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Active Biomaterials for Mechanobiology

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Abstract

Active biomaterials offer novel approaches to study mechanotransduction in mammalian cells. These material systems probe cellular responses by dynamically modulating their resistance to endogenous forces or applying exogenous forces on cells in a temporally controlled manner. Stimuli-responsive molecules, polymers, and nanoparticles embedded inside cytocompatible biopolymer networks transduce external signals such as light, heat, chemicals, and magnetic fields into changes in matrix elasticity (few kPa to tens of kPa) or forces (few pN to several μN) at the cell-material interface. The implementation of active biomaterials in mechanobiology has generated scientific knowledge and therapeutic potential relevant to a variety of conditions including but not limited to cancer metastasis, fibrosis, and tissue regeneration. We discuss the repertoire of cellular responses that can be studied using these platforms including receptor signaling as well as downstream events namely, cytoskeletal organization, nuclear shuttling of mechanosensitive transcriptional regulators, cell migration, and differentiation. We highlight recent advances in active biomaterials and comment on their future impact.

Keywords

dynamic matrices; nanomaterials; actuation; programmable

1. Introduction

The field of biomaterials has made dramatic advances in the last decades, leading to the development of complex material systems with tunable physicochemical properties. The

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Declaration of interests

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physical, chemical and biological properties of a given biomaterial can be engineered to provide distinct manipulative cues for mammalian cells and applications. These cues can be spatially patterned with molecular precision, while scaffolds can be miniaturized to the cellular scale with the adoption of microfabrication tools. Moreover, the incorporation of nanotechnology and stimuli-responsive supramolecular systems into material design has led to multifunctional materials with adaptive functionalities. An emerging group of such material systems is active biomaterials that offer external control over physical and chemical properties in both space and time. These materials have the potential to make significant impact in various biomedical basic research areas and applications.

Active materials are excellent candidates for the study of mechanotransduction in mammalian cells. Mechanotransduction refers to the process by which cells sense and respond to mechanical cues in their microenvironment by transducing these signals into biological responses. Cells constantly interact with their surroundings, and their engagement with other cells and the physical extracellular matrix (ECM) typically involves the formation of dynamic adhesions and application of cellularly-generated (endogenous) forces via these adhesions. The other cells and materials to which these forces are applied typically respond by deforming, and their resistance to a cell's endogenous forces is sensed by the originating cell via the same machinery that enables adhesion and application of its endogenous forces. In addition, cells and the ECM in tissues are subjected to externally applied (exogenous) forces that arise from a variety of sources, including gravity, fluid shear forces, and neighboring or distant cells and tissues. As a result, cells experience the implications of both endogenous and exogenous forces, and these ultimately influence numerous cellular processes, including those related to homeostasis and regeneration [1], [2]. The mechanical interplay between cells and their microenvironment is spatiotemporally regulated, with stresses continuously generated and distributed at multiple length scales. Active biomaterials can recapitulate the dynamic microenvironment within living tissues because they have the ability to convert electromagnetic fields and sound waves into structural reconfiguration and mechanical cues by either changing mechanical properties or generating and transmitting mechanical forces.

In this review, we focus on active biomaterials that can be programmed to apply dynamic mechanical cues to cells and tissues in a controllable manner. In the following sections, we first briefly discuss established *in vitro* methods for the study of mechanotransduction. We then focus on the working principles of active biomaterials and their impact in mechanobiology to date by highlighting seminal work in the field. The article ends with a discussion on a number of challenges and opportunities where materials science and nanotechnology are expected to drive the scientific inquiry as well as potentially provide solutions to pressing clinical problems.

2. Brief background on designer materials for studies of mechanobiology

Several technological platforms and material systems have been developed for the study of how cells perceive and process mechanical cues, leading to the discovery of key mechanosensitive proteins and intracellular signaling pathways. These platforms can be classified either as systems with structural modification, where the propagation and

dissipation of endogenous forces are manipulated through externally controlled changes in the mechanical properties of the substrate, or stress-generating systems where the activated substrate applies exogenous forces to cells.

The study of how cells remodel and respond to their ECM via application of endogenous forces has been aided by the use of a number of synthetic substrates, and hydrogel-based systems have been widely exploited for this purpose. Hydrogels often offer control over mechanical properties while providing physiologically relevant biochemical cues for cells. Hydrogel based synthetic matrices have been utilized to study the effects of changes in matrix stiffness [3], [4], degradation [5], stress relaxation [6], topography [7], and polymer network structure [8] on cell behavior. These studies revealed that alterations in the physical interactions of cells with the ECM are alone sufficient to drive various biological processes such as migration [9], [10], epithelial-mesenchymal transition [11], [12], and stem cell differentiation [13], [14]. For example, 3D cultures of mammary epithelial cells lost their physiological acinar and underwent malignant transformation in stiff collagen gels (2100 Pa) while cells cultured in soft matrices (170 Pa) retained the normal phenotype [11]. Mesenchymal stem cell (MSC) osteogenesis has been shown to be dependent on matrix stiffness as well, with optimal bone tissue formation achieved at 60 kPa in alginate scaffolds [15]. MSCs cultured on polyacrylamide gels with varying stiffness showed neurogenic differentiation at 0.1-1 kPa while intermediate stiffness gels (8-17 kPa) induced myoblast formation and high stiffness gels (25-40 kPa) directed differentiation into osteoblasts [16]. Matrix viscoelasticity is another mechanical property that influences differentiation as demonstrated using alginate matrices with tunable stress relaxation [6]. Faster stress relaxation that was on the order of tens of seconds increased cell spreading, proliferation and subsequent osteogenic differentiation of MSCs for a given matrix stiffness (17 kPa).

The spatiotemporally dynamic nature of exogenous mechanical loads applied to tissues and cells, which include tensile stress, shear, and compression, has led to the use of mechanically active materials to investigate the resulting modes of mechanotransduction. In order to apply forces to cells cultured on planar substrates, micromanipulation techniques such as micropipette aspiration [17], optical tweezers [18], magnetic twisting cytometry [19], atomic force microscopy (AFM) [20], stretch devices, and microfluidics [21] have been employed. The implementation of these techniques in mechanobiology has led to the discovery of force sensing and transducing molecular machinery [22]-[26]. For example, functionalizing the surfaces of end-effector particles with relevant molecules revealed that ECM binding receptor integrins, together with adaptor proteins talin, vinculin, α-actinin and others, are crucial force transducers while nuclear shuttling of transcriptional regulators such as Yes-associated protein (YAP), transcriptional coactivator with PDZ-binding motif (TAZ), and myocardin-related transcription factor-A (MRTF-A) mediate downstream signaling (Figure 1) [19], [27]-[32]. Similarly, mechanical perturbation of mechanosensitive ion channels revealed that ion channel protein Piezo2 regulates the formation and orientation of focal adhesions as well as stress fibers through calcium triggered RhoA activation [33]. At the multicellular scale, cell-cell binding through E-cadherins and adherens junction reinforcement is necessary for mechanosensing [24], [34].

Micromanipulation techniques allow control over the magnitude (tens of pN to a few nN) and timing of exogenous forces [25]. For example, constant exogenous forces of 1.5 nN applied directly to the nucleus of healthy mammary cells using AFM induced nuclear YAP transport by increasing nuclear membrane permeability, whereas indenting the cytoplasm did not induce any changes to nuclear YAP content [20]. Moreover, repeated application of exogenous forces leads to frequency and duration dependent behavior in a variety of cells. Cyclic tensile strain at low frequencies (1-2 Hz) induced cell spreading in human mesenchymal stem cells (hMSCs) [35]. On the other hand, fibroblasts subjected to cyclic stretching exhibited increased cell spreading and stress fiber formation for the first four hours of actuation, with no further response at longer durations of mechanical activation [36]. Spreading and proliferation was highest at 0.1 Hz frequency, and both were mediated by stretch induced nuclear localization of MRTF-A and YAP. Similarly, compressive stress increased cancer cell migration [37] and shear stress induced ATP release in red blood cells [38]. These examples highlight the wide variety of responses and the importance of proper devices for the application of relevant forces.

3. Design and working principles of active biomaterial systems

Mechanically dynamic biomaterials are typically synthesized from cleavable molecules, stimuli responsive polymers, or nanomaterials that are physically and chemically compatible with the physiology of cells of interest. We distinguish active biomaterial systems according to their mechanical function: manipulation of response to cell-endogenous forces via dynamic modulation of matrix elasticity, or application of extrinsic forces on cells upon external stimulation. Reversible elasticity can be achieved with only a single active material, while force generation is typically achieved with composites where nanomaterials serve as the actuators. We explain the fabrication and operation principles of these two classes of mechanically dynamic biomaterials in the following sections.

3.1. Active biomaterials for manipulating resistance to endogenous forces

Biomaterial systems with actively controlled mechanical properties have been developed from synthetic hydrogels, elastomers, proteins, and nucleic acids. These can be triggered by a variety of external stimuli, including light, pH, and enzymes. In these systems, matrix elasticity is typically controlled by actively modulating the network crosslink density. Reducing the crosslinking density decreases the stiffness of the polymerized matrix (i.e. softening) and, likewise, increasing results in stiffening of the matrix. However, alterations in the crosslinking density can lead to variations in network mesh size, which can significantly influence diffusion of soluble factors through the matrix [39]. The specific chemical crosslinking strategy utilized in a particular system typically determines whether these changes are reversible, and whether they can be performed over many cycles.

3.1.1. Optical control of matrix structure—The introduction of photolabile molecules in polymer networks enables externally triggered softening while photoinduced secondary crosslinking leads to temporally controlled stiffening in biomaterials (Figure 2). Functionalizing polymers with photodegradable *o*-nitrobenzyl alcohol derivates and crosslinking methacrylate or thiol groups of polymer chains in the presence of

photoinitiators have been common approaches for softening and stiffening the matrix, respectively (Table 1) [40]-[45]. Combining light activated cleaving and crosslinking strategies in the same material enabled active systems with reversible stiffness. The doubly functionalized HA matrix is a classical example [46]. However, these systems typically allow one cycle of elasticity change and molecules that exhibit reversible transitions or host-guest interactions were used in an effort to enable multiple rounds of cycling between soft-stiff matrix states [47]-[58]. A range of elastic moduli were obtained with these biomaterial systems depending on the polymer and its molecular weight as well as the crosslinking mechanism as summarized in Table 1. We refer the readers to excellent review articles that discuss the working principles of these active biomaterial systems [59]-[64].

Early material systems relied primarily on photocleavable and photo-crosslinker molecules, while recent efforts to engineer active biomaterial platforms have explored optogenetic tools (Figure 2). Genetically engineered proteins with reversible kinetics have been incorporated into polymer networks to control the availability of cell binding sites in synthetic matrices [74], protein [75] and cell release in 3D [76] and recently to achieve cyclic stiffness modulation. One example is the hybrid protein-polymer networks engineered using light, oxygen, and voltage sensing domain 2 (LOV2), a photo-responsive protein that undergoes reversible intramolecular dissociation. With the incorporation of LOV2, the stiffness of PEG hydrogels was reversibly reduced by approximately 8% under 470 nm light exposure. Light triggered softening was relatively fast, occurring within seconds of exposure time, and, using structured illumination, mechanical properties could be spatially patterned [77]. In another study, a near infrared light (NIR) sensitive biomaterial system was developed using bacterial photoreceptor Cph1 as the active element. The protein exists in its monomer form under 740 nm light and switches to a dimeric state when exposed to 660 nm light, leading to a reversible change in crosslinking density within 8-arm PEG hydrogels. Cyclic stiffness modulation was achieved by alternating the excitation wavelength, with the Young’s modulus of the Cph1-PEG network shifting between 2.6 kPa and 4.4 kPa within 10 minutes of illumination [78]. Alternatively, PEG hydrogels functionalized with a photoswitchable crosslinker protein, Dronpa145N, a mutant of fluorescent protein Dronpa, exhibited matrix softening once Dronpa145N shifted from its tetrameric to monomeric state upon exposure to blue light (400-500 nm). This shift in protein configuration led to a reduction of Young's modulus from 2 kPa to 500 Pa within 15 minutes of photoactivation [79].

Optogenetic strategies have the potential to augment material platforms with unprecedent modification capabilities. The wide pool of natural and mutant stimuli responsive proteins provide ample opportunities to designing active biomaterials that respond to various triggers. In parallel, advances in optics can enable fine spatial control over protein distribution and activity. For example, two-photon lasers have overcome the resolution limits of widefield illumination. With this equipment, substrates with precise biomolecular composition can be fabricated in 3D space [80]. The implementation of this approach in active biomaterials has achieved complex physical patterns, such as the microcavities generated in photodegradable PEG matrices by two-photon laser scanning microscopy [65]. Triggering biomaterial platforms via light allows excellent spatial and temporal control when combined with advanced optical manipulation techniques, making these approaches very attractive for time-dependent biological applications that require high precision.

3.1.2. Chemical control of matrix structure—The mechanical properties of biomaterials can be coupled to the chemical composition of their environment with the introduction of chemically responsive transient bonds in the polymer network. An effective way to couple the mechanics of hydrogels with soluble factors exploits materials that possess reversible crosslinking. For example, alginate gels can be formed by mixing the polysaccharide with cations, and the ionically crosslinked hydrogel can be rapidly dissolved with chelating agents. The stiffness of a collagen I and alginate composite scaffold was controlled using calcium chloride and sodium citrate solutions, where reversible stiffening was demonstrated over multiple cycles by simply exchanging the buffer solution [81]. Reversible ionic crosslinking was also applied in pure alginate materials to control the sol-gel transition of 3D hydrogels [82]. Alginate can be ionically crosslinked in the presence of cells without affecting cell viability, and the biopolymer can be functionalized with different click moieties or peptides, making it an excellent candidate for active biomaterial systems [83], [84].

Dynamic hydrogel matrices that rely on chemically responsive non-covalent host-guest reactions have also been developed. Reversible interactions between β-cyclodextrin and adamantane has been exploited in a 4-arm PEG based hydrogel network, where the addition of soluble adamantane functionalized free 4-arm PEG increased the crosslinking density while free β-cyclodextrin reduced it by competing for binding. A long duration of chemical exposure (~40 hours) was necessary to elicit crosslinking alterations leading to a reversible change in matrix stiffness [85]. A similar active biomaterial system requiring a shorter chemical stimulus exposure and providing a wider range of matrix stiffness was recently reported. This β-cyclodextrin and adamantane functionalized acrylamide matrix globally stiffened in the complete absence of soluble β-cyclodextrin within approximately 3 hours, and this was reversed with the addition of β-cyclodextrin to the surrounding media. By alternating β-cyclodextrin concentration, reversible and cyclic changes in matrix stiffness were achieved between 4-11 kPa [86].

Biomolecules such as DNA and enzymes offer alternative methods for generating reversible stiffness in synthetic matrices. Biocompatible polyacrylamide-DNA matrices have been reported to exhibit reversible stiffening behavior by alternating delivery of L and R strands [87]. Similarly, a four-fold increase of stiffness was observed in DNA crosslinked polyacrylamide substrates [88]. Reversible stiffening over several cycles was demonstrated in dynamic protein hydrogels that undergo secondary crosslinking between tyrosine residues due to redox reactions [89], or tyrosinase enzyme [90], [91]. In contrast, sortase enzyme mediated crosslinking led to reversible stiffening in PEG-peptide hydrogels [92]. pH sensitive hydrogels with reversible kinetics have also been engineered, although variations in pH may not be necessarily desired in biological environments, limiting the applications of such systems with live cells [93], [94].

In sum, chemically triggered active biomaterial platforms have been engineered using reversible ionic crosslinking, non-covalent host-guest reactions, conformational changes in proteins, and nucleic acids as crosslinkers. These approaches mostly realize reversible stiffening in a variety of synthetic and natural hydrogels over a range of matrix elasticity that is relevant to biology. Moreover, chemical activation does not require an external energy

source or machinery compared to photoresponsive material systems, which is an attractive feature especially for applications where global material changes are desired in a simple manner. However, it is important to note that the timescale of physical changes is likely diffusion controlled and can be on the order of hours, in contrast to rapid, light triggered activation.

3.1.3. Acoustic control of matrix structure—Sound waves offer an alternative strategy to remotely excite materials and modify their mechanical properties. The internal structure of engineered scaffolds can be controllably disrupted via ultrasound, and this disruption can be transformed into actuation if the polymer network is constructed from self-healing crosslinks. An example of such a material system is ionically crosslinked alginate gels, as cationic bonds can be reversibly broken with ultrasound [95]. The degree of network degradation can be modulated by varying the duration and intensity of acoustic pressure. Millimeter-sized alginate capsules were reversibly disrupted with seconds of acoustic excitation without raising the temperature above physiological conditions [96]. Triggered changes in crosslinking have been primarily used to release therapeutic agents [95], polysaccharides [97], surface functionalized nanoparticles [96], [98], and small molecules [99] for regenerative medicine [96], [99] as well as cancer treatment [95]. In the context of this review, it is noteworthy to highlight recent demonstrations that sound waves can be used to induce reversible matrix softening in hydrogel networks. For example, the storage modulus of cellulose gels was decreased from an initial value of 42 kPa to 4 kPa under 5 minutes of low strain ultrasound actuation, in a reversible fashion. This structural change was attributed to the breakage of hydrogen bonds within the network [100]. Similar observations have been made in colloidal gels composed of a network of inorganic particles such as calcite and silica. The elastic modulus of the calcite colloidal network decreased by a factor of 5 when acoustically actuated [101]. These recent studies suggest that reversible elasticity in hydrogel matrices can be realized with an acoustic trigger. Future work will explore the potential of this technique for mechanobiology research.

3.1.4. Combined strategies to dynamically modulate matrix architecture—The three techniques presented in the previous sections have comparative advantages and disadvantages. A combination of multiple modulation methods may result in superior dynamic control over physical properties of the material. So far, only optical and chemical methods have been combined in the same material platform. For example, in a photochemically crosslinked alginate matrix, UV exposure led to degeneration of a photoacid generator, thereby providing cations for ionic crosslinking. Alginate microstructures and channels on the order of 100 μm were rapidly formed and subsequently dissolved with the addition of chelator ethylenediaminetetraacetic acid (EDTA) [102]. Similarly, a light sensitive calcium cage was used to crosslink alginate on demand upon UV activation, and ionic crosslinking was chemically degraded with EDTA [103]. These active biomaterial systems combine the benefits of chemical and optical activation methods by harnessing the tunability of alginate networks with the speed and spatial specificity of light.

3.2. Application of exogenous forces using actuated active biomaterials

The controlled application of external forces to cells under biomimetic conditions provides another key aspect of mechanobiology. To this end, particles capable of transducing electromagnetic fields and acoustic waves into mechanical work and stimuli responsive materials have been integrated into otherwise static biomaterial systems. Depending on the choice of the inclusion and the design of the scaffold, different strain and stress profiles can be generated in 3D, which translate into mechanical loading at the material-cell interface. Here, we review recent advancements by categorizing the materials according to the applied stimuli, magnetic or optical (Figure 3).

3.2.1. Magnetic actuation for the application of exogenous forces—Magnetic actuation is appealing for the application of local mechanical deformation because magnetic fields provide easy, rapid, and non-invasive control. The most common way of harnessing magnetic forces and torques in mechanobiology research is mixing magnetic nano- or microparticles into hydrogels [104]-[111], synthetic polymers [112]-[115], or elastomers [116]-[118]. Iron oxide (Fe_3O_4) nanoparticles have been the dominant choice due to the favorable properties of the material, including inertness under physiological conditions and tunable magnetic properties. Under the influence of magnetic fields, the embedded particles interact with one another and with the polymer matrices to create rapid and dramatic matrix deformation, while changing mechanical properties such as stiffness in a controlled manner. The magnetically induced deformation can apply local stresses on nearby cells, and the magnitude of the applied force is controlled by tuning the direction, strength, and distribution of the magnetic field. In this section, we review magnetoresponsive biomaterial systems and discuss key aspects of material design for gaining spatiotemporal control over force generation.

There are two distinct strategies for magnetic actuation: culturing cells inside or on magnetized bulk materials and engineering magnetic microactuators that can be interfaced with cells and tissues. Bulk magnetic scaffolds generate high compressive stresses upon actuation with magnetic field gradients (Figure 3). A repertoire of magnetic scaffolds have been fabricated at scales ranging from millimeter to centimeter using hyaluronic acid [104], collagen [105], alginate [106]-[109], cellulose [110], silk [111], starch [112], polycaprylactone [112]-[114], poly(lactic-co-glycolic acid) [113], PEGDA [115], PDMS [116], [117], and liquid crystalline elastomers [118]. Notably, centimeter-sized alginate ferrogels that contain iron oxide nanoparticles provide a biomimetic scaffold for cells and deform up to 70% in volume under magnetic field gradients. The macroporous structure of the network, with ~20-μm pore size, is the main determinant for the high compressibility [107]. Magnetization scales with volume, and sustaining the same deformability at smaller scales is not possible with these nanocomposites. A biphasic version of the scaffold that consisted of a macroporous alginate layer and a magnetic alginate layer addressed the trade-off between compressibility and magnetization. The heterogenous composition increased the bulk contraction from 20% to 55% with an estimated force of 2 N/g inside the body [108], [109]. As demonstrated in these studies, the porosity and internal structure of magnetic scaffolds heavily influence the mechanics of the system. Notably, an increase in porosity

was observed to change material deformation from shrinkage to elongation with actuation [119].

Microfabricated magnetic devices, on the other hand, have the capability of conveying local forces reaching tens of nN. Early work introduced arrays of microscopic PDMS posts containing ferromagnetic cobalt nanowires as an active substrate. The posts were magnetized and bent in the direction of the low-strength homogenous magnetic field, with tip deflection reaching up to 1 μm, which corresponds to 27 nN per post [116]. As an alternative strategy, PDMS-carbonyl iron nanoparticle micropost arrays were actuated using magnetic field gradients, generating tip deflections as high as 26 μm per post [117]. A similar concept was applied in the development of a hydrogel microactuator that was fabricated from poly(ethylene glycol) dimethacrylate (PEGDMA) and iron oxide nanoparticles [120]. Deformation of magnetic polymer devices can be tuned by controlling the distribution and alignment of magnetic nanoparticles prior to casting [115]. Ferrofluid oil microdroplets [121] provide an alternative for harnessing magnetic fields for actuation. Instead of incorporating ferromagnetic nanoparticles inside polymers, fluorocarbon-based biocompatible ferrofluid oil was prepared and used as a microactuator inside living tissues [122], [123]. The application of a controlled, uniform magnetic field on the microdroplet deforms it along the direction of the magnetic field, generating a force dipole of known magnitude and direction. Magnetic stresses up to 100 Pa were applied within tissues, and the droplets showed up to 20% deformation depending on the mechanical properties of the tissue and the capillary stresses.

3.2.2. Photoactivated materials for the application of exogenous forces—

Photothermal heating is an alternative strategy for the application of extrinsic forces, through reversible compaction of thermoresponsive polymers such as poly(N-isopropylacrylamide) (pNIPAM) [124]-[129] and poly(N-vinyl caprolactam) [130]. pNIPAM and its copolymers have been widely used because the temperature at which the material transitions from a hydrophilic to a hydrophobic state can be tuned over a range of physiologically relevant temperatures (32°C - 42°C). Furthermore, the swelling kinetics of the pNIPAM polymer can be modified by introducing ionic functional groups into the polymer chains, as a means to influence the overall network charge density [131]. Thermoresponsive 3D hydrogel scaffolds that exhibit up to 50% volumetric change when subjected to physiological temperatures (37°C) have been fabricated from pNIPAM [125] or co-polymers of pNIPAM with PEG [126]. Notably, compaction in a thermoresponsive polymer network significantly influences the stiffness of the bulk material. For example, it has been reported that a 50% decrease in the volume of pNIPAM films led to a 6-fold increase in the Young’s modulus [124].

Decoupling precise control over generation of stresses during actuation from the mechanical properties of the material is important for many aspects of mechanobiology research. In an effort to address this issue, micro- and nanoscale thermoresponsive elements seeded with plasmonic nanoparticles have been engineered. Metal nanoparticles such as gold and silver exhibit longitudinal surface plasmon resonance upon optical excitation at the resonance wavelength, and the heat generated by the movement of electrons can be used to trigger deformation in thermoresponsive nanocomposites [132]. Gold nanoparticles have been the first choice as nanoscale heating elements due to the inertness of gold in physiological

conditions, ease of surface functionalization, and high photothermal transduction efficiency [133]. Moreover, the excitation wavelength can be tuned by changing nanoparticle shape and size [133]. For example, spherical gold nanoparticles typically exhibit a single maximum absorption peak within 500-550 nm, while nanorods exhibit two maxima with the highest in the NIR range. This maxima can be tuned to values between 600 nm and 1800 nm by changing nanoparticle geometry [133], [134]. When coupled with thermoresponsive polymers, photothermal heating rapidly large forces (Figure 3). The optomechanical nanoactuator platform is an excellent example for this actuation paradigm [135]. The platform consists of nanoactuators in the form of a gold nanorod core and thermoresponsive poly(N-isopropylmethacrylamide) (pNIPMAM) shell, covalently attached to a glass substrate. When triggered by NIR light, heat is generated on the surface of gold nanorods causing the surrounding pNIPMAM layer to collapse by 50% in hydrodynamic size within milliseconds. A single nanoactuator generates 13-50 pN, as measured by a DNA fluorescent tension probe [135].

The force output of these systems can be amplified by storing elastic energy, for example, via reversible clustering of gold-pNIPAM nanoparticles [136]. Van der Waals attractions between gold cores can be very large in the collapsed polymer state, setting up a tightly compressed polymer spring which could be triggered to transition into the inflated state, delivering hundreds of nN of force on the surrounding agarose gel. An alternative strategy to increase forces applied to cells is assembling microscale actuators using nanoparticles as building blocks. Recent work has shown that gold-pNIPMAM nanoactuators could be chemically assembled into larger structures with defined shapes using droplet microfluidics and additive manufacturing techniques [137]. The resulting microactuators contracted rapidly up to 30% in length within tens of milliseconds, and the force generated by a single microactuator was on the order of several μN, which corresponds to a compressive stress of 8.1 kPa. Notably, nanocomposites of sodium alginate and gold-pNIPMAM nanoactuators exhibited tunable deformation, while arbitrarily-shaped soft actuators were printed using capillary extrusion and ionic crosslinking. This suggests that any static biomaterial could be transformed into a force generating active material system with the incorporation of photothermal nanoactuators, a feature that will allow decoupling force generation from mechanical properties of the network.

The distribution of forces at the cell-material interface can be further controlled by assembling microfabricated mechanisms with actuated hydrogels. For example, microfabricated elastomer pillars were suspended into a gold nanorod-pNIPAM nanocomposite, which collapsed and bent the pillars under 808 nm NIR exposure. Tip deformation up to 8 μm was reported as a result of the optimization of gold nanoparticle concentration [138]. Similarly, substrates with strips of gold nanorod-thermoresponsive poly(N-isopropyl acrylamide/N-ethyl acrylamide) copolymer were used to generate local stretching with displacement up to 4.3 μm [139]. Alternatively, photothermal microactuators were attached to PEGDA structures such as lever arms or gripping mechanisms to build micromanipulators capable of converting isotropic contraction of the actuator into various mechanical loading [137]. Heat generation with light is not limited to gold nanoparticles, as photothermal nanocomposites have also been developed from graphene oxide nanoparticles [140], [141], [142], [143] and carbon nanotubes [144]. Graphene nanoplatelet-PDMS

nanocomposite films were able to bend under NIR light, generating forces of tens of nN [145]. Similarly, microcapsules constructed with PEGDA/graphene oxide-pNIPAM hydrogel bilayers were reported to open and close repeatedly [143].

4. Mechanobiology using active biomaterial systems

The composition of the active biomaterial and associated activation mechanism determine the resolution and nature of the generated biomechanical signal. In this section, we discuss the applications of active biomaterial systems in mechanobiology by categorizing the techniques according to the manipulation strategy.

4.1. Manipulation of mechanotransduction associated with cell-endogenous forces

Active biomaterials with dynamically controlled elasticity have been used to study the influence of changing resistance to endogenous forces on various cellular processes. Myofibroblast activation, a biological response that is responsible for loss of tissue function during fibrosis, has been widely studied due to its clinical relevance (Figure 4). For example, one study has shown that hepatic stellate cells cultured on active MeHA hydrogel substrates respond to dynamic changes in matrix stiffness (20-fold) by spreading, changing actin fiber organization to form stress fibers of α-smooth muscle actin (α-SMA), and increasing nuclear YAP content, all indicative of myofibroblast differentiation [43]. Similar observations have been made using other active biomaterials [41], [57], [77], [88], [89], [146]. In contrast, matrix softening was reported to induce valvular myofibroblast de-activation [68].

Temporal control over biomaterial elasticity can be used to investigate mechanobiology of time-sensitive cellular process, such as lineage commitment in stem cells (Figure 4). For example, hMSCs cultured on active MeHA hydrogels were found to favor osteoblast differentiation when stiffening was activated after 1 day in culture. Osteogenic differentiation was gradually replaced by adipogenetic differentiation with delayed stiffening [42]. The response of hMSCs to matrix softening was also shown to be time-sensitive, as cytoplasmic translocation of mechanosensitive transcription factors such as YAP and RUNX2 was significantly reduced when matrix softening was delayed by 10 days [147]. Neural stem cells were reported to respond to stiffness changes within a 12-36 hour time window after adhesion to a substrate, beyond which neurogenesis was not affected by matrix properties [87]. Myoblasts cultured on reversible pH responsive hydrogels retracted when substrate stiffness was decreased, and regained their initial area upon return of the matrix to the original stiffness [93].

Active biomaterials with reversible elasticity have also been used for the study of cell migration. Indeed, cell motility has been studied using a variety of active biomaterials, including photodegradable hydrogels and on-demand stiffening matrices [67], [102], [148]. T cell migration under cyclic application of mechanical cues was investigated using 3D phytochrome-based active matrices [78]. Cells were subjected to softening/stiffening cycles of the substrate for 96 hours, and migration was found to be dependent on the duration at which the materials was kept in a soft state. Notably, active biomaterials that can generate mechanical cues in a cyclic manner allow research into how cells integrate forces over time,

and whether the response is mediated by digital switching mechanisms based on threshold values [78].

4.2. Application of exogenous generated forces on cells

The influence of the magnitude, frequency, and duration of extrinsic forces on cell behavior have been studied using actuated nanocomposites. Early work demonstrated that application of local forces on the order of 13-50 pN to fibroblasts residing on an actuated substrate increased paxilin deposition and focal adhesion organization, reinforcing the importance of force sensing via integrins and transduction into the activity of talin and vinculin (Figure 4). Further, studies have demonstrated that periodic stimulation rather than steady force application can be required to induce a particular cell response, and the mechanosensing process can be frequency dependent. For example, F-actin localization was evident between 10-100 Hz while actuation at lower frequencies did not induce any changes in the actomyosin network (Figure 4) [135]. In contrast, magnetically triggered external forces on the order of 27 nN were shown to increase focal adhesions locally, and this was enhanced by cyclic force application in fibroblasts [116]. Similarly, directional pulling has been reported to guide filipodia generation and to influence of mitotic spindle axis alignment during mitosis in HeLa cells [149].

The amplitude and duration of extrinsic force application significantly influences various other cellular responses, as demonstrated with fibroblasts cultured on photothermally activated deformable nanocomposites [139]. Cyclic stretching with 14% strain at 1 Hz frequency led to a reduction in cell migration speed, while persistence increased and the mechanosensitive myocardin related transcription factor A (MRTF-A) translocated to the nucleus after 8 hours of actuation. MRTF-A nuclear translocation decreased with decreasing laser power and was highest at 1 Hz frequency, showing that the response was dependent on both the magnitude and frequency of applied force.

A key feature of active biomaterial systems is their applicability to a wide range of size scales, from single cells to tissue scale (Figure 4). For example, the application of magnetically triggered external forces on a large population of neurons using a magnetic HA matrix led to the activation of mechanosensitive ion channels PIEZO2 and TRPV4, as quantified from the intracellular calcium influx [104]. By activating a large area, many encapsulated cells can be mechanically conditioned for guiding regenerative processes. For example, microscale magnetically actuated, cell-laden hydrogels were used to induce muscle regeneration *in vitro* under mechanically dynamic conditions [120]. Periodic stretching over 4 weeks with 40% strain for 10 hours per day enhanced myoblast differentiation, with respect to cells cultured under static conditions in a similar 3D environment. Active scaffolds were also used to apply tissue-scale forces for therapeutic purposes *in vivo* (Figure 4). For example, biphasic ferrogels were implanted to apply compressive stresses on an ischemic mouse limb, and it was shown that mechanical stimulation alone decreased inflammation and fibrosis around the damaged muscle tissue while muscle fiber size and corresponding contractile force were both significantly increased over two weeks (Figure 4) [109]. Actuation of similar magnetic scaffolds in vivo enhanced osteogenesis [105], [150] and tendon regeneration [110], [112]. As an alternative strategy, thermoresponsive hydrogel

scaffolds transplanted into mice were used to apply constant compression on embryonic dental MSCs [125]. Constant stress enhanced MSC differentiation, as demonstrated by the increase in the expression of odontogenic factors Pax9, Msx1, and Bmp4 and mineralization levels.

4.3. Key mechanobiology findings in active biomaterial systems

Temporal changes in both resistance to endogenous forces and exogenous stresses trigger distinct mechanoresponses *in vitro*. For example, myoblasts responded to externally induced matrix softening by detaching focal adhesions and retracting protrusions, eventually displaying a round morphology when cultured on 2D active biomaterials [86]. Increasing substrate stiffness *in situ* led to the re-establishment of focal adhesions, F-actin polymerization to generate stress fibers, cell spreading, and increase in cellular traction forces within minutes of external activation [93], [42]. In another work, pulling on integrins of fibroblasts with exogenous forces on the order of 13-50 pN activated talin unfolding and vinculin binding, which together led to F-actin polymerization and focal adhesion maturation within minutes [135]. Unlike sensing of stiffness, this process was independent of myosin contractility and the Rho kinase pathway. Extended stimulation led to the formation of new protrusions in the direction of force application, and within 40 minutes, migration was initiated toward the site of pulling. Similarly, locally applied constant tension above $1 nN/\mu m^{-1}$ on HeLa cells led them to form asymmetric leading-edge type filopodia, a mechanical process mediated by the protein p21-activated kinase (PAK) pathway [149].

Cytoskeletal changes are often followed closely by the nuclear translocation of mechanosensitive transcriptional regulators such as YAP/TAZ, nuclear factor of activated T cells (NFAT), and MRTF-A. For example, reduction in hMSC spreading area in response to matrix softening (from 14.8 kPa to 3.5 kPa) was followed by the translocation of YAP/TAZ to the cytoplasm [46], [128]. Increasing matrix stiffness led to nuclear YAP/TAZ localization, an observation that is consistent with data collected with static biomaterials. ECM stiffness above 5 kPa generated sufficient forces to unfold talin and bind vinculin, leading to force transmission toward the nucleus, triggering nuclear YAP translocation [151]. This mechanism likely plays a major role in cellular responses to cyclic softening/stiffening, and active biomaterials can cross the 5 kPa mechanical threshold repeatedly [88], [152], [153]. Similarly, photo-triggered matrix stiffening led to nuclear translocation of NFAT in cardiac fibroblasts within 80 minutes of biomaterial activation. Interestingly, NFAT translocation was coordinated with intracellular calcium shuttling [57]. This behavior was transient and nuclear NFAT content decreased to baseline as cells adapted to the altered tension on the cell membrane, potentially preventing further calcium uptake. Similarly, repeated exogenous force application over hours to days can activate force transmission to the nucleus via nuclear translocation of MRTF-A and YAP/TAZ [139][110]. When continued over the course of several weeks, cyclic forces influence proliferation and differentiation of adipose stem cells, cardiomyocytes, osteoblasts, and myoblasts [105], [110]-[112], [120]. On the other hand, high strains and forces applied at frequencies above 5 Hz can induce apoptosis [117], [154].

While early responses to changes in endogenous and exogenous forces are typically reversible and follow cyclic changes in ECM elasticity *in situ* [86], [89], force triggered downstream signaling events are sensitive to the timing of stimulation and display memory. Myofibroblast activation and MSC differentiation are two important examples where the timepoint of mechanical dosing can dictate long-term cell behavior. For example, muscle myoblasts that were initially allowed to spread in soft 3D matrices responded to matrix stiffening by exhibiting nuclear YAP translocation, while cells originally cultured in stiff matrices exhibited a round morphology accompanied by decreasing nuclear YAP content [71]. Similarly, upregulation of α-SMA in cardiac fibroblasts and hepatic stellate cells was highest when stiffening was triggered in later stages of cell culture [43], [146]. In the case of neuronal stem cells, neurogenesis increased with matrix softening within the first 3 days of cell culture, beyond which β-III tubulin production was unaffected by changes in ECM elasticity [87]. Interestingly, YAP suppressed neurogenesis through cytosolic interactions by co-precipitating β-catenin. hMSCs underwent predominantly adipogenic differentiation correlated with the duration of culture in soft matrices, while early matrix stiffening promoted osteogenic differentiation [42]. Moreover, hMSCs possessed mechanical memory of previous culture conditions and this memory was mediated through YAP/TAZ shuttling [147]. hMSCs cultured on stiff matrices showed decreasingly less YAP localization in the cytoplasm when matrix softening was delayed up to 10 days, after which YAP shuttling was no longer influenced by elasticity changes. The osteogenic transcription factor RUNX2 followed a similar trend, supporting the observation that MSC differentiation is time sensitive [147]. These findings suggest that cells may need a recovery phase following isolation and active materials which allow *in situ* mechanotransduction observations are likely more suited to investigate temporal processes.

However, future advances resulting from the use of active biomaterials in mechanobiology research will likely depend on better mechanical characterization of these systems in 3D. For example, the effect of changing crosslinking density in dynamic systems on network porosity and ligand density needs further investigation. This is of particular importance in 3D multicellular scaffolds to avoid unappreciated synergistic interactions of different matrix properties which may impact the clarity of research findings. Similarly, matrix stiffness and force generation should be physically decoupled from each other in exogenous stress applying biomaterials. In these material systems, activated nanoparticles may stretch polymer chains during contraction, which can influence matrix elasticity temporarily, and more importantly, these interactions may lead to plastic deformation over extended episodes of actuation. An ideal active biomaterial system is expected to either modulate resistance to cellular endogenous forces or apply exogenous stresses to cells, but not both simultaneously. Moreover, systematic studies on force dissipation in active biomaterial matrices is necessary and could benefit from the adaptation of existing methods for measuring stresses within living tissues.

5. Conclusions and future directions

Active biomaterials that can manipulate resistance to cell-endogenous stresses or apply exogenous forces in temporally controlled manner have allowed unprecedent capabilities to investigate mechanotransduction. Photosensitive and magnetically triggered strategies have

gained significant attention due to their excellent control over the exact timepoint of mechanical activation and tunable force parameters. New insight into the effects of force magnitude, frequency, and duration on cellular decision making has been acquired. The implementation of macroscale magnetic scaffolds in vivo has led to the development of therapies targeting tissue regeneration applications.

However, there are a number of challenges that must be addressed before active biomaterials can be widely used to apply forces for the study of multicellular organization inside 3D fibrous tissues. Mechanotransduction and associated responses take place at the molecular, cellular and multicellular scales, and at a wide range of timescales, from milliseconds to minutes to days [155]. Finding a material system that can address specifications associated with such a broad range of size and time scales is one of the outstanding challenges. Secondly, the actuated elements must co-exist with cells in a minimally invasive manner yet they must generate physiologically relevant signals. For example, a discrete actuator that is significantly softer or stiffer than the surrounding matrix may present static cues that interfere with the dynamic signals. In addition, extended durations of actuation must not lead to excessive heating or release of toxic chemicals due to corrosion. Probes fabricated in the form of thermoresponsive hydrogel beads and ferrofluid droplets opened the doors for the application of local forces in a 3D setting [122], [126], [137], [156]. They already revealed important insights on morphogenesis by reporting mechanical properties of developing tissues inside zebrafish embryos [123]. However, in the existing protocols, these actuators are randomly distributed inside the target tissue. Ideally, the platform is expected to give the scientist the option to apply forces at the desired location with desired waveforms. We anticipate that composite materials that simultaneously transduce multiple different activation stimuli will address some of these challenges.

Moreover, a more streamlined calibration protocol is required to be able compare the mechanical loading induced by different techniques and choose the material formulation that serves best for the chosen scientific problem. To this end, there is a dire need for technologies to map stresses in 3D during the application of exogenous forces at the cellular and subcellular resolutions. 3D traction force microscopy and inclusions such as oil droplets and hydrogel beads with calibrated mechanical properties are exciting developments in the field [122], [156]-[161]. A further complication in the quantification process is the continuous remodeling of ECM by the resident cells. Considering the nonlinear properties of collagen networks and other biological gels, an accompanying computational model may become instrumental for decrypting the collected data. A number of recent reports presented continuum formulations and lattice-based fiber network models for the simulation of force transmission inside fibrous tissues [162]-[164]. A closer collaboration among experts in computational mechanics, materials science, and experimental biomechanics will be essential to develop the toolkit for spatiotemporally control stress distribution inside active biomaterials.

The field of active biomaterials is expected to rapidly evolve as new platforms are engineered with emerging technologies, and applied to a diverse pool of scientific questions. The adaptation of microfluidic systems together with state-of-the-art machine learning tools will likely lead to high-throughput strategies for rapid analysis of cell behavior under

dynamic conditions. Future lab-on-a chip devices may act as diagnostic tools for personalized medicine while functioning as pharmaceutical discovery platforms. Combined with organoids, active biomaterials will likely generate crucial insight on developmental biology and oncology, and help discover effective therapies. We expect to see many exciting developments in the near future.

References

[1]. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SFT, Csiszar K, Giaccia A, Weninger W, Yamauchi M, Gasser DL, and Weaver VM, "Matrix Crosslinking Forces Tumor Progression by Enhancing Integrin Signaling," Cell, vol. 139, no. 5, pp. 891–906, 2009, doi: 10.1016/j.cell.2009.10.027. [PubMed: 19931152]

[2]. Wirtz D, Konstantopoulos K, and Searson PC, "The physics of cancer : the role of physical interactions and mechanical forces in metastasis," Nat. Publ. Gr, vol. 11, no. 7, pp. 512–522, 2011, doi: 10.1038/nrc3080.

[3]. Chaudhuri O, Koshy ST, Branco da Cunha C, Shin J-W, Verbeke CS, Allison KH, and Mooney DJ, "Extracellular matrix stiffness and composition jointly regulate the induction of malignant phenotypes in mammary epithelium," Nat. Mater, vol. 13, pp. 1–35, 2014, doi: 10.1038/nmat4009. [PubMed: 24343503]

[4]. Hadden WJ, Young JL, Holle AW, McFetridge ML, Kim DY, Wijesinghe P, Taylor-Weiner H, Wen JH, Lee AR, Bieback K, Vo BN, Sampson DD, Kennedy BF, Spatz JP, Engler AJ, and Cho YS, "Stem cell migration and mechanotransduction on linear stiffness gradient hydrogels," Proc. Natl. Acad. Sci. U. S. A, vol. 114, no. 22, pp. 5647–5652, 2017, doi: 10.1073/pnas.1618239114. [PubMed: 28507138]

[5]. Gjorevski N, Sachs N, Manfrin A, Giger S, Bragina ME, Ordóñez-Morán P, Clevers H, and Lutolf MP, "Designer matrices for intestinal stem cell and organoid culture," Nature, vol. 539, no. 7630, pp. 560–564, 2016, doi: 10.1038/nature20168. [PubMed: 27851739]

[6]. Chaudhuri O, Gu L, Klumpers D, Darnell M, Bencherif SA, Weaver JC, Huebsch N, Lee H-P, Lippens E, Duda GN, and Mooney DJ, "Hydrogels with tunable stress relaxation regulate stem cell fate and activity," Nat. Mater, vol. 15, pp. 326–334, 2016, doi: 10.1038/nmat4489. [PubMed: 26618884]

[7]. Shao Y and Fu J, "Integrated micro/nanoengineered functional biomaterials for cell mechanics and mechanobiology: A materials perspective," Adv. Mater, vol. 26, no. 10, pp. 1494–1533, 2014, doi: 10.1002/adma.201304431. [PubMed: 24339188]

[8]. Baker BM, Trappmann B, Wang WY, Sakar MS, Kim IL, Shenoy VB, Burdick JA, and Chen CS, "Cell-mediated fibre recruitment drives extracellular matrix mechanosensing in engineered fibrillar microenvironments," Nat. Mater, vol. 14, no. 12, pp. 1262–1268, 2015, doi: 10.1038/nmat4444. [PubMed: 26461445]

[9]. Lo CM, Wang HB, Dembo M, and Wang YL, "Cell movement is guided by the rigidity of the substrate.," Biophys. J, vol. 79, no. 1, pp. 144–152, 2000, doi: 10.1016/S0006-3495(00)76279-5. [PubMed: 10866943]

[10]. Sunyer R, Conte V, Escribano J, Elosegui-artola A, Labernadie A, Valon L, Navajas D, and García-aznar JM, "Collective cell durotaxis emerges from long-range intercellular force transmission," vol. 353, no. 6304, pp. 1157–1162, 2016, doi: 10.5061/dryad.r8h3n.

[11]. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D, Hammer DA, and Weaver VM, "Tensional homeostasis and the malignant phenotype," Cancer Cell, vol. 8, no. 3, pp. 241–254, 2005, doi: 10.1016/j.ccr.2005.08.010. [PubMed: 16169468]

[12]. Leight JL, Drain AP, and Weaver VM, "Extracellular matrix remodeling and stiffening modulate tumor phenotype and treatment response," Annu. Rev. Cancer Biol, vol. 1, no. 1, pp. 313–334, 2017, doi: 10.1146/annurev-cancerbio-050216-034431.

[13]. Shih YRV, Tseng KF, Lai HY, Lin CH, and Lee OK, "Matrix stiffness regulation of integrin-mediated mechanotransduction during osteogenic differentiation of human mesenchymal stem

cells," J. Bone Miner. Res, vol. 26, no. 4, pp. 730–738, 2011, doi: 10.1002/jbmr.278. [PubMed: 20939067]

[14]. Mao AS, Shin JW, and Mooney DJ, "Effects of substrate stiffness and cell-cell contact on mesenchymal stem cell differentiation," Biomaterials, vol. 98, pp. 184–191, 2016, doi: 10.1016/j.biomaterials.2016.05.004. [PubMed: 27203745]

[15]. Huebsch N, Lippens E, Lee K, Mehta M, Koshy ST, Darnell MC, Desai RM, Madl CM, Xu M, Zhao X, Chaudhuri O, Verbeke C, Kim WS, Alim K, Mammoto A, Ingber DE, Duda GN, and Mooney DJ, "Matrix elasticity of void-forming hydrogels controls transplanted-stem-cell-mediated bone formation," Nat. Mater, vol. 14, no. 12, pp. 1269–1277, 2015, doi: 10.1038/nmat4407. [PubMed: 26366848]

[16]. Engler AJ, Sen S, Sweeney HL, and Discher DE, "Matrix elasticity directs stem cell lineage specification," Cell, vol. 126, no. 4, pp. 677–689, 2006, doi: 10.1016/j.cell.2006.06.044. [PubMed: 16923388]

[17]. Hochmuth RM, "Micropipette aspiration of living cells," J. Biomech, vol. 33, no. 1, pp. 15–22, 2000, doi: 10.1016/S0021-9290(99)00175-X. [PubMed: 10609514]

[18]. Zhang H and Liu KK, "Optical tweezers for single cells," J. R. Soc. Interface, vol. 5, no. 24, pp. 671–690, 2008, doi: 10.1098/rsif.2008.0052. [PubMed: 18381254]

[19]. Wang N and Ingber DE, "Probing transmembrane mechanical coupling and cytomechanics using magnetic twisting cytometry.," Biochem. Cell Biol, vol. 73, no. 7–8, pp. 327–335, 1995, doi: 10.1139/o95-041. [PubMed: 8703406]

[20]. Elosegui-Artola A, Andreu I, Beedle AEM, Lezamiz A, Uroz M, Kosmalska AJ, Oria R, Kechagia JZ, Rico-Lastres P, Le Roux AL, Shanahan CM, Trepat X, Navajas D, Garcia-Manyes S, and Roca-Cusachs P, "Force triggers YAP nuclear entry by regulating transport across nuclear pores," Cell, vol. 171, no. 6, pp. 1397–1410, 2017, doi: 10.1016/j.cell.2017.10.008. [PubMed: 29107331]

[21]. Kurth F, Eyer K, Franco-Obregón A, and Dittrich PS, "A new mechanobiological era: Microfluidic pathways to apply and sense forces at the cellular level," Curr. Opin. Chem. Biol, vol. 16, no. 3–4, pp. 400–408, 2012, doi: 10.1016/j.cbpa.2012.03.014. [PubMed: 22525494]

[22]. Kim D-H, Wong PK, Park J, Levchenko A, and Sun Y, "Microengineered platforms for cell mechanobiology," Annu. Rev. Biomed. Eng, vol. 11, no. 1, pp. 203–233, 2009, doi: 10.1146/annurev-bioeng-061008-124915. [PubMed: 19400708]

[23]. Siedlik MJ, Varner VD, and Nelson CM, "Pushing, pulling, and squeezing our way to understanding mechanotransduction," Methods, vol. 94, pp. 4–12, 2016, doi: 10.1016/j.ymeth.2015.08.019. [PubMed: 26318086]

[24]. Pinheiro D and Bellaïche Y, "Mechanical force-driven adherens junction remodeling and epithelial dynamics," Dev. Cell, vol. 47, no. 1, pp. 3–19, 2018, doi: 10.1016/j.devcel.2018.09.014. [PubMed: 30300588]

[25]. Iskratsch T, Wolfenson H, and Sheetz MP, "Appreciating force and shape—the rise of mechanotransduction in cell biology," Nat. Rev. Mol. Cell Biol, vol. 15, no. 12, pp. 825–33, 2014, doi: 10.1038/nrm3903. [PubMed: 25355507]

[26]. Vogel V and Sheetz M, "Local force and geometry sensing regulate cell functions," Nat. Rev. Mol. Cell Biol, vol. 7, no. 4, pp. 265–275, 2006, doi: 10.1038/nrm1890. [PubMed: 16607289]

[27]. Choquet D, Felsenfeld DP, and Sheetz MP, "Extracellular matrix rigidity causes strengthening of integrin- cytoskeleton linkages," Cell, vol. 88, no. 1, pp. 39–48, 1997, doi: 10.1016/S0092-8674(00)81856-5. [PubMed: 9019403]

[28]. Roca-Cusachs P, Iskratsch T, and Sheetz MP, "Finding the weakest link – exploring integrin-mediated mechanical molecular pathways," J. Cell Sci, vol. 125, no. 13, pp. 3025–3038, 2012, doi: 10.1242/jcs.095794. [PubMed: 22797926]

[29]. Wang N, Butler JP, and Ingber DE, "Mechanotransduction across the cell surface and through the cytoskeleton," Science, vol. 260, no. 5111, pp. 1124–1127, 1993, doi: 10.1126/science.7684161. [PubMed: 7684161]

[30]. Giannone G, Jiang G, Sutton DH, Critchley DR, and Sheetz MP, "Talin1 is critical for force-dependent reinforcement of initial integrin-cytoskeleton bonds but not tyrosine kinase

activation," J. Cell Biol, vol. 163, no. 2, pp. 409–419, 2003, doi: 10.1083/jcb.200302001. [PubMed: 14581461]

[31]. Hughes S, McBain S, Dobson J, and El Haj AJ, "Selective activation of mechanosensitive ion channels using magnetic particles," J. R. Soc. Interface, vol. 5, no. 25, pp. 855–863, 2008, doi: 10.1098/rsif.2007.1274. [PubMed: 18077244]

[32]. Brusatin G PS, Panciera t, Gandin A, Citron A, "Biomaterials and engineered microenvironments to control YAP/TAZ-dependent cell behavior," Nat. Mater, vol. 17, pp. 1063–1075, 2018, doi: 10.5281/zenodo.1000462.RES. [PubMed: 30374202]

[33]. Pardo-Pastor C, Rubio-Moscardo F, Vogel-González M, Serra SA, Afthinos A, Mrkonjic S, Destaing O, Abenza JF, Fernández-Fernández JM, Trepat X, Albiges-Rizo C, Konstantopoulos K, and Valverde MA, "Piezo2 channel regulates RhoA and actin cytoskeleton to promote cell mechanobiological responses," Proc. Natl. Acad. Sci. U. S. A, vol. 115, no. 8, pp. 1925–1930, 2018, doi: 10.1073/pnas.1718177115. [PubMed: 29432180]

[34]. Benham-Pyle BW, Pruitt BL, and Nelson WJ, "Mechanical strain induces E-cadherin-dependent Yap1 and β-catenin activation to drive cell cycle entry," Science, vol. 348, no. 6238, pp. 1024–1027, 2015, doi: 10.1126/science.aaa4559. [PubMed: 26023140]

[35]. Gilbert HTJ, Mallikarjun V, Dobre O, Jackson MR, Pedley R, Gilmore AP, Richardson SM, and Swift J, "Nuclear decoupling is part of a rapid protein-level cellular response to high-intensity mechanical loading," Nat. Commun, vol. 10, no. 1, pp. 1–15, 2019, doi: 10.1038/s41467-019-11923-1. [PubMed: 30602773]

[36]. Cui Y, Hameed FM, Yang B, Lee K, Pan CQ, Park S, and Sheetz M, "Cyclic stretching of soft substrates induces spreading and growth," Nat. Commun, vol. 6, pp. 1–8, 2015, doi: 10.1038/ncomms7333.

[37]. Tse JM, Cheng G, Tyrrell JA, Wilcox-Adelman SA, Boucher Y, Jain RK, and Munn LL, "Mechanical compression drives cancer cells toward invasive phenotype," Proc. Natl. Acad. Sci. U. S. A, vol. 109, no. 3, pp. 911–916, 2012, doi: 10.1073/pnas.1118910109. [PubMed: 22203958]

[38]. Wan J, Ristenpart WD, and Stone HA, "Dynamics of shear-induced ATP release from red blood cells," Proc. Natl. Acad. Sci. U. S. A, vol. 105, no. 43, pp. 16432–16437, 2008, doi: 10.1073/pnas.0805779105. [PubMed: 18922780]

[39]. Li J and Mooney DJ, "Designing hydrogels for controlled drug delivery," Nat. Rev. Mater, vol. 1, no. 12, 2016, doi: 10.1038/natrevmats.2016.71.

[40]. Zhao H, Sterner ES, Coughlin EB, and Theato P, "O-Nitrobenzyl alcohol derivatives: Opportunities in polymer and materials science," Macromolecules, vol. 45, no. 4, pp. 1723–1736, 2012, doi: 10.1021/ma201924h.

[41]. Ondeck MG and Engler AJ, "Mechanical characterization of a dynamic and tunable methacrylated hyaluronic acid hydrogel," J. Biomech. Eng, vol. 138, no. 2, pp. 1–6, 2016, doi: 10.1115/1.4032429.

[42]. Guvendiren M and Burdick JA, "Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics," Nat. Commun, vol. 3, 2012, doi: 10.1038/ncomms1792.

[43]. Caliari SR, Perepelyuk M, Cosgrove BD, Tsai SJ, Lee GY, Mauck RL, Wells RG, and Burdick JA, "Stiffening hydrogels for investigating the dynamics of hepatic stellate cell mechanotransduction during myofibroblast activation," Sci. Rep, vol. 6, no. 2, pp. 1–10, 2016, doi: 10.1038/srep21387. [PubMed: 28442746]

[44]. Brown TE, Carberry BJ, Worrell BT, Dudaryeva OY, McBride MK, Bowman CN, and Anseth KS, "Photopolymerized dynamic hydrogels with tunable viscoelastic properties through thioester exchange," Biomaterials, vol. 178, pp. 496–503, 2018, doi: 10.1016/j.biomaterials.2018.03.060. [PubMed: 29653871]

[45]. Yeh YC, Corbin EA, Caliari SR, Ouyang L, Vega SL, Truitt R, Han L, Margulies KB, and Burdick JA, "Mechanically dynamic PDMS substrates to investigate changing cell environments," Biomaterials, vol. 145, pp. 23–32, 2017, doi: 10.1016/j.biomaterials.2017.08.033. [PubMed: 28843064]

[46]. Rosales AM, Vega SL, DelRio FW, Burdick JA, and Anseth KS, "Hydrogels with reversible mechanics to probe dynamic cell microenvironments," Angew. Chemie - Int. Ed, vol. 56, no. 40, pp. 12132–12136, 2017, doi: 10.1002/anie.201705684.

[47]. Lee IN, Dobre O, Richards D, Ballestrem C, Curran JM, Hunt JA, Richardson SM, Swift J, and Wong LS, "Photoresponsive hydrogels with photoswitchable mechanical properties allow time-resolved analysis of cellular responses to matrix stiffening," ACS Appl. Mater. Interfaces, vol. 10, no. 9, pp. 7765–7776, 2018, doi: 10.1021/acsami.7b18302. [PubMed: 29430919]

[48]. Rosales AM, Mabry KM, Nehls EM, and Anseth KS, "Photoresponsive elastic properties of azobenzene-containing poly(ethylene-glycol)-based hydrogels," Biomacromolecules, vol. 16, no. 3, pp. 798–806, 2015, doi: 10.1021/bm501710e. [PubMed: 25629423]

[49]. Tabet A, Forster RA, Parkins CC, Wu G, and Scherman OA, "Modulating stiffness with photo-switchable supramolecular hydrogels," Polym. Chem, vol. 10, no. 4, pp. 467–472, 2019, doi: 10.1039/c8py01554f.

[50]. Truong VX, Li F, Ercole F, and Forsythe JS, "Wavelength-selective coupling and decoupling of polymer chains via reversible [2 + 2] photocycloaddition of styrylpyrene for construction of cytocompatible photodynamic hydrogels," ACS Macro Lett, vol. 7, no. 4, pp. 464–469, 2018, doi: 10.1021/acsmacrolett.8b00099.

[51]. Zhao F, Bonasera A, Nöchel U, Behl M, and Bléger D, "Reversible modulation of elasticity in fluoroazobenzene-containing hydrogels using green and blue light," Macromol. Rapid Commun, vol. 39, no. 1, pp. 1–5, 2018, doi: 10.1002/marc.201700527.

[52]. Pennacchio FA, Fedele C, De Martino S, Cavalli S, Vecchione R, and Netti PA, "Three-dimensional microstructured azobenzene-containing gelatin as a photoactuable cell confining system," ACS Appl. Mater. Interfaces, vol. 10, no. 1, pp. 91–97, 2018, doi: 10.1021/acsami.7b13176. [PubMed: 29260543]

[53]. Tamesue S, Takashima Y, Yamaguchi H, Shinkai S, and Harada A, "Photoswitchable supramolecular hydrogels formed by cyclodextrins and azobenzene polymers," Angew. Chemie - Int. Ed, vol. 49, no. 41, pp. 7461–7464, 2010, doi: 10.1002/anie.201003567.

[54]. Zhao YL and Fraser Stoddart J, "Azobenzene-based light-responsive hydrogel system," Langmuir, vol. 25, no. 15, pp. 8442–8446, 2009, doi: 10.1021/la804316u. [PubMed: 20050041]

[55]. Wang D, Wagner M, Butt HJ, and Wu S, "Supramolecular hydrogels constructed by red-light-responsive host-guest interactions for photo-controlled protein release in deep tissue," Soft Matter, vol. 11, no. 38, pp. 7656–7662, 2015, doi: 10.1039/c5sm01888a. [PubMed: 26292617]

[56]. Rosales AM, Rodell CB, Chen MH, Morrow MG, Anseth KS, and Burdick JA, "Reversible control of network properties in azobenzene-containing hyaluronic acid-based hydrogels," Bioconjug. Chem, vol. 29, no. 4, pp. 905–913, 2018 doi: 10.1021/acs.bioconjchem.7b00802. [PubMed: 29406696]

[57]. Günay KA, Ceccato TL, Silver JS, Bannister KL, Bednarski OJ, Leinwand LA, and Anseth KS, "PEG-anthracene hydrogels as an on-demand stiffening matrix to study mechanobiology," Angew. Chemie, vol. 131, no. 29, pp. 10017–10021, 2019, doi: 10.1002/ange.201901989.

[58]. Truong VX, Li F, and Forsythe JS, "Versatile bioorthogonal hydrogel platform by catalyst-free visible light initiated photodimerization of anthracene," ACS Macro Lett., vol. 6, no. 7, pp. 657–662, 2017, doi: 10.1021/acsmacrolett.7b00312.

[59]. Rosales AM and Anseth KS, "The design of reversible hydrogels to capture extracellular matrix dynamics," Nat. Rev. Mater, vol. 1, no. 2, pp. 1–15, 2016, doi: 10.1038/natrevmats.2015.12.

[60]. Levalley PJ and Kloxin AM, "Chemical approaches to dynamically modulate the properties of synthetic matrices," ACS Macro Lett., vol. 8, no. 1, pp. 7–16, 2019, doi: 10.1021/acsmacrolett.8b00808. [PubMed: 32405440]

[61]. Zhang YS and Khademhosseini A, "Advances in engineering hydrogels," Science, vol. 356, no. 6337, 2017, doi: 10.1126/science.aaf3627.

[62]. Li L, Scheiger JM, and Levkin PA, "Design and applications of photoresponsive hydrogels," Adv. Mater, vol. 31, no. 26, 2019, doi: 10.1002/adma.201807333.

[63]. Ruskowitz ER and Deforest CA, "Photoresponsive biomaterials for targeted drug delivery and 4D cell culture," Nat. Rev. Mater, vol. 3, 2018, doi: 10.1038/natrevmats.2017.87.

[64]. K. Hand Das C TJ, Lucia MS, “Dynamically tunable cell culture platforms for tissue engineering and mechanobiology,” Prog. Polym. Sci, vol. 65, pp. 53–82, 2017, doi: 10.1016/j.physbeh.2017.03.040. [PubMed: 28522885]

[65]. Kloxin AM, Kasko AM, Salinas CN, and Anseth KS, “Photodegradable hydrogels for dynamic tuning of physical and chemical properties,”, vol. 324, no. 3, pp. 59–63, 2009.

[66]. Käpylä E, Delgado SM, and Kasko AM, “Shape-changing photodegradable hydrogels for dynamic 3D cell culture,” ACS Appl. Mater. Interfaces, vol. 8, no. 28, pp. 17885–17893, 2016, doi: 10.1021/acsami.6b05527. [PubMed: 27322508]

[67]. DeForest CA and Anseth KS, “Cytocompatible click-based hydrogels with dynamically tunable properties through orthogonal photoconjugation and photocleavage reactions,” Nat. Chem, vol. 3, no. 12, pp. 925–931, 2011, doi: 10.1038/nchem.1174. [PubMed: 22109271]

[68]. Wang H, Haeger SM, Kloxin AM, Leinwand LA, and Anseth KS, “Redirecting valvular myofibroblasts into dormant fibroblasts through light-mediated reduction in substrate modulus,” PLoS One, vol. 7, no. 7, 2012, doi: 10.1371/journal.pone.0039969.

[69]. Peng K, Tomatsu I, Van Den Broek B, Cui C, Korobko AV, Van Noort J, Meijer AH, Spaink HP, and Kros A, “Dextran based photodegradable hydrogels formed via a Michael addition,” Soft Matter, vol. 7, no. 10, pp. 4881–4887, 2011, doi: 10.1039/c1sm05291h.

[70]. Truong VX, Tsang KM, Simon GP, Boyd RL, Evans RA, Thissen H, and Forsythe JS, “Photodegradable gelatin-based hydrogels prepared by bioorthogonal click chemistry for cell encapsulation and release,” Biomacromolecules, vol. 16, no. 7, pp. 2246–2253, 2015, doi: 10.1021/acs.biomac.5b00706. [PubMed: 26056855]

[71]. Brown TE, Silver JS, Worrell BT, Marozas IA, Yavitt FM, Günay KA, Bowman CN, and Anseth KS, “Secondary photocrosslinking of click hydrogels to probe myoblast mechanotransduction in three dimensions,” J. Am. Chem. Soc, vol. 140, no. 37, pp. 11585–11588, 2018, doi: 10.1021/jacs.8b07551. [PubMed: 30183266]

[72]. Keating M, Lim M, Hu Q, and Botvinick E, “Selective stiffening of fibrin hydrogels with micron resolution via photocrosslinking,” Acta Biomater, vol. 87, pp. 88–96, 2019, doi: 10.1016/j.actbio.2019.01.034. [PubMed: 30660778]

[73]. Hui E, Gimeno KI, Guan G, and Caliari SR, “Spatiotemporal control of viscoelasticity in phototunable hyaluronic acid hydrogels,” Biomacromolecules, vol. 20, no. 11, pp. 4126–4134, 2019, doi: 10.1021/acs.biomac.9b00965. [PubMed: 31600072]

[74]. Baaske J, Mühlhäuser WWD, Yousefi OS, Zanner S, Radziwill G, Hörner M, Schamel WWA, and Weber W, “Optogenetic control of integrin-matrix interaction,” Commun. Biol, vol. 2, no. 1, pp. 1–8, 2019, doi: 10.1038/s42003-018-0264-7. [PubMed: 30740537]

[75]. Shadish JA, Strange AC, and Deforest CA, “Genetically encoded photocleavable linkers for patterned protein release from biomaterials,” J. Am. Chem. Soc, vol. 141, no. 39, pp. 15619–15625, 2019, doi: 10.1021/jacs.9b07239. [PubMed: 31525979]

[76]. Wang R, Yang Z, Luo J, Hsing I, and Sun F, “B12-dependent photoresponsive protein hydrogels for controlled stem cell/protein release,” Proc. Natl. Acad. Sci, vol. 114, no. 23, pp. 5912–5917, 2017, doi: 10.1073/pnas.1621350114. [PubMed: 28533376]

[77]. Liu L, Shadish JA, Arakawa CK, Shi K, Davis J, and DeForest CA, “Cyclic stiffness modulation of cell-laden protein-polymer hydrogels in response to user-specified stimuli including light,” Adv. Biosyst, vol. 2, no. 12, pp. 1–9, 2018, doi: 10.1002/adbi.201800240.

[78]. Hörner M et al., “Phytochrome-based extracellular matrix with reversibly tunable mechanical properties,” Adv. Mater, vol. 31, no. 12, pp. 1–11, 2019, doi: 10.1002/adma.201806727.

[79]. Wu X, Huang W, Wu WH, Xue B, Xiang D, Li Y, Qin M, Sun F, Wang W, Bin Zhang W, and Cao Y, “Reversible hydrogels with tunable mechanical properties for optically controlling cell migration,” Nano Res., vol. 11, no. 10, pp. 5556–5565, 2018, doi: 10.1007/s12274-017-1890-y.

[80]. Hahn MS, Miller JS, and West JL, “Three-dimensional biochemical and biomechanical patterning of hydrogels for guiding cell behavior,” Adv. Mater, vol. 18, no. 20, pp. 2679–2684, 2006, doi: 10.1002/adma.200600647.

[81]. Gillette BM, Jensen JA, Wang M, Tchao J, and Sia SK, “Dynamic hydrogels: Switching of 3D microenvironments using two-component naturally derived extracellular matrices,” Adv. Mater, vol. 22, no. 6, pp. 686–691, 2010, doi: 10.1002/adma.200902265. [PubMed: 20217770]

[82]. Stowers RS, Allen SC, and Suggs LJ, "Dynamic phototuning of 3D hydrogel stiffness," Proc. Natl. Acad. Sci. U. S. A, vol. 112, no. 7, pp. 1953–1958, 2015, doi: 10.1073/pnas.1421897112. [PubMed: 25646417]

[83]. Lee KY and Mooney DJ, "Alginate: properties and biomedical applications," Prog Polym Sci, vol. 37, no. 1, pp. 106–126, 2013, doi: 10.1016/j.progpolymsci.2011.06.003.Alginate.

[84]. Desai RM, Koshy ST, Hilderbrand SA, Mooney DJ, and Joshi NS, "Versatile click alginate hydrogels crosslinked via tetrazine--norbornene chemistry," Biomaterials, vol. 50, pp. 30–37, 2015, doi: 10.1016/j.biomaterials.2015.01.048. [PubMed: 25736493]

[85]. Shih H and Lin CC, "Tuning stiffness of cell-laden hydrogel: Via host-guest interactions," J. Mater. Chem. B, vol. 4, no. 29, pp. 4969–4974, 2016, doi: 10.1039/c6tb00890a. [PubMed: 32264023]

[86]. Hörning M, Nakahata M, Linke P, Yamamoto A, Veschgini M, Kaufmann S, Takashima Y, Harada A, and Tanaka M, "Dynamic mechano-regulation of myoblast cells on supramolecular hydrogels cross-linked by reversible host-guest interactions," Sci. Rep, vol. 7, no. 1, pp. 1–3, 2017, doi: 10.1038/s41598-017-07934-x. [PubMed: 28127051]

[87]. Rammensee S, Kang MS, Georgiu K, Kumar S, and Schaffer DV, "Dynamics of mechanosensitive neural stem cell differentiation," Stem Cells, vol. 35, pp. 497–506, 2017, doi: 10.1634/stem-cells.2005-0640. [PubMed: 27573749]

[88]. Jiang FX, Yurke B, Schloss RS, Firestein BL, and Langrana NA, "The relationship between fibroblast growth and the dynamic stiffnesses of a DNA crosslinked hydrogel," Biomaterials, vol. 31, no. 6, pp. 1199–1212, 2010, doi: 10.1016/j.biomaterials.2009.10.050. [PubMed: 19931905]

[89]. Fu L, Haage A, Kong N, Tanentzapf G, and Li H, "Dynamic protein hydrogels with reversibly tunable stiffness regulate human lung fibroblast spreading reversibly," Chem. Commun, vol. 55, no. 36, pp. 5235–5238, 2019, doi: 10.1039/c9cc01276a.

[90]. Liu HY, Greene T, Lin TY, Dawes CS, Korc M, and Lin CC, "Enzyme-mediated stiffening hydrogels for probing activation of pancreatic stellate cells," Acta Biomater., vol. 48, pp. 258–269, 2017, doi: 10.1016/j.actbio.2016.10.027. [PubMed: 27769941]

[91]. Liu HY, Korc M, and Lin CC, "Biomimetic and enzyme-responsive dynamic hydrogels for studying cell-matrix interactions in pancreatic ductal adenocarcinoma," Biomaterials, vol. 160, pp. 24–36, 2018, doi: 10.1016/j.biomaterials.2018.01.012. [PubMed: 29353105]

[92]. Arkenberg MR, Moore DM, and Lin CC, "Dynamic control of hydrogel crosslinking via sortase-mediated reversible transpeptidation," Acta Biomater, vol. 83, pp. 83–95, 2019, doi: 10.1016/j.actbio.2018.11.011. [PubMed: 30415064]

[93]. Yoshikawa HY, Rossetti FF, Kaufmann S, Kaindl T, Madsen J, Engel U, Lewis AL, Armes SP, and Tanaka M, "Quantitative evaluation of mechanosensing of cells on dynamically tunable hydrogels," J. Am. Chem. Soc, vol. 133, no. 5, pp. 1367–1374, 2011, doi: 10.1021/ja1060615. [PubMed: 21218794]

[94]. Guo W, Lu CH, Qi XJ, Orbach R, Fadeev M, Yang HH, and Willner I, "Switchable bifunctional stimuli-triggered poly-N-isopropylacrylamide/DNA hydrogels," Angew. Chemie - Int. Ed, vol. 53, no. 38, pp. 10134–10138, 2014, doi: 10.1002/anie.201405692.

[95]. Huebsch N, Kearney CJ, Zhao X, Kim J, Cezar CA, Suo Z, and Mooney DJ, "Ultrasound-triggered disruption and self-healing of reversibly cross-linked hydrogels for drug delivery and enhanced chemotherapy," Proc. Natl. Acad. Sci. U. S. A, vol. 111, no. 27, pp. 9762–9767, 2014, doi: 10.1073/pnas.1405469111. [PubMed: 24961369]

[96]. Kennedy S, Hu J, Kearney C, Skaat H, Gu L, Gentili M, Vandenburgh H, and Mooney D, "Sequential release of nanoparticle payloads from ultrasonically burstable capsules," Biomaterials, vol. 75, pp. 91–101, 2016, doi: 10.1016/j.biomaterials.2015.10.008. [PubMed: 26496382]

[97]. Moncion A, Arlotta KJ, O'Neill EG, Lin M, Mohr LA, Franceschi RT, Kripfgans OD, Putnam AJ, and Fabiilli ML, "In vitro and in vivo assessment of controlled release and degradation of acoustically responsive scaffolds," Acta Biomater, vol. 46, pp. 221–233, 2016, doi: 10.1016/j.actbio.2016.09.026. [PubMed: 27686040]

[98]. Kearney CJ, Skaat H, Kennedy SM, Hu J, Darnell M, Raimondo TM, and Mooney DJ, "Switchable release of entrapped nanoparticles from alginate hydrogels," Adv. Healthc. Mater, vol. 4, no. 11, pp. 1634–1639, 2015, doi: 10.1002/adhm.201500254. [PubMed: 26044285]

[99]. Moncion A, Lin M, O'Neill EG, Franceschi RT, Kripfgans OD, Putnam AJ, and Fabiilli ML, "Controlled release of basic fibroblast growth factor for angiogenesis using acoustically-responsive scaffolds," Biomaterials, vol. 140, pp. 26–36, 2017, doi: 10.1016/j.biomaterials.2017.06.012. [PubMed: 28624705]

[100]. Noguchi S and Takaomi K, "Ultrasound response of viscoelastic changes of cellulose hydrogels triggered with Sono-deviced rheometer," Ultrason. - Sonochemistry, vol. 67, p. 105143, 2020.

[101]. Gibaud T, Dagès N, Lidon P, Jung G, Ahouré LC, Sztucki M, Poulesquen A, Hengl N, Pignon F, and Manneville S, "Rheoacoustic gels: Tuning mechanical and flow properties of colloidal gels with ultrasonic vibrations," Phys. Rev. X, vol. 10, no. 1, pp. 1–21, 2020, doi: 10.1103/PhysRevX.10.011028.

[102]. Valentin TM, Leggett SE, Chen PY, Sodhi JK, Stephens LH, McClintock HD, Sim JY, and Wong IY, "Stereolithographic printing of ionically-crosslinked alginate hydrogels for degradable biomaterials and microfluidics," Lab Chip, vol. 17, no. 20, pp. 3474–3488, 2017, doi: 10.1039/c7lc00694b. [PubMed: 28906525]

[103]. Cui J, Wang M, Zheng Y, Rodriguez Muniz GM, and Del Campo A, "Light-triggered cross-linking of alginates with caged Ca2+," Biomacromolecules, vol. 14, no. 5, pp. 1251–1256, 2013, doi: 10.1021/bm400022h. [PubMed: 23517470]

[104]. Tay A, Sohrabi A, Poole K, Seidlits S, and Di Carlo D, "A 3D magnetic hyaluronic acid hydrogel for magnetomechanical neuromodulation of primary dorsal root ganglion neurons," Adv. Mater, vol. 30, no. 29, pp. 1–8, 2018, doi: 10.1002/adma.201800927.

[105]. Yuan Z, Memarzadeh K, Stephen AS, Allaker RP, Brown RA, and Huang J, "Development of a 3D collagen model for the in vitro evaluation of magnetic-assisted osteogenesis," Sci. Rep, vol. 8, no. 1, pp. 1–11, 2018, doi: 10.1038/s41598-018-33455-2. [PubMed: 29311619]

[106]. Sapir Y, Cohen S, Friedman G, and Polyak B, "The promotion of in vitro vessel-like organization of endothelial cells in magnetically responsive alginate scaffolds," Biomaterials, vol. 33, no. 16, pp. 4100–4109, 2012, doi: 10.1016/j.biomaterials.2012.02.037. [PubMed: 22417620]

[107]. Zhao X, Kim J, Cezar CA, Huebsch N, Lee K, Bouhadir K, and Mooney DJ, "Active scaffolds for on-demand drug and cell delivery," Proc. Natl. Acad. Sci, vol. 108, no. 1, pp. 67–72, 2011, doi: 10.1073/pnas.1007862108. [PubMed: 21149682]

[108]. Cezar CA, Kennedy SM, Mehta M, Weaver JC, Gu L, Vandenburgh H, and Mooney DJ, "Biphasic Ferrogels for Triggered Drug and Cell Delivery," Adv. Healthc. Mater, vol. 3, pp. 1–8, 2014, doi: 10.1002/adhm.201400095.

[109]. Cezar CA, Roche ET, Vandenburgh HH, Duda GN, Walsh CJ, and Mooney DJ, "Biologic-free mechanically induced muscle regeneration," Proc. Natl. Acad. Sci. U. S. A, vol. 113, no. 6, pp. 1534–9, 2016, doi: 10.1073/pnas.1517517113. [PubMed: 26811474]

[110]. Tomás AR, Goncąlves AI, Paz E, Freitas P, Domingues RMA, and Gomes ME, "Magneto-mechanical actuation of magnetic responsive fibrous scaffolds boosts tenogenesis of human adipose stem cells," Nanoscale, vol. 11, no. 39, pp. 18255–18271, 2019, doi: 10.1039/c9nr04355a. [PubMed: 31566629]

[111]. Chouhan Di., Mehrotra S, Majumder O, and Mandal BB, "Magnetic actuator device sssisted modulation of cellular behavior and tuning of drug release on silk platform," ACS Biomater. Sci. Eng, vol. 5, no. 1, pp. 92–105, 2019, doi: 10.1021/acsbiomaterials.8b00240.

[112]. Gonçalves AI, Rodrigues MT, Carvalho PP, Bañobre-López M, Paz E, Freitas P, and Gomes ME, "Exploring the potential of starch/polycaprolactone aligned magnetic responsive scaffolds for tendon regeneration," Adv. Healthc. Mater, vol. 5, no. 2, pp. 213–222, 2016, doi: 10.1002/adhm.201500623. [PubMed: 26606262]

[113]. Chen H, Sun J, Wang Z, Zhou Y, Lou Z, Chen B, Wang P, Guo Z, Tang H, Ma J, Xia Y, Gu N, and Zhang F, "Magnetic cell-scaffold interface constructed by superparamagnetic IONP enhanced osteogenesis of adipose-derived stem cells," ACS Appl. Mater. Interfaces, vol. 10, no. 51, pp. 44279–44289, 2018, doi: 10.1021/acsami.8b17427. [PubMed: 30499649]

[114]. Kim JJ, Singh RK, Seo SJ, Kim TH, Kim JH, Lee EJ, and Kim HW, "Magnetic scaffolds of polycaprolactone with functionalized magnetite nanoparticles: Physicochemical, mechanical, and biological properties effective for bone regeneration," RSC Adv, vol. 4, no. 33, pp. 17325–17336, 2014, doi: 10.1039/c4ra00040d.

[115]. Kim J, Chung SE, Choi S-E, Lee H, Kim J, and Kwon S, "Programming magnetic anisotropy in polymeric microactuators," Nat. Mater, vol. 10, no. 10, pp. 747–752, 2011, doi: 10.1038/nmat3090. [PubMed: 21822261]

[116]. Sniadecki NJ, Anguelouch A, Yang MT, Lamb CM, Liu Z, Kirschner SB, Liu Y, Reich DH, and Chen CS, "Magnetic microposts as an approach to apply forces to living cells," Proc. Natl. Acad. Sci. U. S. A, vol. 104, no. 37, pp. 14553–14558, 2007, doi: 10.1073/pnas.0611613104. [PubMed: 17804810]

[117]. Gao Y, Zhou B, Wu X, Gao X, Zeng X, Xie J, Wang C, Ye Z, Wan J, and Wen W, "Three dimensional and homogenous single cell cyclic stretch within a magnetic micropillar array (mMPA) for a cell proliferation study," ACS Biomater. Sci. Eng, vol. 2, no. 1, pp. 65–72, 2016, doi: 10.1021/acsbiomaterials.5b00381.

[118]. Herrera-Posada S, Mora-Navarro C, Ortiz-Bermudez P, Torres-Lugo M, McElhinny KM, Evans PG, Calcagno BO, and Acevedo A, "Magneto-responsive liquid crystalline elastomer nanocomposites as potential candidates for dynamic cell culture substrates," Mater. Sci. Eng. C, vol. 65, pp. 369–378, 2016, doi: 10.1016/j.msec.2016.04.063.

[119]. Sapir-Lekhovitser Y, Rotenberg MY, Jopp J, Friedman G, Polyak B, and Cohen S, "Magnetically actuated tissue engineered scaffold: Insights into mechanism of physical stimulation," Nanoscale, vol. 8, no. 6, pp. 3386–3399, 2016, doi: 10.1039/c5nr05500h. [PubMed: 26790538]

[120]. Li Y, Huang G, Gao B, Li M, Genin GM, Lu TJ, and Xu F, "Magnetically actuated cell-laden microscale hydrogels for probing strain-induced cell responses in three dimensions," NPG Asia Mater, vol. 8, no. 1, pp. e238–e238, 2016, doi: 10.1038/am.2015.148.

[121]. Zhang X, Sun L, Yu Y, and Zhao Y, "Flexible ferrofluids: design and applications," Adv. Mater, vol. 31, no. 51, pp. 1–35, 2019, doi: 10.1002/adma.201903497.

[122]. Serwane F, Mongera A, Rowghanian P, Kealhofer DA, Lucio AA, Hockenbery ZM, and Campàs O, "In vivo quantification of spatially varying mechanical properties in developing tissues," Nat. Methods, vol. 14, no. 2, pp. 181–186, 2017, doi: 10.1038/nmeth.4101. [PubMed: 27918540]

[123]. Mongera A, Rowghanian P, Gustafson HJ, Shelton E, Kealhofer DA, Carn EK, Serwane F, Lucio AA, Giammona J, and Campàs O, "A fluid-to-solid jamming transition underlies vertebrate body axis elongation," Nature, vol. 561, no. 7723, pp. 401–405, 2018, doi: 10.1038/s41586-018-0479-2. [PubMed: 30185907]

[124]. Schmidt S, Zeiser M, Hellweg T, Duschl C, Fery A, and Möhwald H, "Adhesion and mechanical properties of PNIPAM microgel films and their potential use as switchable cell culture substrates," Adv. Funct. Mater, vol. 20, no. 19, pp. 3235–3243, 2010, doi: 10.1002/adfm.201000730.

[125]. Hashmi B, Zarzar LD, Mammoto T, Mammoto A, Jiang A, Aizenberg J, and Ingber DE, "Developmentally-inspired shrink-wrap polymers for mechanical induction of tissue differentiation," Adv. Mater, vol. 26, no. 20, pp. 3253–3257, 2014, doi: 10.1002/adma.201304995. [PubMed: 24550068]

[126]. Hackelbusch S, Rossow T, Steinhilber D, Weitz DA, and Seiffert S, "Hybrid microgels with thermo-tunable elasticity for controllable cell confinement," Adv. Healthc. Mater, vol. 4, no. 12, pp. 1841–1848, 2015, doi: 10.1002/adhm.201500359. [PubMed: 26088728]

[127]. He X, Aizenberg M, Kuksenok O, Zarzar LD, Shastri A, Balazs AC, and Aizenberg J, "Synthetic homeostatic materials with chemo-mechano-chemical self-regulation," Nature, vol. 487, pp. 214–218, 7 2012, doi: 10.1038/nature11223. [PubMed: 22785318]

[128]. Zhang J, Cheng C, Cuellar-Camacho JL, Li M, Xia Y, Li W, and Haag R, "Thermally responsive microfibers mediated stem cell fate via reversibly dynamic mechanical stimulation," Adv. Funct. Mater, vol. 28, no. 47, pp. 1–12, 2018, doi: 10.1002/adfm.201804773.

[129]. Liu Y, Zhang K, Ma J, and Vancso GJ, "Thermoresponsive semi-IPN hydrogel microfibers from continuous fluidic processing with high elasticity and fast actuation," ACS Appl. Mater. Interfaces, vol. 9, no. 1, pp. 901–908, 2017, doi: 10.1021/acsami.6b13097. [PubMed: 28026935]

[130]. Shi K, Liu Z, Yang C, Li XY, Sun YM, Deng Y, Wang W, Ju XJ, Xie R, and Chu LY, "Novel biocompatible thermoresponsive poly(n-vinyl caprolactam)/clay nanocomposite hydrogels with macroporous structure and improved mechanical characteristics," ACS Appl. Mater. Interfaces, vol. 9, no. 26, pp. 21979–21990, 2017, doi: 10.1021/acsami.7b04552. [PubMed: 28603958]

[131]. Das M, Sanson N, Fava D, and Kumacheva E, "Microgels loaded with gold nanorods: Photothermally triggered volume transitions under physiological conditions," Langmuir, vol. 23, no. 1, pp. 196–201, 2007, doi: 10.1021/la061596s. [PubMed: 17190504]

[132]. Pastoriza-Santos I, Kinnear C, Pérez-Juste J, Mulvaney P, and Liz-Marzán LM, "Plasmonic polymer nanocomposites," Nat. Rev. Mater, vol. 3, no. 10, pp. 375–391, 2018, doi: 10.1038/s41578-018-0050-7.

[133]. Tabish TA, Dey P, Mosca S, Salimi M, Palombo F, Matousek P, and Stone N, "Smart gold nanostructures for light mediated cancer theranostics: Combining optical diagnostics with photothermal therapy," Adv. Sci, vol. In press, pp. 1–28, 2020, doi: 10.1002/advs.201903441.

[134]. Ye X, Zheng C, Chen J, Gao Y, and Murray CB, "Using binary surfactant mixtures to simultaneously improve the dimensional tunability and monodispersity in the seeded growth of gold nanorods," Nano Lett, vol. 13, no. 2, pp. 765–771, 2013, doi: 10.1021/nl304478h. [PubMed: 23286198]

[135]. Liu Z, Liu Y, Chang Y, Seyf HR, Henry A, Mattheyses AL, Yehl K, and Zhang Y, "Nanoscale optomechanical actuators for controlling mechanotransduction in living cells," Nat. Methods, vol. 13, pp. 143–146, 2016, doi: 10.1038/nmeth.3689. [PubMed: 26657558]

[136]. Ding T, Valev VK, Salmon AR, Forman CJ, Smoukov SK, Scherman OA, Frenkel D, and Baumberg JJ, "Light-induced actuating nanotransducers," Proc. Natl. Acad. Sci, vol. 113, pp. 5503–5507, 2016, doi: 10.1073/pnas.1524209113. [PubMed: 27140648]

[137]. Özkale B, Parreira R, Bekdemir A, Pancaldi L, Özelçi E, Amadio C, Kaynak M, Stellacci F, Mooney DJ, and Sakar MS, "Modular soft robotic microdevices for dexterous biomanipulation," Lab Chip, vol. 19, no. 5, pp. 778–788, 2019, doi: 10.1039/c8lc01200h. [PubMed: 30714604]

[138]. Sutton A, Shirman T, Timonen JVI, England GT, Kim P, Kolle M, Ferrante T, Zarzar LD, Strong E, and Aizenberg J, "Photothermally triggered actuation of hybrid materials as a new platform for in vitro cell manipulation," Nat. Commun, vol. 8, p. 14700, 2017, doi: 10.1038/ncomms14700. [PubMed: 28287116]

[139]. Chandorkar Y, Castro Nava A, Schweizerhof S, Van Dongen M, Haraszti T, Köhler J, Zhang H, Windoffer R, Mourran A, Möller M, and De Laporte L, "Cellular responses to beating hydrogels to investigate mechanotransduction," Nat. Commun, vol. 10, no. 1, pp. 1–13, 2019, doi: 10.1038/s41467-019-11475-4. [PubMed: 30602773]

[140]. Yang Y, Tan Y, Wang X, An W, Xu S, Liao W, and Wang Y, "Photothermal nanocomposite hydrogel actuator with electric-field-induced gradient and oriented structure," ACS Appl. Mater. Interfaces, vol. 10, no. 9, pp. 7688–7692, 2018, doi: 10.1021/acsami.7b17907. [PubMed: 29363307]

[141]. Mu J, Hou C, Wang H, Li Y, Zhang Q, and Zhu M, "Origami-inspired active graphene-based paper for programmable instant self-folding walking devices," Sci. Adv, vol. 1, no. 10, pp. 1–9, 2015, doi: 10.1126/sciadv.1500533.

[142]. Li W, Wang J, Ren J, and Qu X, "3D graphene oxide-polymer hydrogel: Near-infrared light-triggered active scaffold for reversible cell capture and on-demand release," Adv. Mater, vol. 25, no. 46, pp. 6737–6743, 2013, doi: 10.1002/adma.201302810. [PubMed: 24123218]

[143]. Fusco S, Sakar MS, Kennedy S, Peters C, Bottani R, Starsich F, Mao A, Sotiriou GA, Pané S, Pratsinis SE, Mooney D, and Nelson BJ, "An integrated microrobotic platform for on-demand, targeted therapeutic interventions," Adv. Mater, vol. 26, no. 6, pp. 952–957, 2014, doi: 10.1002/adma.201304098. [PubMed: 24510666]

[144]. Zeng Y and Lu JQ, "Optothermally responsive nanocomposite generating mechanical forces for cells enabled by few-walled carbon nanotubes," ACS Nano, vol. 8, no. 11, pp. 11695–11706, 2014, doi: 10.1021/nn505042b. [PubMed: 25327464]

[145]. Jiang W, Niu D, Wei L, Ye G, Wang L, Liu H, Chen P, Luo F, and Lu B, "Controllable actuation of photomechanical bilayer nanocomposites for in vitro cell manipulation," Carbon, vol. 139, pp. 1048–1056, 2018, doi: 10.1016/j.carbon.2018.07.074.

[146]. Yeh YC, Corbin EA, Caliari SR, Ouyang L, Vega SL, Truitt R, Han L, Margulies KB, and Burdick JA, “Mechanically dynamic PDMS substrates to investigate changing cell environments,” Biomaterials, vol. 145, pp. 23–32, 2017, doi: 10.1016/j.biomaterials.2017.08.033. [PubMed: 28843064]

[147]. Yang C, Tibbitt MW, Basta L, and Anseth KS, “Mechanical memory and dosing influence stem cell fate,” Nat. Mater, vol. 13, no. 6, pp. 645–652, 2014, doi: 10.1038/NMAT3889. [PubMed: 24633344]

[148]. Liu HY, Korc M, and Lin CC, “Biomimetic and enzyme-responsive dynamic hydrogels for studying cell-matrix interactions in pancreatic ductal adenocarcinoma,” Biomaterials, vol. 160, pp. 24–36, 2018, doi: 10.1016/j.biomaterials.2018.01.012. [PubMed: 29353105]

[149]. Tseng P, Judy JW, and Di Carlo D, “Magnetic nanoparticle-mediated massively parallel mechanical modulation of single-cell behavior,” Nat. Methods, vol. 9, no. 11, pp. 1113–1119, 2012, doi: 10.1038/nmeth.2210. [PubMed: 23064517]

[150]. Henstock JR, Rotherham M, Rashidi H, Shakesheff KM, and El Haj AJ, “Remotely activated mechanotransduction via magnetic nanoparticles promotes mineralization synergistically with bone morphogenetic protein 2: applications for injectable cell therapy,” Stem Cells Transl. Med, vol. 3, pp. 1363–1374, 2014. [PubMed: 25246698]

[151]. Elosegui-Artola A, Oria R, Chen Y, Kosmalska A, Pérez-González C, Castro N, Zhu C, Trepat X, and Roca-Cusachs P, “Mechanical regulation of a molecular clutch defines force transmission and transduction in response to matrix rigidity,” Nat. Cell Biol, vol. 18, no. 5, pp. 540–548, 2016, doi: 10.1038/ncb3336. [PubMed: 27065098]

[152]. Engler AJ, Griffin MA, Sen S, Bönnemann CG, Sweeney HL, and Discher DE, “Myotubes differentiate optimally on substrates with tissue-like stiffness: Pathological implications for soft or stiff microenvironments,” J. Cell Biol, vol. 166, no. 6, pp. 877–887, 2004, doi: 10.1083/jcb.200405004. [PubMed: 15364962]

[153]. Solon J, Levental I, Sengupta K, Georges PC, and Janmey PA, “Fibroblast adaptation and stiffness matching to soft elastic substrates,” Biophys. J, vol. 93, no. 12, pp. 4453–4461, 2007, doi: 10.1529/biophysj.106.101386. [PubMed: 18045965]

[154]. Li Y, Huang G, Li M, Wang L, Elson EL, Jian Lu T, Genin GM, and Xu F, “An approach to quantifying 3D responses of cells to extreme strain,” Sci. Rep, vol. 6, no. 6 2015, pp. 1–9, 2016, doi: 10.1038/srep19550. [PubMed: 28442746]

[155]. Ladoux B and Mège RM, “Mechanobiology of collective cell behaviours,” Nat. Rev. Mol. Cell Biol, vol. 18, no. 12, pp. 743–757, 2017, doi: 10.1038/nrm.2017.98. [PubMed: 29115298]

[156]. Mok S, Al Habyan S, Ledoux C, Lee W, MacDonald KN, McCaffrey L, and Moraes C, “Mapping cellular-scale internal mechanics in 3D tissues with thermally responsive hydrogel probes,” Nat. Commun, vol. 11, no. 1, 2020, doi: 10.1038/s41467-020-18469-7.

[157]. Lee W, Kalashnikov N, Mok S, Halaoui R, Kuzmin E, Putnam AJ, Takayama S, Park M, McCaffrey L, Zhao R, Leask RL, and Moraes C, “Dispersible hydrogel force sensors reveal patterns of solid mechanical stress in multicellular spheroid cultures,” Nat. Commun, vol. 10, no. 1, pp. 1–14, 2019, doi: 10.1038/s41467-018-07967-4. [PubMed: 30602773]

[158]. Träber N, Uhlmann K, Girardo S, Kesavan G, Wagner K, Friedrichs J, Goswami R, Bai K, Brand M, Werner C, Balzani D, and Guck J, “Polyacrylamide bead sensors for in vivo quantification of cell-scale stress in zebrafish development,” Sci. Rep, vol. 9, no. 1, pp. 1–14, 2019, doi: 10.1038/s41598-019-53425-6. [PubMed: 30626917]

[159]. Dolega ME, Delarue M, Ingremeau F, Prost J, Delon A, and Cappello G, “Cell-like pressure sensors reveal increase of mechanical stress towards the core of multicellular spheroids under compression,” Nat. Commun, vol. 8, no. 5 2016, 2017, doi: 10.1038/ncomms14056.

[160]. Campàs O, Mammoto T, Hasso S, Sperling RA, O’Connell D, Bischof AG, Maas R, Weitz DA, Mahadevan L, and Ingber DE, “Quantifying cell-generated mechanical forces within living embryonic tissues.,” Nat. Methods, vol. 11, no. 2, pp. 183–9, 2014, doi: 10.1038/nmeth.2761. [PubMed: 24317254]

[161]. Gómez-González M, Latorre E, Arroyo M, and Trepat X, “Measuring mechanical stress in living tissues,” Nat. Rev. Phys, vol. 2, no. 6, pp. 300–317, 2020.

[162]. Ronceray P, Broedersz CP, and Lenz M, “Fiber networks amplify active stress,” Proc. Natl. Acad. Sci. U. S. A, vol. 113, no. 11, pp. 2827–2832, 2016, doi: 10.1073/pnas.1514208113. [PubMed: 26921325]

[163]. Wang H, Abhilash AS, Chen CS, Wells RG, and Shenoy VB, “Long-range force transmission in fibrous matrices enabled by tension-driven alignment of fibers,” Biophys. J, vol. 107, no. 11, pp. 2592–2603, 2015, doi: 10.1016/j.bpj.2014.09.044.

[164]. Beroz F, Jawerth LM, Münster S, Weitz DA, Broedersz CP, and Wingreen NS, “Physical limits to biomechanical sensing in disordered fibre networks,” Nat. Commun, vol. 8, no. 5, 2017, doi: 10.1038/ncomms16096.

[165]. Yoon C, Choi C, Stapleton S, Mirabella T, Howes C, Dong L, King J, Yang J, Oberai A, Eyckmans J, and Chen CS, “Myosin IIA-mediated forces regulate multicellular integrity during vascular sprouting,” Mol. Biol. Cell, vol. 30, no. 16, pp. 1974–1984, 2019, doi: 10.1091/mbc.E19-02-0076. [PubMed: 31318321]

[166]. Gjorevski N, Piotrowski AS, Varner VD, and Nelson CM, “Dynamic tensile forces drive collective cell migration through three-dimensional extracellular matrices.,” Sci. Rep, vol. 5, no. 2, p. 11458, 2015, doi: 10.1038/srep11458. [PubMed: 26165921]

[167]. Labernadie A, Kato T, Brugués A, Serra-Picamal X, Derzsi S, Arwert E, Weston A, González-Tarragó V, Elosegui-Artola A, Albertazzi L, Alcaraz J, Roca-Cusachs P, Sahai E, and Trepat X, “A mechanically active heterotypic E-cadherin/N-cadherin adhesion enables fibroblasts to drive cancer cell invasion,” Nat. Cell Biol, vol. 19, no. 3, pp. 224–237, 2017, doi: 10.1038/ncb3478. [PubMed: 28218910]

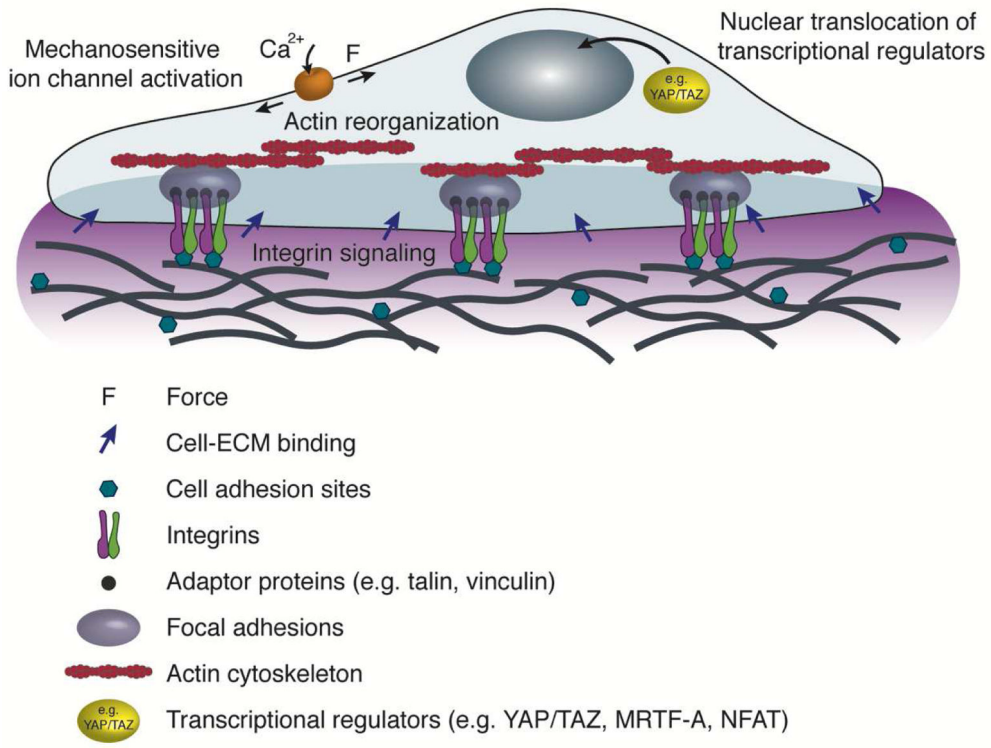

Figure 1.

Cell responses to mechanical forces can involve integrin signaling, focal adhesion assembly, stress fiber formation, calcium signaling and nuclear translocation of mechanosensitive transcriptional regulators.

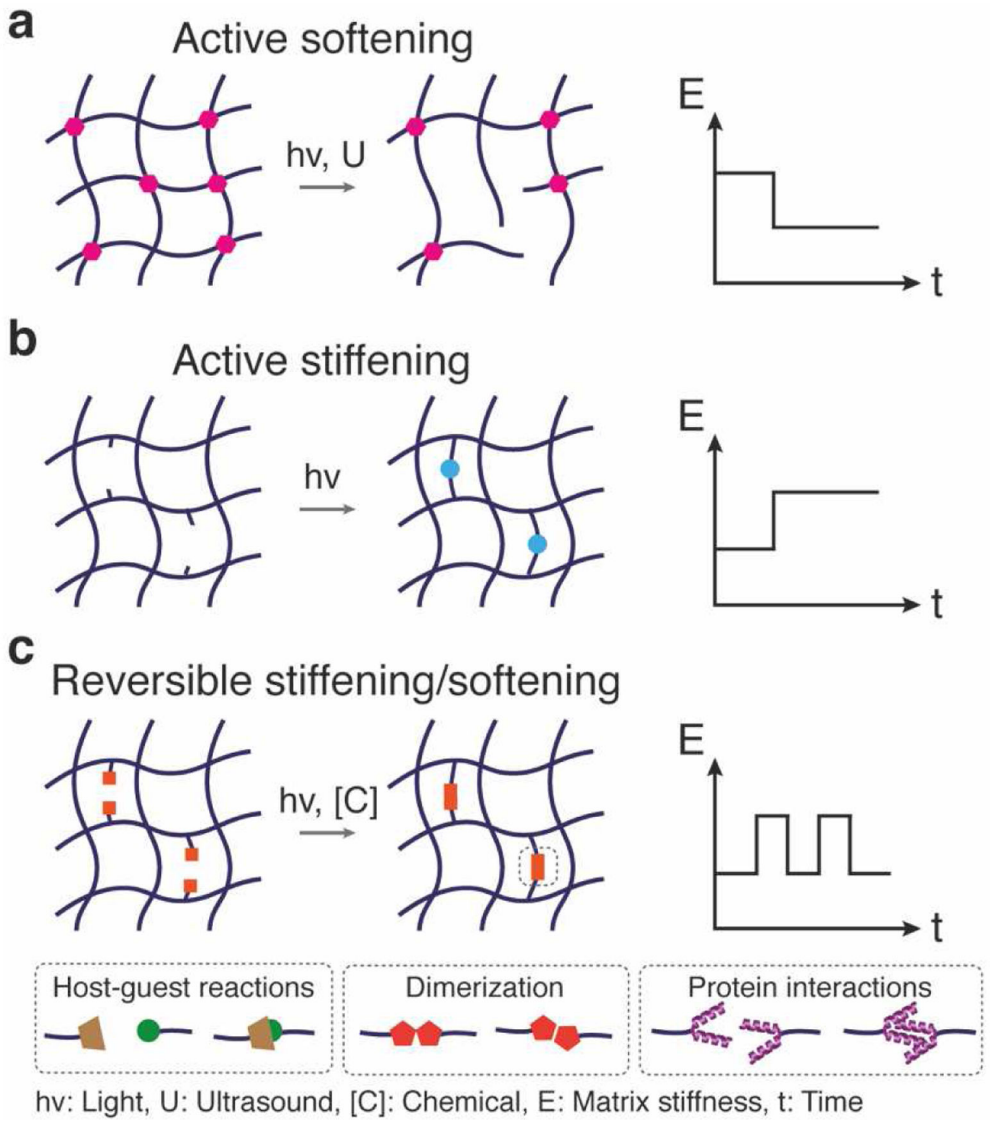

Figure 2.

Dynamic modulation of response to endogenous forces is typically achieved by changing the network crosslinking density. Matrix softening is initiated by (a) reducing crosslinking via light or ultrasound activation while (b) increasing crosslinks results in matrix stiffening. (c) Emerging approaches relying on reversible reactions enable cyclic control over matrix elasticity. These interactions are triggered typically by light although chemical control is also established in several material systems.

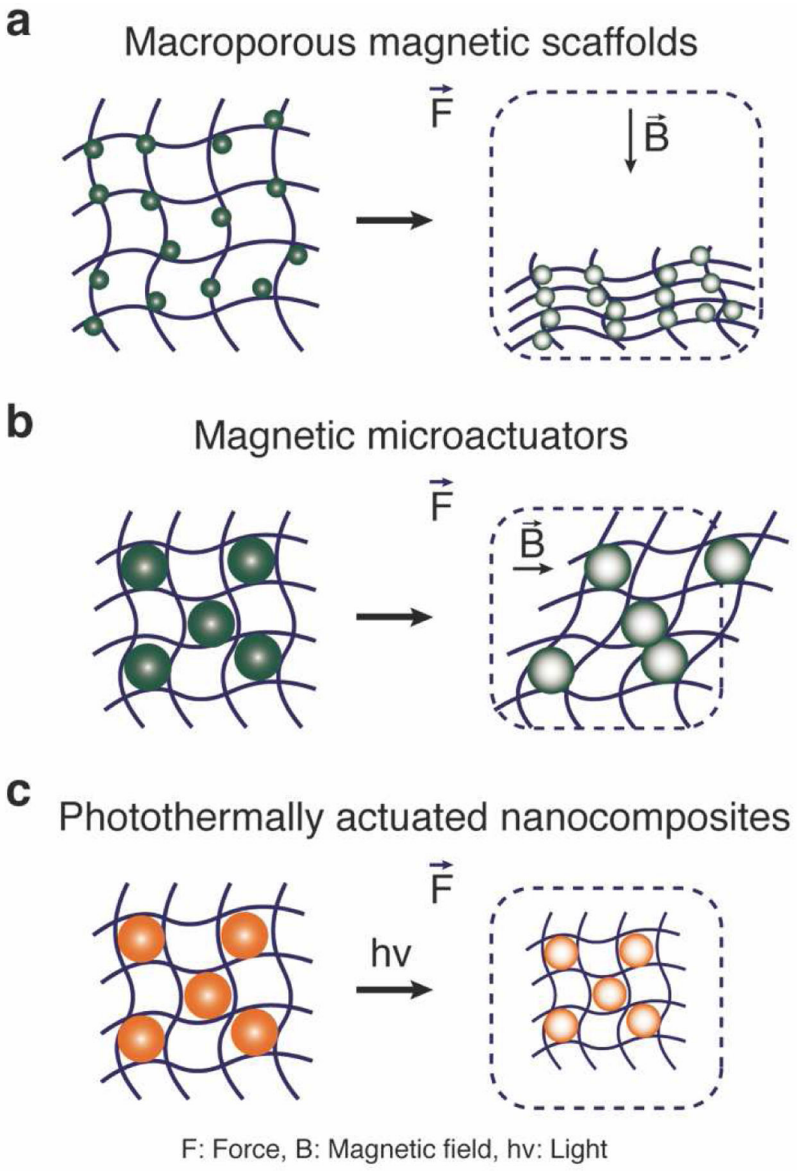

Figure 3.

Exogenous forces can be generated using magnetic fields and photothermal effects. (a) Macroporous magnetic scaffolds allow the application of compressive forces on large populations of cells. (b) Microfabricated substrates enable other modes of actuation through magnetic torque such as bending or twisting. (c) Photothermally activated nanocomposites generate rapid and large deformation that can be harnessed to apply tensile or compressive loads.

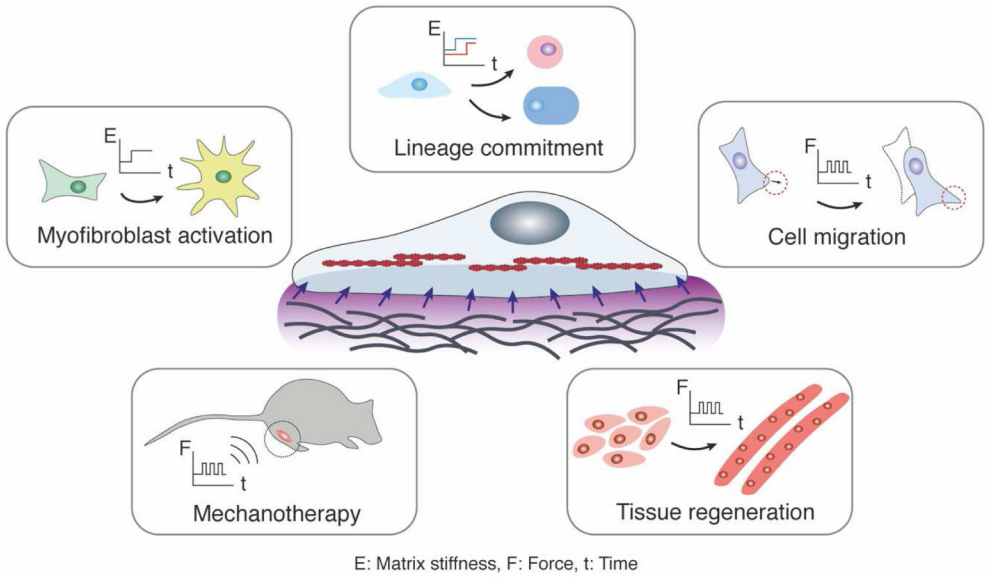

Figure 4.

Active biomaterial systems offer a wide range of mechanobiology applications and have been used to investigate fibrosis, stem cell differentiation, cell migration, signaling, and muscle regeneration *in vitro* as well as for *in vivo* mechanotherapy.

Table 1.

Optical manipulation strategies to achieving temporally controlled softening and/or stiffening. The functionality is categorized with respect to functional groups, choice of polymer, and corresponding change in network stiffness. Downward arrow (↓) indicates decrease and upward arrow (↑) indicates increase in storage modulus (SM) and Young's modulus (YM). Double arrows (↓↑) refer to reversible material systems which allow one cycle of change. Double arrows with an *x* (↓↑*x*) indicate active biomaterials that can cycle between the soft and stiff states several times.

	Functional groups	**Polymer**	**Change in matrix stiffness**
Softening via photodegradation	*o*-nitrobenzyl	Polyethylene glycol (PEG) [65]-[68], dextran [69], gelatin [70]	↓ 32 kPa to 7 kPa (YM) [68]
Stiffening via photocrosslinking	Methacrylate	Hyaluronic acid (HA) [41]-[43]	↑ 5-47 kPa to 38-724 (YM) [41]
	Thiol	PEG [44], polydimethylsiloxane (PDMS) [45]	↑ 3 kPa to 50 kPa (YM) [45]
	DBCO-azide	PEG	↑ 4 kPa to 9 kPa (SM) [71]
	Tyrosine	Fibrin	↑ 10-fold change (nN/μm) [72]
Reversible softening/stiffening	*o*-nitrobenzyl and Methacrylate	HA	↓↑ 15 kPa to 4 kPa to 28 kPa (YM) [46]
	Azobenzene	Polyacrylamide [47], PEG [48], [51], gelatin [52]	↓↑*x* 10 kPa to 6 kPa (YM) [47]
	Cyclodextrin-Azobenzene or Adamantane (host-guest)	Polyacrylic acid [53]-[55], HA [56], [73]	↓↑*x* 1000 Pa to 600 Pa (SM) [56]
	Coumarin	HA	↑↓ 80 Pa to 350 Pa (SM) [49]
	Anthracene	PEG [57], [58]	↑ 10 kPa to 50 kPa (YM) [57]
	Styrylpyrene	PEG	↑↓*x* 30 Pa to 600 Pa (SM) [50]