Title: Active Biomaterials for Mechanobiology

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Keywords: dynamic matrices, nanomaterials, actuation, programmable

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Abstract

Active biomaterials offer novel approaches to study mechanotransduction in mammalian cells. These material systems can either modulate the resistance cells sense to endogenous forces or apply exogenous forces on cells in a temporally controlled manner. The ability to dynamically control the mechanical cues cells receive allows one to mimic various aspects of the native microenvironment. The implementation of active biomaterials in mechanobiology has generated valuable insight relevant to a variety of biological processes including but not limited to stem cell lineage commitment, disease progression, and tissue regeneration. The field is rapidly evolving as emerging technologies and materials are introduced and will continue to develop in the near future.

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 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 dissipated at multiple length scales. Active biomaterials can recapitulate the dynamic microenvironment within living tissues because they have the ability to convert wireless energy into structural reconfiguration and mechanical cues by either changing their mechanical properties or directly applying mechanical forces to cells.

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 (Figure 1). 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.

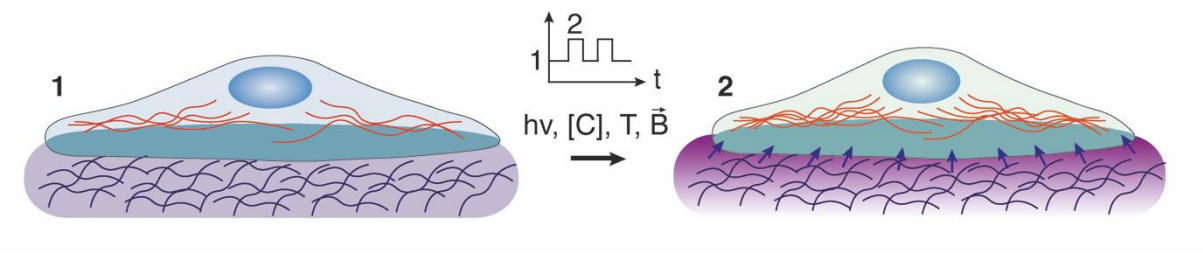

Figure 1. Active biomaterials are externally triggered to either modulate resistance to endogenous stresses or to apply exogenous forces on cells. Wireless activation stimuli such as light and magnetic fields ensure fine temporal control over the applied mechanical cues.

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], degradation [4], and stress relaxation [5] as well as the structure of the polymer network [6] on cell behavior. The adoption of micropatterning techniques enabled interrogation of the impacts of spatial stiffness gradients [7] and topography [8]. Three-dimensional (3D) biomimetic hydrogel scaffolds have also been developed to study the effect of local geometry [9]. Taken together, 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 [10], epithelial-mesenchymal transition [11], [12], and control over stem cell fate [13], [14].

The spatiotemporally dynamic nature of exogenous mechanical loads applied to tissues and cells, which include stretching, 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 [15], optical tweezers [16], magnetic twisting cytometry [17],

stretch devices, and microfluidics [18] have been employed. We refer the readers to excellent review articles that discuss the working principles of these micromanipulation techniques and their implementation in mechanobiology research [19]–[22]. Functionalizing the surfaces of end-effector particles with relevant molecules revealed the contribution of ECM binding receptor integrins, Talin proteins, mechanically gated ion channels, and transcriptional regulators such as YAP/TAZ on mechanotransduction [17], [23]–[27]. Forces on the order of tens of pN to a few nN are generated with the existing technologies, and these values correspond well to physiologically relevant force magnitudes [22].

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 resistance cells to endogenous forces via dynamic modulation of matrix elasticity, or application of extrinsic forces on cells upon external stimulation. Dynamic 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 [28]. 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

A common chemical approach for generating dynamic softening in biomaterials relies on photolabile *o*-nitrobenzyl alcohol derivatives (Figure 2) [29]. A classic example is the photodegradable polyethylene glycol (PEG) hydrogel. The light sensitive component, a nitrobenzyl ether derivative, is cleaved when activated by 365 nm light, decreasing the

crosslinking density of the hydrogel matrix in a cytocompatible manner [30]. Photodegradation decreased material stiffness from 32 kPa to 7 kPa within 5 minutes of light activation [31]. Similarly, a photodegradable methacrylated hyaluronic acid network was developed using o-nitrobenzyl-acrylates which exhibited matrix softening from 15 kPa to 3 kPa under 365 nm light [32]. This method has been extended to other biomaterials such as dextran [33] and gelatin [34]. Interestingly photodegradation can be used to induce deformation in hydrogels by taking advantage of crosslinking gradients, as demonstrated with polyethylene glycol diacrylate (PEGDA) films which bent into a scroll shape due to a crosslink density gradient in the z-axis [35].

In contrast, photoinduced crosslinking has been widely implemented over a range of materials as a dynamic stiffening strategy (Figure 2). For example, the stiffness of methacrylated hyaluronic acid (MeHA) hydrogels could be increased as much as 7-fold within several minutes with 365 nm light exposure in the presence of a photoinitator Irgacure [36]. An increase in stiffness from 3 kPa to approximately 30 kPa was achieved in MeHA hydrogels when photocrosslinking was initiated [37].

An alternative approach to controlled matrix stiffening in biomaterials utilizes thiol-ene polymerization. PEG hydrogels were on-demand stiffened via thiol-ene chemistry in the presence of the photoinitiator lithium phenyl-2,4,6- trimethylbenzoylphosphinate (LAP) [38]. Thiol-ene photochemistry was recently extended to polydimethylsiloxane (PDMS), a cytocompatible elastomer often used in the development of flexible devices. Light triggered stiffening in PDMS substrates was achieved under UV activation in the presence of 2,2-dimethoxy-2-phenylacetophenone (DMPA) resulting in an increase of compressive modulus from 3 kPa to 50 kPa. The stiffened material state was stable up to a week in an aqueous environment, maintaining a modulus of 40-50 kPa [39]. Click PEG hydrogels have also been reported to undergo dynamic stiffening due to light initiated secondary crosslinking between excess cyloocytyne moeities [40]. Other crosslinking strategies, including ruthenium crosslinked hydrogels have been recently shown to degrade under exposure to 400-500 nm light [41], [42].

Triggering dynamic elasticity in synthetic matrices with visible light promises a more physiologically relevant route for controlling local mechanics, as compared to UV activation. Fortunately, UV sensitive photoinitiators can be exchanged with alternatives that are activated at higher wavelengths. For example, MeHA hydrogels were photocrosslinked with blue light in the presence of lithium acylphosphinate, and this did not significantly affect the stiffening kinetics of the hydrogel as Young's moduli were found to approximately double [43]. Similarly, PEG based networks can be crosslinked using eosin Y under green light [44]. As an alternative strategy, secondary crosslinking moieties caged with a photolabile molecule can be controllably activated with visible light [45]. This method was implemented

in a fibrin hydrogel network, with a 25-fold increase in stiffness through photocrosslinking between tyrosine residues under blue light illumination [46].

Combining light activated cleaving and crosslinking strategies in the same material enables active systems with reversible stiffness. Doubly functionalized PEG [38], [44] and MeHA [32] hydrogels have been shown to complete a single cycle of stiffening and softening via light activation. The ability to exhibit dynamic elasticity over many cycles would potentially enable one to recapitulate the dynamics of living systems in engineered materials. Chemical approaches that rely on reversible states of light sensitive molecules have been introduced to address this point. Azobenzene is a well known example that reversibly shifts between cis and trans forms when triggered by light, which leads to a change in molecule length within picoseconds [47]. The wavelength at which this transformation occurs can be tuned [48], [49]. For example, in polyacrylamide hydrogels synthesized with the crosslinker 4,4'-(diacrylamido)azobenzene, light activated isomerization led to reversible reduction in matrix stiffness. This strategy allowed cycling between different stiffness states by alternating photoisomerization wavelengths between UV and blue light, in a cytocompatible manner [50]. Similar dynamic elasticity matrices have been synthesized with other polymers such as PEG [49], [51], HA [52], gelatin [53], and polyacrylic acid [54]. Alternatively, reversible cyclodextrin-azobenzene host-guest reactions have been implemented for reversible crosslinking [52], [54]–[56]. An important advantage of such biomaterial systems is that the activation wavelengths can be tuned towards near-infrared, which allows greater light penetration in tissue for in vivo applications [54].

Reversible crosslinking via dimerization of photosensitive molecules such as coumarin [57], [58], anthracene [59], [60], and styrylpyrene [61], [62] have also been realized in dynamic hydrogels. Anthracene functionalized PEG hydrogels exhibited 5 fold stiffening under 365 nm light exposure [59]. The photodimerization wavelength can be shifted to 400-500 nm by using triazole anthracenes, and this shift can ensure reversible crosslinking under cytocompatible conditions in PEG hydrogels [60]. Similarly, styrylpyrene functionalized PEG hydrogels could be photocrosslinked with 400-500 nm light, and crosslinking was reversed by 340 nm light, which allowed cycling between soft and stiff states of the hydrogel network [62].

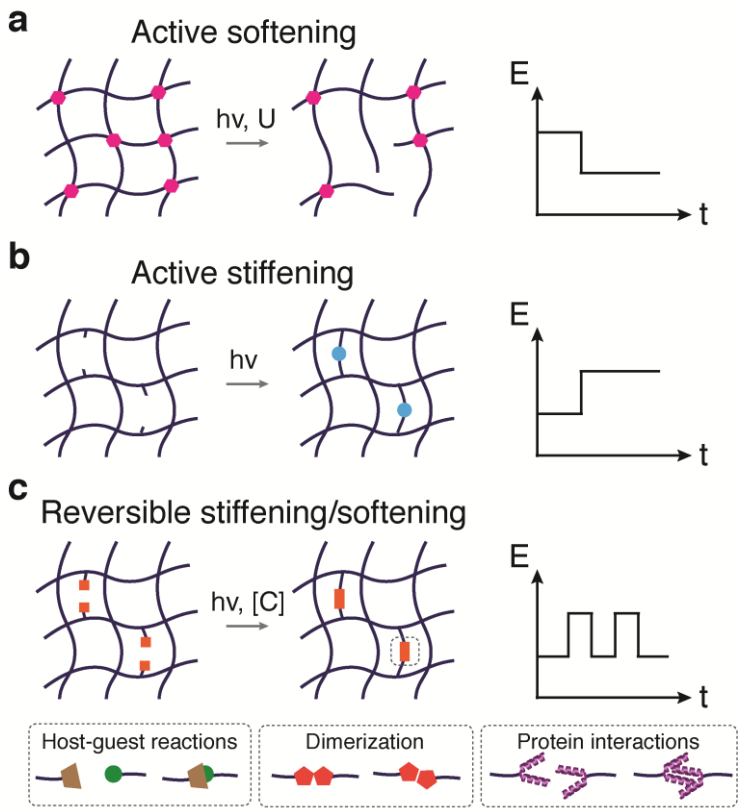

Figure 2. Dynamic modulation of resistance to endogenous forces is typically achieved by changing the network crosslinking density. Matrix softening is initiated by (a) reducing crosslinking via light or ultrasound activation while (b) increasing crosslinks results in matrix stiffening. (c) Emerging 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.

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 [63], protein [64] and cell release in 3D [65] and recently to achieve dynamic 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 [66]. 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.

Dynamic 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 [67]. 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 [68].

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 [69]. 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 [30]. 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 [70]. Reversible ionic crosslinking was also applied in pure alginate materials to control the sol-gel transition of 3D hydrogels [71]. 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 [72], [73].

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 [74]. 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 [75].

Biomolecules such as DNA and enzymes offer alternative methods for generating dynamic stiffness in synthetic matrices. Biocompatible polyacrylamide-DNA matrices have been reported to exhibit reversible stiffening behavior by alternating delivery of L and R strands [76]. Similarly, a four-fold increase of stiffness was observed in DNA crosslinked polyacrylamide substrates [77]. Reversible stiffening over several cycles was demonstrated in dynamic protein hydrogels that undergo secondary crosslinking between tyrosine residues due to redox reactions [78], or tyrosinase enzyme [79], [80]. In contrast, sortase enzyme mediated crosslinking led to reversible stiffening in PEG-peptide hydrogels [81]. 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 [82], [83].

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 wirelessly 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 [84]. 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 [85]. Triggered changes in crosslinking have been primarily used to release therapeutic agents [84], polysaccharides [86], surface functionalized nanoparticles [85], [87], and small molecules [88] for regenerative medicine [85], [88] as well as cancer treatment [84]. 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 [89]. 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 [90]. These recent studies suggest that dynamic 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) [91]. Similarly, a light sensitive calcium cage was used to crosslink alginate on demand upon UV activation, and ionic crosslinking was chemically degraded with EDTA [92]. 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 wireless energy 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 translates 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 [93]–[100], synthetic polymers [101]–[104], or elastomers [105]–[107]. 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 [93], collagen [94], alginate [95]–[98], cellulose [99], silk [100], starch [101], polycaprylactone [101]–[103], poly(lactic-co-glycolic acid) [102], PEGDA [104], PDMS [105], [106], and liquid crystalline elastomers [107]. 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 [96]. 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 [97], [98]. 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 [108].

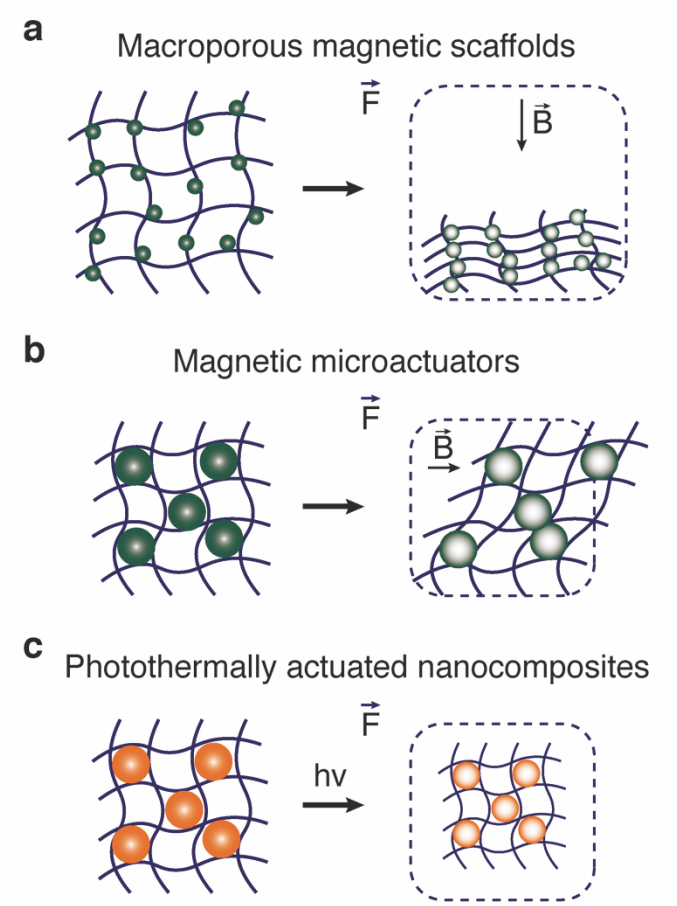

Figure 3. Exogenous forces can be generated using magnetic fields and photothermal effects. (a) Macroporous magnetic scaffolds allow the application of compressive forces on large populations of cells. (b) Microfabricated substrates enable other modes of actuation through magnetic torque such as bending or twisting. (c) Photothermally activated nanocomposites generate rapid and large deformation that can be harnessed to apply tensile or compressive loads.

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 [105]. 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 [106]. 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 [109]. Deformation of magnetic polymer devices can be tuned by controlling the distribution and alignment of magnetic nanoparticles prior to casting [104]. Ferrofluid oil microdroplets [110] 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 [111], [112]. 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) [113]–[118] and poly(N-vinyl caprolactam) [119]. 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 [120]. Thermoresponsive 3D hydrogel scaffolds that exhibit up to 50% volumetric change when subjected to physiological temperatures (37°C) have been fabricated from pNIPAM [114] or co-polymers of pNIPAM with PEG [115]. 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 [113].

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 [121]. 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 [122]. Moreover, the excitation wavelength can be

tuned by changing nanoparticle shape and size [122]. 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 [122], [123]. When coupled with thermoresponsive polymers, photothermal heating rapidly large forces (Figure 3). The optomechanical nanoactuator platform is an excellent example for this actuation paradigm [124]. 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 [124].

The force output of these systems can be amplified by storing elastic energy, for example, via reversible clustering of gold-pNIPAM nanoparticles [125]. 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 [126]. 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 [127]. 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 [128]. 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 [126]. Heat generation with light is not limited to gold nanoparticles, as photothermal nanocomposites have also been developed from graphene oxide nanoparticles [129], [130], [131], [132] and carbon nanotubes [133]. Graphene nanoplatelet-PDMS nanocomposite films were able to bend under NIR light, generating forces of tens of nN [134]. Similarly, microcapsules constructed with PEGDA/graphene oxide-pNIPAM hydrogel bilayers were reported to open and close repeatedly [132].

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 [36], [59], [66], [77], [78], [135]. In contrast, matrix softening was reported to induce valvular myofibroblast de-activation [31].

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). Human MSCs 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 [37]. 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 [136]. 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

[76]. 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 [82].

Active biomaterials with dynamic 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 [44], [91], [137]. T cell migration under cyclic application of mechanical cues was investigated using 3D phytochrome-based dynamic elasticity matrices [67]. 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 [67].

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) [124]. 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 [105]. Similarly, directional pulling has been reported to guide filipodia generation and to influence of mitotic spindle axis alignment during mitosis in HeLa cells [138].

The amplitude and duration of extrinsic force application significantly influences various other cellular responses, as demonstrated with fibroblasts cultured on photothermally activated deformable nanocomposites [128]. 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 (MRTFA) translocated to the nucleus after 8 hours of actuation. MRTFA 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 [93]. 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 [109]. 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) [98]. Actuation of similar magnetic scaffolds in vivo enhanced osteogenesis [94], [139] and tendon regeneration [99], [101]. As an alternative strategy, thermoresponsive hydrogel scaffolds transplanted into mice were used to apply constant compression on embryonic dental MSCs [114]. Constant stress enhanced MSC differentiation, as demonstrated by the increase in the expression of odontogenic factors Pax9, Msx1, and Bmp4 and mineralization levels.

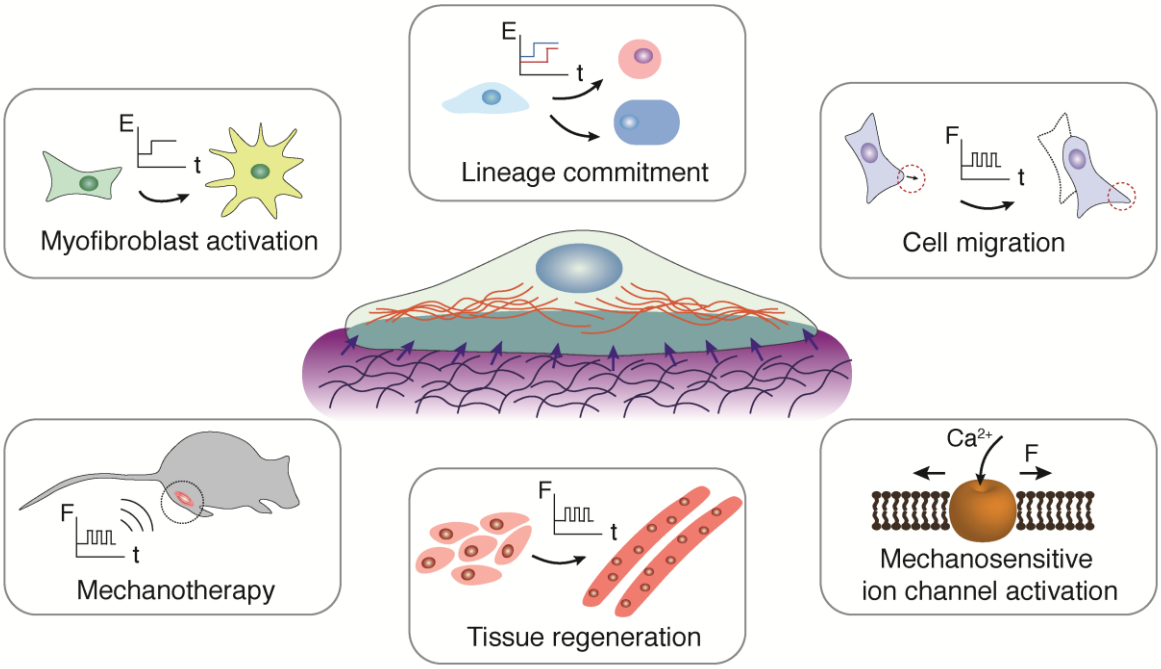

Figure 4. Active biomaterial systems offer a wide range of mechanobiology applications and have been used to investigate fibrosis, stem cell differentiation, cell migration, signaling, and muscle regeneration *in vitro* as well as for *in vivo* mechanotherapy.

5. Conclusions and future directions

Active biomaterials that can manipulate resistance to cell-endogenous stresses or apply exogenous forces in temporally controlled manner have allow 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 mechanically-based therapies targeting tissue regeneration applications.

However, the future success of active biomaterials in mechanobiology research will likely depend on better mechanical characterization of these systems. For example, the effect of changing crosslinking density in dynamic systems on other network properties, and their subsequent influence on cells need further investigation. This is of particular importance in 3D multicellular scaffolds to avoid unappreciated synergistic interactions of different matrix properties which impact the clarity of research findings. Similarly, the synergistic relationship between matrix stiffness and force generation should be deciphered in extrinsic stress applying biomaterials. In these material systems, activated nanoparticles may stretch polymer chains during contraction, which can influence matrix elasticity temporarily and potentially can also lead to plastic deformation over long durations of actuation. An ideal active biomaterial system for basic research studies should 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 cells/tissues.

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 cells/tissues and biological 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. The implementation of active biomaterials to a range of human cells will further advance therapeutic and diagnostic medicine. We expect to see many exciting developments in the near future.

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