasmic tails of integrins do not have any intrinsic enzymatic activity. Therefore, for signal transduction, integrins couple with several adapter proteins. The outside-in signaling regulates various cellular processes such as migration and differentiation.\(^{40}\)

The affinity of integrins to extra-cellular ligands is modulated by conformational changes of the extra-cellular domains of the integrins, which occur in response to the signals that impinge upon the integrin cytoplasmic tails. This process is termed inside-out signaling or activation of integrins.\(^{41}\) Different integrins bind to different ECM ligands with varying affinity. The shortest peptide sequence identified as recognizable by many integrins is Arg-Gly-Asp (RGD).\(^{42}\)

Integrins cluster locally upon binding to the ECM ligands. The clustering of integrins can occur from binding to ligands with multiple binding sites and/or cytoskeletal interactions within the cell.\(^{40}\) The clustering of integrins leads to the formation of dynamic adhesion structures called focal adhesions, which are involved in regulating force transmission. It has been demonstrated that integrin-mediated cell adhesion requires a minimum ligand spacing of 58–73 nm for focal adhesion formation.\(^{43}\) Also, it has been suggested that a minimum of four integrins within the same cluster is needed for the growth and maturation of focal adhesions.\(^{44}\)

#### 2.3. Cytoskeleton

The cytoskeleton maintains the shape of cells and is present in the cytoplasm from the nucleus to the plasma membrane. The
cytoskeleton of cells is composed of three main components: actin filaments, microtubules, and intermediate filaments. The actin filaments are formed by an adenosine triphosphate (ATP)-driven polymerization of globular actin (G-actin) monomers with α-actinin serving as the cross-linker. F-actin is continuously created through polymerization at the front edge of the cell, thereby polarizing the cell. When the cells experience physical forces, F-actin assembles into bundles termed stress fibers. The binding to, and pulling on, F-actin by myosin-II proteins results in contractile forces. For instance, during cell migration, a cell must polarize, and the leading edge is formed through actomyosin cytoskeletal assembly, which produces protrusions called lamellipodia. This makes the cells propel forward and there is a net backward flow of the F-actin referred to as the retrograde flow. During cell locomotion, nascent adhesions develop at the site of lamellipodia. The nascent adhesions incorporate integrins, talin, vinculin, and other proteins. As the cell propels forward, nascent adhesions may disassemble when they encounter the zone of actin depolymerization, or grow into mature adhesions by recruiting various other proteins. It has been suggested that the maturation of nascent adhesions depends mainly on the ECM rigidity. Adhesions differ in composition, size, location, and lifetime and can be classified as nascent adhesions, focal adhesions, and fibrillar adhesions. Nascent adhesions are short-lived and are about 1 μm in diameter. Focal complexes serve as precursors to focal adhesions which mainly consist of talin, vinculin, paxillin, focal adhesion kinase (FAK), and so on. Focal adhesions reside at the ends of stress fibers and are typically 1–5 μm in length. Focal adhesions can further mature into fibrillar adhesions, present in the central portion of the cell involved in fibronectin deposition and remodeling.

2.4. Nucleus

Finally, the nucleus contains the genetic information and is isolated from the rest of the cell by the nuclear membrane. The genetic information is stored in the DNA in the nucleus. The DNA is wrapped around histone proteins. The resulting compact structure is called chromatin, which, in turn, is compacted into the chromosomes. The nucleus is connected to the cytoskeleton of the cell through the linker of nucleoskeleton and cytoskeleton (LINC) complex. The LINC complex contains nesprin proteins that can interact with the cytoskeleton of the cell and transmit this force through the nuclear membrane, via SUN proteins (which, in turn, link to the lamina and nuclear scaffold). It is through this machinery that mechanotransduction can impact gene expression. When the plasma membrane of the cell experiences force, the force is propagated along the cytoskeleton and reaches the nucleus via the LINC complex. The LINC complex transmits the force across the nuclear membrane to the nuclear lamina and to chromatin.

2.5. Molecular Clutch Model of Mechanotransduction

The molecular clutch hypothesis is a model of mechanotransduction that describes the mechanical forces exerted on focal adhesions during cell migration. This model has constantly evolved over decades of research, and further additions with each iteration have contributed to what is currently the most accurate model of the forces at play during cell migration, and the behavior of the proteins involved. An accurate description of the physical and molecular mechanisms by which cells respond to their environment—and subsequently migrate—is critical in overall broadening our understanding of how cells migrate and...
transduce signals to elicit cell responses. This can, in turn, help us understand important and complex topics such as cancer growth and regenerative medicine—both of which rely heavily on how cells bind to their environment.

Initially proposed by Mitchison et al. in 1988, the molecular clutch hypothesis is an analogy that describes the mechanisms by which cells migrate using FAs. The analogy depicts FAs, which are the structures coupling the ECM to actin, as the “clutch” being engaged. The actin polymerization force subsequently diminishes retrograde flow and protrudes the leading edge, generating a rearward tractional force through which the cell is mobilized forwardly.

Odde et al. conceived a stochastic physical model of the molecular clutch to determine the clutch’s dynamic response to ECMs of different stiffness in 2008. Two dynamic regimes were observed, being “frictional slippage” with stiff substrates, and “load-and-fail” with flexible substrates, shown in Figure 2A, with the difference being those flexible substrates give clutches more time to form as the substrate stretches accommodatingly, and failure is brought upon by force becoming high enough that failure of one clutch results in failure of all clutches—this cycle repeating results in force fluctuations with time. Subsequent research has supported Odde’s model on flexible substrates, wherein traction force microscopy on neuronal growth cones and migrating fibroblasts expectedly uncovered that forces at FAs are low and steady on stiff substrates but fluctuate on flexible substrates. However, experiments showed a monophasic increase in tractional force with increased ECM stiffness, contrary to the biphasic growth predicted by Odde’s model.

Roca-Cusachs and co-workers accounted for this discrepancy in the most modern iteration of the molecular clutch model (Figure 2B). This was done by considering integrin, talin, and vinculin interactions, and incorporating this effect into their mathematical model. The talin linkers contain many vinculin binding sites that only become uncovered through the force-induced unfolding of talin proteins. This force-induced recruitment of vinculin reinforces the clutch and accounts for the high stiffness substrate experimental observations of a monophasic increase in tractional force. The stiffness threshold observed by which talin unfolding occurs was 5 kPa, and therefore beyond this value, vinculin recruitment was observed as well as the formation of vinculin-rich FAs. As further evidence for their model, the authors demonstrated that when talin was silenced by incorporating short interfering RNA (ShRNA), the traction force and substrate stiffness exhibited the biphasic relationship predicted by Odde et al.

More recently, Cheng et al. added to the understanding of how integrin cluster dynamics modulate mechanotransduction via FAKY397 phosphorylation, which was known to be the case; however, the mechanism through which this occurred remained elusive. This was achieved by developing a mathematical Monte Carlo model of integrin clustering kinetics. It was found that integrin clustering mechanics dictates the cell’s ability to convert substrate stiffness cues to FAKpY397, therefore mediating how cells can form these molecular clutches and transduce mechanical signals. This model was supported by existing reports using MDCK and HT1080 cells, as well as their follow-up experiment utilizing 3T3 fibroblasts.

3. Signaling Cascades in Mechanotransduction

Cell sensing is the means through which integrin-ECM binding influences intracellular processes such as gene expression. The signal transducing kinase, FAK, is a molecule responsible for many early stages in cellular signaling cascades and is bound directly or indirectly to integrin β subunits through vinculin or paxillin. Mechanical stresses have been found to enhance FAK activation, thus suggesting its role in mechanotransduction. Of the many signaling pathways involved in mechanotransduction,
the following are the most significant and well researched. The downstream signaling cascades FAK are responsible for including Src (nonreceptor tyrosine kinase family), Rho (small GTPase, activating Rho-associated protein kinase [ROCK]), and ERK (extra-cellular signal-regulated kinases).[67]

The Rho/ROCK mechanotransduction pathway regulates cytoskeletal dynamics, cellular contractility, and mobility by affecting actin stress fibers.[68] To probe this signaling pathway, inhibitors, such as blebbistatin (myosin inhibitor), Cytochalasin-D (actin polymerization inhibitor), and Y27632 (ROCK inhibitor), are commonly used.[69–71] When integrin engages with the ECM through FAs, the ERK and Src downstream signaling cascades activate Gef-H11 and LARG, respectively, which are Rho guanine nucleotide exchange factors. This function is to propagate the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP), which causes the direct activation of Rho signaling.[72] RhoA is a significant form of Rho signaling involved in actin mediation and myosin contractility through ROCK activation.[73] ROCK is responsible for the phosphorylation of substrates such as LIM kinase, myosin light chain (MLC), and MLC phosphatase. LIM kinase phosphorylation through the RhoA/ROCK pathway can then further phosphorylate coflin. Coflin affects actin depolymerization activity, thus regulating the cell’s cytoskeleton.[74,75] Interestingly, ROCK can also have an opposing effect through its phosphorylation of MLC kinase which, in turn, regulates actomyosin assembly and contraction.[76] The signaling from Rho-PTase to the cytoskeleton can also occur through the downstream effector, mammalian homolog of diaphanous (mDia), which belongs to the formin family of proteins. mDia regulates the cytoskeleton by directly affecting the actin nucleation and elongation or through a protein complex called Arp 2/3.[77,78] The RhoA/Rock signaling pathway, as well as the complimentary ERK, Src, coflin, and mDia events described are paramount in the mechanotransductive signaling pathways dictating how cells sense stiffness and tension by mediating FA maturation.[79] Figure 3 shows the important signaling pathways involved in mechanotransduction.

The Hippo pathway is a significant signaling pathway known to be mediated by mechanotransduction to affect gene transcription. Important entities which are involved in this signaling pathway are Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ).[80] They operate by acting as a committer between the cytoplasm and nucleus, wherein YAP/TAZ can translocate into the nucleus to aid in gene transcription. This is done by interacting with various transcription factors to transmit mechanical signals back to the nucleus to trigger the transcription of genes regulating cell fate, organ size growth, carcinogenesis, apoptosis, and proliferation.[81,82] It has been shown that both YAP and TAZ are negatively regulated by the Hippo pathway[83] and that they share many redundant functions.[84] YAP can be activated within the nucleus by different mechanical stimuli; however, phosphorylation of YAP/TAZ causes them to migrate from the nucleus to the cytoplasm, where they degrade. This phosphorylation is mediated by the Hippo signaling pathway to precisely mediate cellular events such as organ size control, apoptosis, and proliferation.[85]

4. Hydrogels and Strategies for Modulating Mechanical Properties

In this section, different material systems and synthetic strategies used for recapitulating the ECM properties are discussed. First, different synthetic strategies are reviewed. This is followed by specific strategies to modulate the mechanical properties with spatial resolution and strategies to add dynamic (time-resolved) control.

4.1. Synthetic Strategies and Properties

The various macromolecules that constitute the ECM, such as proteoglycans and fibrous networks such as collagen, give rise to interesting properties such as strain stiffening and time-dependent viscoelasticity.[86,87] Fibrous networks such as collagen have been identified as long-range force transmitters between cells.[88] Different aspects of the complex mechanical properties of the ECM have been recapitulated in hydrogel systems. For example, to add a fibrous structure to hydrogels, materials such as hyaluronic acid have been electrospun to mimic native ECM.[89–91] The Rowan group has also presented an elegant synthetic system based on self-assembling polysaccharide-polypeptides. These peptides have been shown to self-assemble into hydrogels that consist of fibrous networks and that strain stiffening properties, similar to that of native ECM.[92,93]

A commonly adopted hydrogel system for mechanotransduction studies to present viscoelasticity is the poly(acrylamide) (PAAm) hydrogel. In PAAm gels, the viscoelasticity is modulated by adjusting the monomer (acrylamide) to cross-linking (bis-acrylamide) ratio. Ammonium persulfate (APS) and tetramethylthelylenediamine (TEMED) act as radical initiator and accelerant, and the hydrogel network is formed by radical polymerization through the vinyl groups.[94] PAAm gels can also be produced by UV-induced polymerization using photoinitiators.[95,96] The ease of producing a wide range of stiffnesses has made PAAm gels a widely adopted system. Highly elastic gels are produced by using a higher concentration of cross-linkers. Gels of varying viscous properties exhibiting recoverable energy (viscoelastic solid-like) have been produced by entrapping linear PAAm inside a PAAm cross-linked network.[97]

Alginate is a biopolymer commonly used in tissue engineering. Alginate hydrogels can be produced by ionic cross-linking with divalent ions such as calcium or magnesium.[98,99] These ionically cross-linked gels generally exhibit viscoelastic behaviors where the plastic deformation is caused by breaking and reforming of some of the ionic cross-links under load. In one study using alginate hydrogels[127] (Figure 4C), gels with the same initial storage modulus but different stress relaxation times were produced by altering the molecular weight of alginate and increasing the ionic cross-links. Furthermore, by coupling poly(ethylene glycol) (PEG) to alginate by carbodiimide chemistry, faster relaxation was produced.[100] This was explained by the PEG acting as steric spacers hindering the ionic cross-linking. Through these methods, the half-stress-relaxation time $\tau_{1/2}$ (time taken for stress to drop to half of its initial value) was tuned from 1 min to 2 h.

Reversible covalent cross-linking chemistries such as hydrazone and imine, among more, have also been used for tuning
the viscoelasticity of hydrogels. For instance, McKinnon et al.\textsuperscript{[101]} produced PEG hydrogels by reversible hydrazone covalent cross-linking, which exhibited stress relaxation of the order of seconds to hours. In these gel networks, the ability of the covalent networks to break and reform depends on the dynamic equilibrium constant, which gives a measure of active cross-links in the system. The rate at which the bonds form and break also becomes important, as these rate constants directly influence viscoelastic properties.\textsuperscript{[102]} However, these dynamic covalent chemistries generally require harsh environmental conditions.\textsuperscript{[103]}

Interpenetrating double networks have also been explored to tune the viscoelasticity of hydrogels. For instance, hydrogels formed by an interpenetrating network of PAAm and alginate were demonstrated to exhibit stress relaxation.\textsuperscript{[104]} The mechanism of energy dissipation observed in these gels was thought to arise from two factors: stretching of the covalent portion of the network and breaking/reforming of ionic cross-links in the alginate network. Lou et al.\textsuperscript{[105]} produced interpenetrating networks of hyaluronic acid (HA) and collagen-I (Figure 4D). The authors found that the stress relaxation was tuned by varying the HA molecular weight and concentration. Double networks composed of gellan gum and PEGDA gels have been found to exhibit unique stress-relaxation behavior resembling the native ECM and has been applied to assess the spreading behavior of bone mesenchymal stem cells.\textsuperscript{[106]}

\textbf{Figure 3.} Outline of main signaling pathways in mechanotransduction starting from focal adhesions and propagating through to the nucleus. For simplification, some focal adhesion proteins are not shown here.
4.2. Spatial Modulation of Mechanical Properties

Spatial control of mechanical properties is desired to recapitulate the ECM structure, wherein gradients in mechanical properties exist. One such example found in vivo is the osteochondral interface that allows the transition from bone to cartilage.\textsuperscript{[107]} The differences in the ECM composition and arrangement of fibrils give rise to gradients in mechanical properties across the interface.\textsuperscript{[108]} For instance, the subchondral bone transitions from an elastic modulus of $3.9 \pm 1.5$ to $0.32 \pm 0.25$ GPa in the mineralized cartilage.\textsuperscript{[107]} Local variations in mechanical properties also exist in diseased tissues. Thus, to mimic both physiological and pathophysiological conditions, spatial control of mechanical properties is desired. When mimicking local variations in mechanical properties, length scales are usually chosen as 10–100 μm\textsuperscript{[109]} or 1 mm as the response of single cell or group of cells can be studied in this range.

A common method to achieve stiffness gradients in photopolymerized hydrogels is to use a mask with an opacity gradient. For instance, Tse et al.\textsuperscript{[110]} produced PAAm hydrogels with stiffness gradients in the range of physiological cardiac tissue gradients (0.8–10 kPa mm$^{-1}$) using such an approach. In a...
subsequent work by Sunyer et al.\cite{111} (Figure 3E), a moving photomask was used to fabricate steep stiffness gradients. By moving the mask at a constant speed, a linear gradient in stiffness up to 115 kPa/μm was achieved. A straightforward approach was demonstrated by Vincent et al.\cite{112} who produced gradients in poly(acrylamide) gels by using a printed black to white gradient on a transparent sheet. They were able to generate stiffness gradients in the range of 1 kPa mm\(^{-1}\). The limit of gradients achieved in mask-based systems is set by the depth of penetration of the UV source and the inhomogeneous diffusion of free radicals produced. Also, these methods are restricted only to gels that can be phototriggered.

Changes in the geometry of the substrates on which the hydrogels are allowed to gelate can also produce stiffness gradients in the hydrogels. Kuo et al.\cite{113} published an example of this approach. They cast hydrogels on three different substrates made of steps, beads, and groove patterns to produce complex gradients in the stiffness of the hydrogels. Chao et al.\cite{114} produced poly(dimethylsiloxane) (PDMS) microstructures using soft lithography and immobilized collagen gels onto such surfaces. Gradients were observed due to the differences in the thickness of the collagen gels attached on hard substrates that exhibited variations in mechanical properties when characterized using a nanoindentation approach. In a similar approach, Choi et al.\cite{115} micropatterned silicon substrates to produce pillar-like structures with distinct spacings acting as a mold for PAAm gelation. After polymerization, the imprinted gel was removed from the mold and used as a substrate for another layer of PAAm, resulting in a gradient hydrogel. The limit for micropatterend substrates is posed by the diffraction limit of light used in the lithography steps. Norris et al.\cite{116} used a maskless lithography technique for creating stiffness gradients in photodegradable PEG hydrogels by using projection optics. The input image had two attributes, intensity and position, which is correlated to stiffness and stiffness gradient. The advantage of this method is that it eliminates the mask alignment problem faced during lithography, but limits relating to the diffraction of light still remain. To overcome this, recent work by Hadden et al.\cite{117} adopted a two-step polymerization without using lithography to produce PAAm hydrogels with stiffness gradients (Figure 4F). The first step limited the gel formation to a gradient in height. This was achieved by covering the pregel solution with a tilted coverslip. After gelation, the gel and the coverslip were removed. In the second step, another pregel solution was poured into a flat-based mold and covered with the already formed hydrogel wedge. The resultant hydrogel with two layers displayed gradients in stiffness that covered physiological (~2 kPa mm\(^{-1}\)) and pathological ranges (~7 kPa mm\(^{-1}\)). However, the stiffness gradients produced with this approach exhibited poor reproducibility.\cite{117}

Control of stiffness gradients may also be achieved by using microfluidics-based techniques. Isenberg et al.\cite{109} used a three-inlet Christmas-tree microfluidic gradient generator\cite{118} for fabricating phototriggered PAAm hydrogels. Gradients were generated over time by diffusive mixing of solutions with different concentrations of cross-linkers. Gradients in stiffness of 0–4 kPa/100 μm were achieved using this setup. Hartman et al.\cite{119} also used a diffusion-based microfluidics setup to produce PAAm gels with stiffness gradients. In this case, a dumbbell-shaped mask was reproduced on a glass slide by maskless lithography. The two weights of the dumbbell shape were designed as reservoirs for pregel solutions with high and low concentrations of cross-linkers, respectively. The gradient was then produced by allowing the pregel solutions to mix at the center with the gelation initiated via UV-light exposure. Stiffness gradients of 2.9–142.6 kPa/μm were achieved with this method.\cite{119} Diederich et al.\cite{120} used programmable syringe pumps to vary the concentration of monomers fed to a mold in which a hydrogel is produced, which resulted in gradients in mechanical properties in the range of 150–20 kPa over a length scale of 10–2.5 mm. This method is comparatively less complex than fabricating complex gradient generators using microfluidics.

### 4.3. Strategies for Mimicking Dynamic Features of ECM

As the ECM is a dynamic entity, it is also interesting to incorporate some active features into the materials design space to understand the mechanotransduction of cells to vary properties over time. Light is one of the most used stimulants for controlling the dynamic properties of hydrogels. A common strategy used in such systems is to use azobenzene, a photosensitive molecule, as a cross-linker that can switch from \( \text{trans} \) to \( \text{cis} \) form when irradiated with UV light and vice versa when irradiated with visible light, leading to changes in mechanical properties of the hydrogel it is cross-linking. Such a strategy has been applied to PAAm and PEG gels.\cite{121,122} Recently, gold nanorod-incorporated hydrogels have been used to produce spatially controlled actuating hydrogels when excited with near-infrared laser.\cite{123} The hydrogels were produced by random copolymerization of temperature-responsive N-isopropylacrylamide (NIPAM) and N-ethylacrylamide (NEAM), whose individual lower critical solution temperatures are 32 and 82 °C, respectively. The ratio of the two monomers was chosen such that the phase transition occurs at physiological conditions (37 °C), and the temperature is imparted by the photothermal heating of the gold nanorods. Such hydrogel systems are interesting for studying cellular mechanotransduction in response to varying levels of deformations.

### 5. Physicochemical Considerations

Before applying engineered hydrogels to cell culture, it is important to take several parameters into consideration. The mechanical properties may change in response to cell culture media or the gel may experience differential swelling, the gel may leach toxic chemicals, and the gel will need to either allow for protein adhesion, or be functionalized with proteins or peptides for integrin binding.

Hydrogels used in cell culture should not be toxic to the cells cultured on/in them. The presence of any unreacted monomers or certain photoinitiators can be toxic to cells.\cite{124} Therefore, unreacted or diffusing small molecules should be removed before seeding the cells. For 3D photo-polymerizing hydrogel systems, the cytotoxicity of photoinitiators is critical, and UV dose needs to be regulated so that the radiation does not kill the cells.\cite{125}

Swelling is another important property to be considered when preparing substrates for mechanotransduction studies. Loosely
cross-linked synthetic PAAm gels have been shown to exhibit about 33% reduction in stiffness after 1 day of swelling compared with highly cross-linked gels as measured by atomic force microscopy (AFM).[126] To avoid swelling-induced changes in mechanical properties, it is therefore desirable to use higher cross-linking of gels to ensure less swelling over time. However, higher cross-linking will result in stiffer gels; therefore, the concentration of monomers needs to be adjusted accordingly.[127] Fitzgerald et al.[104] observed a reduction in stiffness and elimination of stress-relaxation behavior for PAAm-alginate gels after swelling for 3 days. The long-term mechanical integrity of hydrogels is relevant for mechanotransduction studies investigating the differentiation of cells and secretion of matrix proteins by cells. It is also important to note that cells secrete matrix proteins that would alter the mechanical properties of hydrogels over time.[128]

Cell culture media contains various salts, and it is crucial to bear in mind that osmotic swelling can happen for polyelectrolyte gels which may affect the mechanical properties.[129] Copolymers made of poly(N-isopropylacrylamide) and poly(2-acylamido-2-methylpropanesulfonic acid) exhibit a decrease in swelling in cell culture media/fetal bovine serum (FBS) due to the presence of salts and the media composition, which lowers the osmotic driving force.[130]

Hydrogels with predictable and reliable physical properties are a prerequisite for mechanotransduction studies. A recent work by Richbourg et al.[131] explored the influence of synthetic variables in hydrogel design across hydrogels made with different materials. Specifically, poly(ethylene glycol) diacrylate (PEGDA), cross-linked poly(vinyl alcohol) (PVA) gels, and methacrylamide-modified gelatin (GelMA) hydrogels were compared and contrasted. Both PEGDA and PVA gels exhibited linear synthesis–swelling relationships governed by two synthesis variables: the initial polymer volume fraction and the degree of polymerization between cross-links with significantly less batch variability. Furthermore, the study also revealed a relationship between mechanical properties (shear modulus) and synthesis variables, indicating hydrogels with predictable equilibrium swelling and mechanical properties are possible. However, hydrogels made from GelMA did not exhibit such relationships, which was attributed to the differences in conformations of gelatin with different temperatures and batch-to-batch variability of GelMA.

Different studies use different methods to quantify the mechanical properties of hydrogels. The measured mechanical property differs with each technique, and their relevance in cell-mechanotransduction behavior is still a topic of debate. A recent work by Tassieri et al. points out that rheology may overestimate the stiffness of synthetic hydrogels when high normal forces are used for measurements.[132] Another example is the overestimation of gel modulus in AFM measurements due to the underlying substrate.[113] The overestimation occurs due to the influence of gel thickness and the failure of contact mechanics models due to the indenter geometries.[134] A detailed discussion on mechanical properties measured using different techniques and factors to consider in such measurements is reviewed elsewhere.[113,136]

For cell attachment to hydrogels, the gels should either contain adhesion domains or need to be covalently or noncovalently functionalized with adhesion proteins. For synthetic gels containing amide group such as PAAm, adhesion proteins are usually presented using Sulfo-SANPAH chemistry.[135,136] Synthetic poly(ethylene glycol) diacrylate (PEG-DA) hydrogels can be functionalized with RGD peptides modified with acryloyl-PEG-N-hydroxysuccinimide.[137] In the case of biopolymer gels made of gelatin, the cells attach by interacting with the RGD domains of gelatin,[138] and the intrinsic binding domains in gelatin, make the GelMA hydrogels popular despite issues arising from batch-to-batch variation. For hyaluronic acid hydrogels, cells can interact directly with the CD44 binding domains of hyaluronic acid,[139] but for integrin-mediated mechanotransduction studies, hyaluronic acid is usually modified with chemical moieties such as methacrylates or norbornene for conjugating adhesion proteins such as collagen or RGD peptides by chemistries such as thiol–Michael addition.[140,141] EDC/NHS carbodiimide chemistry is commonly used for both synthetic and biopolymer gels.[142,143]

Another important factor that needs to be addressed to compare the results in cell mechanotransduction studies is the effect of cell density. Stiff matrices tend to promote osteogenesis and inhibit adipogenesis of MSCs.[144] However, at high cell seeding density, both osteogenesis and adipogenesis lineage commitment have been observed to be favored, and a potential role of cell–cell contact was implied as a result.[144] Cell–cell contacts in the form of adherent junctions consisting of proteins such as cadherins have been shown to modulate the influence of substrate stiffness.[145] Contrary to the previous studies that point to cell–cell interactions, the cell spreading on soft and stiff hydrogels as a function of seeding density was studied in a recent work by Venugopal et al.[146] The authors found that on soft 500 Pa gels, cell spreading was increased by three times when the seeding density was changed from 1000 to 8000 cells per cm², which contrasts to cell spreading behavior on soft hydrogels with low seeding density where the cells exhibited a round morphology and less spreading area. For stiffer 2 kPa gels, the cells exhibited low spreading area for high seeding density. For intermediate stiffness 1 kPa gels, the cell spreading did not show any effect with seeding density. Such behavior was attributed to the local stiffening of the matrix when the seeding density was increased. Apart from cell density, cell shape, matrix dimensions, and cell type can also contribute to the ability of cells to transduce mechanical cues.

6. Cell Responses to Viscoelastic Cues

Initially, the use of model substrates with a range of stiffness focused on elastic hydrogel substrates.[41] In this case, an elastic hydrogel substrate is defined as one that does not exhibit time-dependent changes in storage modulus (or) gels where the Young’s modulus is taken from the linear portion for the load applied. Studies on cell spreading revealed that for elastic substrates, the cell spreading increased with increasing stiffness of the substrates and has been correlated to the molecular clutch model.[54,147]

The response of cells to purely viscous substrates was studied by Bennett et al.[148] The response to the substrate viscosity was found to follow the same trend as for elastic substrates, i.e., for
increasing viscosity, the cell spreading was found to increase. This is also in agreement with the molecular clutch model, which predicted the observed behavior, including viscosities above which the clutch reinforcement occurs. In this case, the molecular clutch model was simulated by presenting adhesion ligands to viscous dashpots instead of Hookean springs. Therefore, the talin unfolding occurs at a threshold level of viscosity instead of a threshold level of elasticity as proposed in the original molecular clutch model.[57]

Cameron et al.[149] investigated PAAm gels with high loss modulus (about 10 times more than control samples) exhibiting viscoelastic liquid-like behavior. When mesenchymal stem cells were cultured on these substrates, the area of the cells was found to increase for gels with higher loss modulus. However, the focal adhesions were found to be nascent. This contrasts with cells on elastic substrates where the substrate stiffness enhances the traction forces of cells and thereby increases spreading by forming a high proportion of mature focal adhesions.[150] The authors suggested that the observed cell spreading on high loss modulus substrates could be explained by a cellular tensile model.[151] This model states that there is always an active tension in cells generated by the actomyosin machinery, while the mechanical properties of the surroundings generate a passive tension. For substrates with a high loss modulus, there is a time-dependent loss in passive tension, and in response to this, the active tension of the cells is suggested to increase, resulting in increased cell spreading.[149]

Time-dependent mechanical properties have also been shown to affect mechanotransduction. Chaudhuri et al.[152] studied the influence of time-dependent changes in the elastic modulus on cell adhesion and spreading. In that study, alginate cross-linked with calcium ions was used to produce stress relaxing hydrogels. As stress relaxation implies a drop in the elastic modulus over time, it was expected that the matrix sensed by the cells would appear softer over time and lead to decreased cell spreading. However, when fibroblasts were cultured on these gels, the cells were found to spread more when compared with the fibroblasts cultured on purely elastic gels. When the substrates were observed under a fluorescent microscope, the fluorescently labeled integrin ligands (RGD) were found to be locally clustered on stress relaxing hydrogels even at high ligand densities (1500 μM RGD).[152] This suggests that the stress relaxing hydrogels locally modify the ligand density, thereby enhancing cell spreading. When tested with the molecular clutch hypothesis by including ligand density and stress relaxation in the model, a regime presenting low stiffness and high ligand density with increased cell spreading was predicted.[57] Experimentally, it was verified that the cell response to stress relaxation was mediated through integrin adhesions, via Rho activation, actomyosin-based contractility, and nuclear translocation of YAP. A subsequent publication by the same group[17] found similar cell behavior in 3D cell culture as well, where gels with faster relaxation times resulted in increased spreading of the cells. Thus, a softer matrix with stress relaxation shows similar cell spreading as a stiffer elastic matrix. The time scale of relaxing hydrogels as a parameter was investigated by Gong et al.[153] Remarkably, when the relaxation time of hydrogel substrates matched the time scale for molecular clutch binding and focal adhesions lifetime, cell spreading was maximized.

Charrier et al.[97] studied the influence of viscoelastic solid-like substrates on mechanotransduction. The gels used in this work had two components: viscous linear PAAm and cross-linked PAAm. As both these components exhibit different mechanical properties, the attachment of adhesion ligands to any of these components exclusively is expected to make the cells sense different mechanical properties. Utilizing this unique approach, the authors found that presenting collagen-I to the viscous part inhibited fibroblast spreading, while cell spreading was observed when collagen-I was presented to the cross-linked part. When collagen-I was presented to both the components, the cell spreading was found to be similar to that of elastic hydrogels. The observed cell spreading differences may be attributed to the existence of distinct molecular clutches in operation for different mechanical cues presented and adds validity to the notion that cells do indeed sense the mechanical properties of their surroundings through integrin complexes.

Energy in viscoelastic substrates may be derived from the elastic (stored) or the viscous (dissipated) energy. Two studies by Sacco et al.[154] and Panzetta et al.[155] studied the cells’ response to different levels of deformation to elastic substrates and dissipating substrates, respectively. To study the influence of elastic energy, substrates were prestretched in the linear region of the stress–strain curve, and cells were seeded on these substrates[154]. When compared with unstretched elastic substrates, cells seeded on prestretched elastic substrates exhibited stiffer cytoskeleton as probed by particle tracking microrheology. The authors conclude that, as stiffness is not affected by prestretch, it can be understood that cells sense the substrate elastic energy.[154] Gels exhibiting different levels of dissipation had a marked impact on cellular mechanotransduction.[155] Specifically, with moderate dissipation gels, the cells lose their ability to spread, and with higher dissipation levels, the cells stop attaching to gels. Here, dissipation corresponds to substrates exhibiting significant nonlinear strain softening. From these studies, it is evident that energy is also an important parameter that cannot be discarded with respect to mechanosensing.

Sensing of mechanical properties of substrates by cells is a complex process. In the past decade, a range of mechanical parameters affecting stem cell fate has been explored. While the molecular clutch model accounts well for stiffness and viscosity sensing, subsequent incorporation of parameters such as energy into the model helps in obtaining better mechanistic insights. A summary of all the explored substrate properties described above is shown in Figure 5 regarding how cell spreading is affected.

7. Recent Mechanotransduction Studies Incorporating Various Biophysical and Biochemical Cues

It is well understood now that single factors such as substrate topography, substrate stiffness, the spatial presentation of ligands, and so on are paramount in guiding cell behavior in vivo. For instance, the idea that cell behaviors can be mediated through topographical cues from their surroundings dates back as early as 1911.[156] Research techniques have evolved over decades such that we can precisely tune these biophysical and
biochemical cues in vitro. What remains elusive, however, is the mechanisms responsible for the synergy between multiple of these factors occurring spatiotemporally, as evidence suggests the result, when occurring simultaneously, is not simply the sum of each part. Understanding the underlying mechanisms behind this is critical in allowing us to understand and manipulate cell behavior; as within the natural cellular environment, the ECM presents a multitude of cues simultaneously in a specific and intentional manner.

7.1. Ligand Presentation and Stiffness

It is important to note that while the mechanical properties of gels are crucially important to cells grown on them, the cell adhesion and spreading to the gel are mediated via integrins binding to ligands present on (or in) the gel. Thus, in addition to the mechanical properties of the substrate, the type and density of integrin ligands have also been identified to play an essential role in how cells behave on elastic gels. To investigate the influence of ligands versus stiffness, Trappmann et al. studied mesenchymal stem cells cultured on both PAAm gels and poly(dimethoxysiloxane) (PDMS) substrates of varying stiffness. The authors found that mesenchymal stem cells cultured on PDMS did not differentiate, while vastly increased cell differentiation was observed on PAAm gels with the same stiffness. It was hypothesized that the pore size of PAAm gels decreased with increasing stiffness, leading to an increase in the density of adhesion points (collagen coupled to the gel). This suggests that the cells sense the mechanical feedback of adhesion proteins by applying force to them and that the effect of substrate stiffness may be overridden by the density of anchoring points. In a recent work by Stanton et al., the conjugation efficiency of adhesive ligands on PAAm gels was increased by adding 2-aminoethyl methacrylate along with PAAm gel precursor solution which introduces primary amine groups on the gel surface. The work further showed that human mesenchymal stem cells (hMSCs) respond to varied ligand density (fibronectin) alone without altering the substrate stiffness. Specifically, stem cells exhibited YAP nuclear translocation as a function of ligand density. In another study by the same group, the effect of density of adhesion ligands of four different ECM adhesion ligands (fibronectin, collagen-I, collagen-IV, and laminin) on stem cell mechanotransduction was studied. While the ligand density seemed to be a determinant factor for inducing YAP nuclear translocation across the four ECM ligands, the density varied across the four different ligands. For instance, collagen-I required 5 μg mL⁻¹ for nuclear YAP, while collagen-IV required 50 μg mL⁻¹ on soft 3 kPa hydrogels. Furthermore, the study

Figure 5. Representation of how the substrate properties affect the spreading of cells. Inclusive of hydrogel substrates with elastic properties, viscoelastic properties (solid and liquid), stored elastic energy (prestretched), and energy dissipation.
found that different integrin subunits were engaged for different ECM ligands. For example, fibronectin ligand required both α5- and α2β1 integrins for YAP translocation, while collagen-I and collagen-IV required solely α5 integrins. Thus, both ligand density and ECM ligand type were found to determine the YAP nuclear translocation and therefore modulate stem cell differentiation, superseding the effects imparted by substrate stiffness in this case.\textsuperscript{161,162} As demonstrated in this work, substrate stiffness can be overridden by a range of ligand densities, and it has been further shown that this range changes for different ECM adhesion ligands.\textsuperscript{162,161}

Zhang et al. have studied the influence of ordered ligand spacing, superimposed on gels, on stem cell differentiation.\textsuperscript{164} To produce ordered ligand spacing, the authors first used block copolymers loaded with gold nanoparticles to obtain ordered gold nanoarrays of spacings 70, 150, and 230 nm between the gold nanoparticles on glass coverslips. Then, PAAm gels were cast on these glass coverslips consisting of the gold nanoarrays to enable pattern transfer to gels. This was followed by incubation in thiol-modified cyclo-RGDK to facilitate the attachment of adhesive ligands (cyclo-RGDK to gold nanoparticles on the gels). The study found that osteogenic differentiation of stem cells was possible on soft hydrogels (3 kPa) with large ordered ligand spacing (230 nm). This was in contrast to previous work, where soft hydrogels with randomly distributed ligands did not promote osteogenic differentiation. The authors argued that this may be due to instances of decreased local ligand distances in the case of randomly distributed ligands, something that would lead to reduced force generation on molecular clutches, limiting the recruitment of extra integrins. Thus, in addition to ligand density and stiffness, the spacing of ligands has also emerged to be an important contributor in determining cell fate.

### 7.2. Topography and Stiffness

Cells have been found to respond to topography and patterns. Such studies were motivated by the fact that cells encounter complex microenvironments during development and processes such as wound healing and cancer.\textsuperscript{163–167} Some cells have been shown to be sensitive to topographical features as small as 5 nm,\textsuperscript{168} so topographical cues down to nanoscale resolution have been paramount in engineered cell scaffolds. In the late 1990s and early 2000s, a series of research works by the Ingber group showed that cells respond to cues such as adhesion islands and geometry.\textsuperscript{169–171} However, these studies predominantly used hard substrates, with stiffnesses in the range cells cannot sense as different from each other. In recent years, following these pioneering works, there has been considerable research interest in investigating various patterns and topographical cues—such as surface roughness, anisotropic patterning, and isotropic patterning—in tandem with substrate mechanical properties.

Hou et al.\textsuperscript{172} investigated the influence of surface roughness in combination with hydrogel stiffness. The roughness, in this case, was in the range of 200 nm to 1.2 µm. Previous research showed that the spreading area of stem cells decreased when cultured on soft hydrogels\textsuperscript{173}; however, on high roughness regions, the spreading area increased with better aligned actin stress fibers and matured focal adhesions. For stiff hydrogels (31.3 kPa), mature focal adhesions were only observed on intermediate roughness regions and not on high roughness regions. In both cases (soft 3.8 kPa hydrogels with high roughness and stiff hydrogels with intermediate roughness), YAP nuclear accumulation was found to be increased. The authors interpret these results as the synergistic effect of roughness and substrate stiffness and demonstrated that this was mediated by the FAK-RhoA/ROCK-actomyosin-lamin-YAP/TAZ pathway.

Isotropic and anisotropic surface patterning has also been explored within the context of topographical patterning with mediated stiffness. Isotropic patterns include pits, pillars, random fibers, and so on and are characterized by their lack of orientation (randomness), while anisotropic surface patterning, characterized by the clear orientation of surface features, includes grooves/ridges. Random and regular patterns of mechanical properties in regulating the mesenchymal stem cell fate were recently studied by Anseth et al.\textsuperscript{174} In this study, YAP activation was found to only occur beyond a certain threshold of stiffness (5 kPa) and to deactivate below this threshold (on soft substrates). The study was motivated by the question of how the stem cell fate would be affected by the presentation of random or regular patterns of activating and deactivating signals. The size of the patterns was chosen to be comparable with that of mature focal adhesions (1–5 µm²). Surprisingly, osteogenic differentiation of stem cells cultured on regularly patterned regions was higher than that of cells cultured on random patterns, the opposite of what was observed previously for mesenchymal stem cells on random nanotopographic hard surfaces.\textsuperscript{175} This suggests that the way that cells sense patterns may be regulated by the underlying mechanical properties. Also, stem cells have been shown to possess mechanical memory, which can guide long-term fate decisions.\textsuperscript{176} The fact that cells responding to spatial mechanical dosing by YAP activation in this study may corroborate the stem cell’s role in mechanical memory. Anisotropic microgroove surface patterns were used in tandem with stiff and soft substrates in a recent study by Parandakh et al.\textsuperscript{177} to explore the dualistic effects of substrate stiffness and substrate topography, along with cell culture time of hMSCs. Ordered microgrooves (10 µm wide, 10 µm apart, 5 µm deep) were induced on silicon substrates via lithography, and atop this was a stiff (1500 kPa) and soft (90 kPa) PDMS layer. In general, substrate topography and substrate stiffness showed significant interactions, whereby microgrooved, stiff substrates resulted in the greater cell stiffness and gene expression of α-actin and h1-caldesmon. These same values, which are indicative of the extent of gene expression, were found to be at a minimum on smooth, soft substrates. A significant conclusion of this research was that there was no significant three-way interaction between substrate stiffness, substrate topography, and culture time, and the synergy seemed to be solely within the interaction between topography and stiffness.\textsuperscript{177}
of cells on stiffness gradients has been investigated across various cell lines.\cite{109,166,178} These studies suggest that the migration of cells toward higher stiffness regions ( durotaxis) depends only on the gradient strength and not on absolute stiffness. However, a recent study on invasive breast cancer cells cultured on an anisotropic stiffness gradient revealed that migration of invasive cancer cells depended on absolute stiffness as well as on cell contractility.\cite{179} In the study, the invasive cell line (MDA-MB-231) was compared against noninvasive breast cells (MCF-10A cell line). While the invasive cell line exhibited guided migration, the normal breast cell line did not display guided migration. While the protein complex Arp2/3 has been shown to be a mediator for directional migration of cells on stiffness gradients,\cite{166} the relationship of Arp2/3 with substrate mechanical properties is still not completely clear, and further research must be conducted to understand the migration processes of invasive cancer cell lines. Also, more sophisticated mechanical models need to be developed to understand the migration processes. For instance, this could include tackling questions such as the optimal stiffness ranges required for directed migration and the role of underlying viscosity of substrates.

8. Conclusions and Outlook

Over the past few years, researchers have used various material systems and characterization tools to study stem cell differentiation and understand the mechanotransduction processes. In recent years of research, the understanding of tunable substrate properties has evolved, from initially being solely substrate stiffness, to more complex models involving strain stiffening and time-dependent properties. There has also been continuous research on understanding the mechanical memory of stem cells and its regulation. Various material systems have been explored to impart controlled mechanical properties to cells, and recently some of the dynamic features of the ECM have been explored.

The cellular response to the stiffness of the substrate is well explained by the motor clutch model of mechanotransduction. Many current studies attempt to elucidate the influence of viscoelasticity on cells using the motor clutch model. The effect of parameters, such as elastic and dissipative energy on mechanotransduction, is starting to be explored. Whether the motor clutch model can be used to describe the influence of such new parameters is still a question.

Recently, there has been a move toward combining substrate mechanical properties with various biophysical and biochemical cues. Such studies provide insights into understanding the complex behavior of cells and also to design a material environment to control the differentiation processes of cells that more effectively mimic the elaborate presentation of cues to cells by the ECM. An important point to note in such studies is that various parameters such as cell lines, cell density, culture conditions, and ligand presentation can influence the observed behavior. Accompanying the rapidly growing trajectory in this field are many complex, yet important questions. Among these include answering the effect of viscoelasticity on the cell’s migratory processes and mechanical memory. Indeed, recent research works head in the direction of pattern sensing of cells and its relation to various emergent behaviors.\cite{180,181} Another important question to answer is how multiple physical cues synergistically affect cellular processes. Moving forward, further research on material systems to design and implement multiphysical cues need to be conducted. As cells reside inside ECM, translating such systems to 3D would be a future challenge to address. Finally, understanding the influence of such cues on complex cellular processes will help in controlling cell fate and also in clinical translation.

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Conflict of Interest

The authors declare no conflict of interest.

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[1] J. P. Vacanti, R. Langer, Lancet 1999, 354, 32.
[2] M. D. Davidson, J. A. Burdick, R. G. Wells, Adv. Healthcare Mater. 2020, 9, 1901682.
[3] S. Mobini, Y. H. Song, M. W. McCrary, C. E. Schmidt, Biomaterials 2019, 198, 146.
[4] A. J. Engler, S. Sen, H. L. Sweeney, D. E. Discher, Cell 2006, 126, 677.
[5] F. Han, C. Zhu, Q. Guo, H. Yang, B. Li, J. Mater. Chem. B 2016, 4, 9.
[6] B. Trappmann, C. S. Chen, Curr. Opin. Biotechnol. 2013, 24, 948.
[7] L. Wong, A. Kumar, B. Gabela-Zuniga, J. Chua, G. Singh, C. L. Happe, A. J. Engler, Y. Fan, K. E. McCloskey, Acta Biomater. 2019, 96, 321.
[8] N. D. Leipzig, M. S. Shoichet, Biomaterials 2009, 30, 6867.
[9] M. Cantini, H. Donnelly, M. J. Dalby, M. Salmeron-Sanchez, Adv. Healthcare Mater. 2020, 9, 1901259.
[10] J. Almodóvar, T. Crouzier, Š. Selimović, T. Boudou, A. Khademhosseini, C. Picart, Lab Chip 2013, 13, 1562.
[11] K. U. Clausen, T. Scheibel, H. W. Schmidt, R. Giesa, Macromol. Mater. Eng. 2012, 297, 938.
[12] A. Seidi, M. Ramalingam, I. Elloumi-Hannachi, S. Ostroviod, A. Khademhosseini, Acta Biomater. 2011, 7, 1441.
[13] J. Malmström, B. Christensen, H. P. Jakobsen, J. Lovmand, R. Foldbjerg, E. S. Sørensen, D. S. Sutherland, Nano Lett. 2010, 10, 686.
[14] D. Lehnert, B. Wehrle-Haller, C. David, U. Weiland, C. Ballestrem, B. A. Imhof, M. Baste Mey er, J. Cell Sci. 2004, 117, 41.
[15] J. A. Deeg, I. Louban, D. Aydin, C. Selhuber-Unkel, H. Kessler, J. P. Spatz, Nano Lett. 2011, 11, 1469.
[16] L. Kuen Yong, D. J. Mooney, Chem. Rev. 2001, 101, 1869.
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