The Art of Engineering Biomimetic Cellular Microenvironments

Ross C. Bretherton[1,2], **Cole A. DeForest**[1,2,3,4,5,*]

[1]Department of Bioengineering, University of Washington, Seattlfe, WA 98105, USA

[2]Institute of Stem Cell & Regenerative Medicine, University of Washington, Seattle, WA 98109, USA

[3]Department of Chemical Engineering, University of Washington, Seattle, WA 98195, USA

[4]Department of Chemistry, University of Washington, Seattle, WA 98195, USA

[5]Molecular Engineering & Sciences Institute, University of Washington, Seattle, WA 98195, USA

Abstract

Cells and their surrounding microenvironment exist in dynamic reciprocity, where bidirectional feedback and feedforward crosstalk drives essential processes in development, homeostasis, and disease. With the ongoing explosion of customizable biomaterial innovation for dynamic cell culture, an ever-expanding suite of user-programmable scaffolds now exists to probe cell fate in response to spatiotemporally controlled physiochemical cues. Here, we highlight emerging trends in these efforts, emphasizing strategies that offer tunability over complex network mechanics, present biomolecular cues anisotropically, and harness cells as physiochemical actuators of the pericellular niche. Altogether, these material advances will lead to breakthroughs in our basic understanding of how cells interact with, integrate signals from, and influence their surrounding microenvironment.

Graphical Abstract
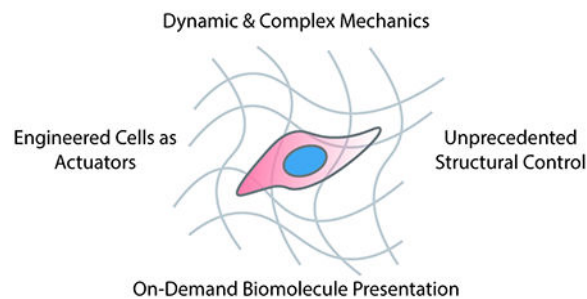
Keywords

Dynamic Biomaterials; Hydrogel; Microenvironment; Cell Fate

Introduction

It is now widely accepted that the extracellular matrix (ECM) evolves in space and time, harboring persistent recollections of past cellular states. These biological memories are most distinctly present as state-dependent cell-secreted proteins tethered to the ECM and anisotropic variations in matrix mechanics.[1,2] Accompanying progression of many diseases, particularly those with a fibrotic element, the ECM binds an altered set of secreted growth factors and develops distinct mechanics from healthy tissue.[3,4] With seminal studies highlighting the role of ECM-presented cues in driving significant changes in cell fate,[5] researchers now appreciate why seeding healthy cells onto a diseased matrix is often sufficient to induce unhealthy cell phenotypes.[6] Therefore, cells exist in dynamic reciprocity with their environment: extracellular cues alter cell behavior, and cells in turn shape their surroundings through secreted bioactive and structural proteins.[7]

Studies over the past many decades underscore the need to further decouple the ECM's role in guiding cell behavior throughout development, health, and disease. Engineered microenvironments can provide a user-defined platform in which to precisely tune individual aspects of the ECM to probe and direct encapsulated cell response, increasingly with four-dimensional (4D) and reversible control. To this end, the community has innovated and established a variety of modular hydrogel biomaterial designs that recapitulate critical complexities of the native cellular niche. Here, we highlight recent advances in the synthesis and manipulation of dynamic biomaterials and discuss future strategies to mimic complex biological microenvironments *in vitro*.

Engineering Tissue Mechanics Beyond the Modulus

Tissue mechanics clearly play an important role in development and disease, and the varied mechanical properties of tissues cannot be fully captured by a single elastic modulus. While most covalently crosslinked polymeric hydrogels are linearly elastic, whole tissues exhibit complex mechanical properties such as strain stiffening/softening and viscoelasticity.[8] Though substrate stiffness is often seen as the classic mechanical parameter to characterize and manipulate in an engineered biomaterial, recent efforts have moved to decouple ECM elasticity, viscosity, and fiber thickness/architecture towards elucidating their individual roles on influencing cell function (Figure 1).

Underpinning the importance of complex mechanics when engineering cellular microenvironments is the understanding that cellular mechanosensation on and within soft materials is inherently dynamic.[9,10] Cells adhere to the matrix through membrane-bound integrins that are clustered into focal adhesions linking the actin cytoskeleton to the ECM. As cells exert spatiotemporally varied forces on their surroundings, time-dependent microenvironmental viscous behaviors complement substrate stiffness in establishing dynamic mechanical reciprocity between intracellular and extracellular tension.

Tuning Viscoelasticity and Viscoplasticity

Non-degradable synthetic polymer hydrogels exhibit dominantly elastic mechanics. This is in stark contrast with the varying stress relaxation responses of soft tissues that can be

on the order of several minutes.[8] In addition, the viscoelastic properties of tissue have been reported to change throughout the course of disease; patients with cardiomyopathies exhibit increased cardiac muscle viscosity that further contributes to progressive diastolic dysfunction throughout the disease course.[11] Conversely, cancerous tissues will also stiffen, but with a lower degree of stress relaxation.[12] The viscous behavior of biomaterials has been tuned primarily independent from the storage modulus through encapsulation of non-crosslinked entrapped polymer elements. For example, linear polyacrylamide can be incorporated within a crosslinked polyacrylamide gel to endow this popular and linearly elastic biomaterial platform with tunable viscosity.[13] Increasing polyacrylamide viscosity attenuated seeded hepatic myofibroblasts spreading, restoring hallmarks of quiescent hepatic stellate cell phenotype. By incorporating poly(ethylene glycol) (PEG) spacers into an alginate hydrogel, stress relaxation rates can be increased with faster relaxation that drives cell spreading.[14] In hyaluronan, the introduction of noncovalent guest-host crosslinks has been used to independently increase the loss modulus of the hydrogel with the same viscosity-dependent effect on cell spreading.[15]

In addition to the reversible elastic deformation of substrates, cells can also sense the plasticity or irreversible deformation of a biomaterial.[16,17] Many natural biomaterials that are noncovalently crosslinked (e.g., gels based on collagen, fibrin, reconstituted basement membrane, agarose, alginate) exhibit some degree of time-dependent plasticity – viscoplasticity.[16] Cells encapsulated in these types of materials can plastically remodel their surroundings over time in a manner dependent on integrin-based cellular force transmission and the strength of material crosslinks within the gel. Plasticity can be modulated in a cell adhesion-independent manner through interpenetrating networks of varied molecular weight alginate and ionic crosslinking embedded in a reconstituted basement membrane.[17] In this system, highly plastic networks promoted the spreading and invasive behavior of cancer cells independent of matrix modulus or enzymatic degradability. The effects of material plasticity on cell function have also been explored by incorporating PEG spacer sidechains into an alginate hydrogel that are either covalently tethered, dynamically bound and able to rearrange, or free sliding within the network.[18] Increased plasticity had profound effects on the transcriptome of mesenchymal stem cells (MSCs) seeded on the gel, especially with respect to pathways regulating focal adhesion remodeling and cell spreading. Gels with intermediate substrate plasticity promoted optimal spreading of MSCs, whereas cell spreading on highly plastic gels could be improved by attenuating cell contraction with the myosin inhibitor blebbistatin.

Whereas many tissues stiffen with compressive strain and soften with extension or shear, natural polymeric hydrogels such as collagen or fibrin do the opposite. These findings highlight the often-overlooked contribution of cells to the overall stiffness and mechanical behaviors of a tissue. Especially at lower strains, the passive stiffness of cells and their cytoskeleton plays a dominant role in dictating tissue stiffness.[19] As determined rheologically through progressive decellularization of otherwise intact tissues, cells also contribute to the compressive strain stiffening behavior through both passive stiffness and active contraction.[19] These findings offer some explanation as to why many of the most successful hydrogel systems for engineering functional and interconnected constructs are far softer than the tissues they aim to recapitulate.

Independent Control over Gel Mechanics and Network Properties

Another interesting development in the space of engineered tissue mechanics has been the decoupling of stiffness, fiber architecture, and crosslink density in cell-compatible hydrogels. In collagen gels, stiffening the microenvironment by increasing the collagen weight percentage was shown to decrease angiogenic sprouting but stiffening the microenvironment without increasing fiber density through nonenzymatic glycation does the opposite.[20] A similar study using pulmonary fibroblasts found that while cells cultured on stiffer gels were more prone to myofibroblastic activation, increased crosslinking density diminished such phenotypic change when cultured in three-dimensional (3D) materials.[21] The authors were then able to supplement the hydrogel system with electrospun polymeric fibers in a manner that did not impact bulk storage modulus, demonstrating that increased fiber density promoted fibroblast proliferation and primed for activation.[21] Together, these studies reveal the distinct and sometimes opposing effects of fiber density, crosslink density, and substrate stiffness in an engineered biomaterial – three parameters that are often taken for granted as interchangeable in the field. Network crosslink concentration and cell-degradability have also been decoupled in a hydrogel system in which elastin-like polypeptides with varying rates of proteolytic degradation were crosslinked by copper-free click reaction with a suite of non-degradable PEG macromers ranging from 2 to 8 arms.[22] To form functional endothelial networks from encapsulated brain microvascular cells, both a low crosslink density and rapid cleavage kinetics were necessary.[22]

Biomaterials with Dynamic and Reversible Mechanical Control

Temporal evolution of stiffness has been another evolving locus of dynamic biomaterial development. As disease pathophysiology is progressive and chronic, simply lifting cells from a substrate mimicking a healthy mechanical environment and placing them in a diseased environment may not be sufficient to recapitulate the gradual compensation of cells to their changing microenvironment. To overcome this barrier, dynamic materials whose crosslinking density can altered *in situ* have been the subject of great interest from the field. Many hydrogels that irreversibly stiffen have already been developed, including those based on release of calcium for alginate crosslinking,[23] Michael-type addition,[24] radical polymerization,[25] photoinitiated thiol-ene reaction,[26] enzymatic crosslinking,[27,28] and anthracene dimerization.[29] Similarly, bioorthogonal softening or material degradation can be accomplished through inclusion of a photodegradable moiety (e.g., *ortho*-nitrobenzyl, allyl sulfide, ruthenium complexes) within a crosslink,[30–33] passive crosslinker hydrolysis,[34] or even enzymatic transpeptidation *in situ*.[35]

With unidirectional control over substrate moduli well established, the field has since turned towards materials capable of reversible stiffening and softening.[36] One reversible stiffening cycle can be readily programmed into materials through progressive crosslinking with a subsequently cleavable crosslink, resulting in a system where unidirectional stiffening and softening are controlled orthogonally to one another.[37] When true reversibility is desired, the conformational change of a photoresponsive chemical group such as an azobenzene can be exploited for repeat cycling of hydrogel stiffness.[38] Through site-specific modification of both the N- and C- termini, full-length proteins may be incorporated as functional crosslinks within a biomaterial. Since the end-to-end translational movement

lengths associated with stimuli-sensitive proteins is typically much larger than that obtained by small molecules, systems utilizing photoactivatable proteins can enable cyclic control over stiffness spanning a significantly larger dynamic range.[39,40] Using the conformational change of the optogenetic protein pair LOV2-Jα, our group developed one such gel which softens in response to cytocompatible blue light and rapidly recovers its native stiffness in the dark.[39] Intriguingly, fibroblast activation was enhanced within these gels when subjected to pulsatile stiffening relative to both persistently soft and stiff controls; these results suggest that cells are not only sensitive towards the static substrate stiffness, but the temporal element of stiffening as well.

Crosslinking gel materials with photoresponsive proteins containing an engineered phytochrome that reversibly dimerize upon red/near-IR light exposure has also provided a route to dynamic mechanical control.[40] In this work, human MSCs were seeded onto the gel and subjected to 24 hrs of mechanical priming in the soft configuration, followed by an additional day of either static or dynamic photoswitching of the material. Transcriptomic analysis of cells on the static and dynamic substrates revealed that material stiffness for the first 24 hrs after seeding was more important than any subsequent dynamic alterations to the modulus. Notably, transforming growth factor-β1 (TGF-β1) and yes-associated protein (YAP) pathways were influenced by mechanical priming and less sensitive to more recent modulus switching, underscoring their known roles underpinning longer-term cellular mechanical memory. In contrast, mitogen-activated protein kinase (MAPK) signaling was a key pathway distinguishing slow (160 min) and fast (10 min) cycling of the substrate modulus, supporting its role as a more acute downstream effector of cell mechanotransduction.

With recent efforts to characterize the effects of more complex mechanical and physical properties (e.g., viscoelasticity, material plasticity, strain softening/stiffening, network architecture) on cell fate and function, we anticipate that the field's next step will be to innovate strategies to reversibly control these material properties as has been done for substrate stiffness. Dynamic control over substrate viscoelasticity poses an interesting challenge, as properties such as stress relaxation are already time-dependent and reliant on non-covalent interactions within the hydrogel network.

Controlling Dynamic Presentation of Bioactive Ligands

Biological tissues are dynamic, not just mechanically, but also biochemically (Figure 2). Cells are exposed to tightly regulated cues in the form of secreted proteins and factors from other cells and the extracellular environment, which in turn influence cell phenotype. Uniform decoration of engineered microenvironment with small molecules, peptides, and whole proteins has become fairly straightforward; several chemical strategies are now in existence to covalently functionalize hydrogels with bioactive elements.[41–46] For nonspecific tethering of proteins to a scaffold, custom and commercially available small molecules (e.g., activated esters) can be used to stochastically install functional handles for biomaterial tethering [e.g., azides, alkynes, maleimides, (meth)acrylates] onto a protein under gentle, aqueous conditions. For controlled tethering to a scaffold and minimal impact on protein activity, site-specific modification techniques including through the use of sortase,[42] N-

myristoyltransferase (NMT),[43] and the SpyCatcher/Tag pair[44] have proven useful. Further flexibility of chemical group placement within a protein can be achieved with noncanonical amino acid tagging and genetic code expansion.[45]

User-Controlled Presentation of Bioactive Ligands

Heterogenous presentation of biochemical cues within hydrogels has most frequently been achieved using photopatterning, whereby directed light exposure can be used to dictate when and where biomolecules are presented. Photomediated ligations, including those based on acrylates,[47,48] thiol-ene,[49] oxime,[50] and enzymatic chemistries,[51] have proven particularly useful for immobilizing small molecules, peptides, and even proteins into hydrogels. Photodegradation reactions, primarily based off of *ortho*-nitrobenzyl ester,[30] coumarin,[52] or photocleavable proteins,[53] have found benefit for stimulating biomolecule release. These unidirectional material patterning approaches have enabled spatiotemporal control over proliferation, outgrowth, differentiation, and other complex cell fates within 3D gels.

Reversible biochemical control uniquely enables researchers to probe feedback loops between cells and their environment, which may be informative to identify tipping points in disease pathophysiology.[36] The first path to reversible payload tethering and release from biomaterial is simply combining an additive chemistry with an orthogonal subtractive one, and this strategy has been successfully used to sequentially tether and release whole proteins to create complex and temporally evolving patterns capable of directing cell fate.[50,54,55] However, this approach only allows one cycle of reversion, and current efforts seek to identify fully reversibly chemistries. One of the most promising approaches to date employs an allyl sulfide chain transfer, in which active radicals can help to trade one network-bound thiolated biomolecule for another.[56] While this reversible chemistry offers some repeatability, nonspecific reactions associated with free-radicals limit reversibility and may be undesirable in the presence of cells. The reversible association of protein binding pairs has also been exploited through the optogenetic LOVTRAP system in hydrogels, which also enabled repetitive cycles of protein patterning and release.[57] While fully reversible, this strategy relies on non-covalent protein association with the gel that is comparatively unstable. Ongoing efforts seek to identify truly reversible and covalent strategies for biomolecule patterning.

Cell-Dictated Release of Bioactive Factors

Beyond user-directed ligand presentation, an emergent line of materials development focuses on systems that present biochemical cues in a cell-directed manner. The extracellular matrix acts not just as a structural scaffold but also as a reservoir for sequestered growth factors that become available to the cell upon matrix strain or remodeling.[58,59] One well-characterized example is the sequestration of TGF-β1 in the form of a large latent complex in the ECM, which when activated by strain or ECM degradation, causes fibroblast activation in the initiation of tissue repair.[60] Disrupting this sequestration capacity leads to dysregulated TGF-β1 signaling, in turn causing developmental defects, cardiac disease, and cancers.[61] The context of growth factor presentation is also incredibly important; when cells release sequestered growth factors from the ECM, receptor clustering with integrins can alter the

nature of downstream intracellular signaling cascades. Therefore, mimicking cell-mediated release of bioactive factors from the ECM has been of great interest both for researchers wishing to deliver these factors therapeutically, as well as for those studying growth factor signaling through disease.

Growth factor sequestration within an engineered matrix for subsequent cell-mediated release can be accomplished through the inclusion of natural ECM components with growth factor affinity (e.g., fibronectin, heparin) or engineered components (e.g., antibodies, binding peptides).[58] More recently, synthetic aptamers have been designed to bind growth factors with high affinity and tether them to the matrix.[62] By conjugating a cell-adhesive peptide to the free end of the aptamer, growth factors may be released from their bind by cell-mediated traction forces. The aptamer design of these Traction-Activated Payloads (TrAPs) could be modified to accommodate nearly any protein payload, whereas cell-selective release can also be achieved by modifying the cell-adhesive ligand through which the TrAP unravels. TrAP delivery of platelet-derived growth factor-BB (PDGF-BB) promoted denser cell growth in serum-free conditions that persisted for two weeks, suggesting that the growth factor could be stabilized by the aptamer in a manner similar to that of native ECM sequestration.

One of the most important aspects in designing a dynamic microenvironment for cells is the selection of an exogenous or cell-mediated trigger to induce material dynamics.[63] The number of triggers available to a researcher is ever-increasing and now includes bioorthogonal mechanisms including remote fields (e.g., light, ultrasound, magnetism)[64] and engineered enzymes[35]. Utilization of many of these exogenous triggers frequently requires specialty chemistries that are synthetic inaccessible and are limited in their capacity for multiplexing. Technologies for gene editing, specifically Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas, are now easily adaptable to target nearly any DNA sequence of interest, allowing researchers to quickly customize the DNA sequence which Cas targets by changing the sequence of the guide RNA. This complete customizability is highly desired for programming the stimulus response of a biomaterial, and for this reason the CRISPR/Cas12a system has been recently adapted to the materials space.[65] Cas12a specifically recognizes a double-stranded DNA (dsDNA) trigger, but upon sequence recognition collaterally cleaves nearby single-stranded DNA (ssDNA) nonspecifically.[65] By incorporating a payload tethered to a hydrogel backbone by ssDNA or incorporating ssDNA into the material crosslink, stimulus-responsive payload release or bulk material degradation was induced upon introduction of a specific dsDNA trigger. By customizing the guide RNA and dsDNA trigger sequences, this strategy is easily adapted to any oligonucleotide trigger; since the mechanism for degradation is the nonspecific collateral cleavage of ssDNA downstream to dsDNA recognition, payload release is not entirely specific. Exploring material dynamics reliant only on specific dsDNA cleavage could open the door to near limitless multiplexing potential based on distinct target sequences and gRNAs.

Fabricating Complex Tissue Structures

The heterogeneous and hierarchical structures of biological tissues provide both an inspiration and a challenge to engineers looking to recapitulate structurally facilitated

tissue functions. The human body is full of branching structures scaling many orders of magnitude in size for gas, liquid, and nutrient transport, anisotropic tissues optimized for the generation of force, and gradients of stiffness or biochemical factors. Appropriate structures are often necessary for the function of bioengineered tissues; as one example, engineered heart muscles must align to mature and generate appreciable force, and large tissues are limited by nutrient diffusion and require perfusable vasculature. Recent advances in spatially controlling material structural properties have opened the door to tissues with greater functionality (Figure 3).

Additive Manufacturing of Engineered Tissues

Additive manufacturing of complex architectures with native and specialty biomaterials has hit maturity, and several advances to the field over the past few years now enable facile printing of exquisitely complex geometries. Specialty bioinks are no longer required to print high resolution structures – with the development of freeform reversible embedding of suspended hydrogels (FRESH), native collagen can be sculpted down to a resolution of 20 micrometers through extrusion into a buffered gelatin slurry that is washed away upon incubation at 37 °C.[66] This technique has enabled perfusable vessels, a contractile ventricular model, and an at-scale heart to be printed using unmodified collagen.[66,67] Though extrusion is perhaps the simplest method for bioprinting, stereolithographic photopolymerization of materials enables highly parallelized fabrication of structures. While this approach is much faster and offers fewer architectural constraints, cytotoxic photoinitiators/photoabsorbers have limited its use for bioprinting.[68] Recently, the cytocompatible food dye tartrazine was identified as an alternative photoabsorber for stereolithography, leading to the development of a technique termed stereolithography apparatus for tissue engineering (SLATE). By minimizing unwanted out-of-plane photopolymerization, SLATE has proven useful for the fabrication of complex and interlocking void spaces within hydrogels to recapitulate vascular geometries, and can be used to rapidly construct engineered tissues containing a wide variety of cell types, including primary human stem cells.[69]

While stereolithographic polymerization of tissues enables precise and parallelized deposition of voxels of a single material by layer, it does not readily enable voxel-by-voxel control of material composition. To this end, multimaterial multinozzle arrays have been reported, capable of switching between up to 8 materials that can be switched at the nozzle head level at a frequency of up to 50Hz.[70] Applied to bioprinting, this approach could provide unprecedented control over the composition of a biomaterial in 3D, with the benefits of parallelized material deposition. To further expand the palette of bioinks that can be 3D printed, eliminating requirements of photopolymerization and material extrudability, open-microfluidic well-plate inserts can be exploited to sculpt gel precursors by capillary action, facilitating controlled deposition of 3D structures with nearly any hydrogel chemistry.[71]

Another limitation of conventional stereolithography for additive manufacture is its resolution (typically tens to hundreds of microns), which may not be small enough for applications exploring subcellular patterning of topographical and biological cues.[72] An alternative means for additive manufacture is multiphoton polymerization, a versatile

strategy in which a femtosecond-pulsed laser can initiate polymerization on a voxel-by-voxel basis, enabling a much finer resolution (hundreds of nanometers).[73] Many of the conventionally used bioinks used for stereolithography also have multiphoton absorbance, and thus can be readily used with this technique. Though these methods offer unmatched spatial patterning resolution, one critical limitation lies in fabrication speed; as polymerization occurs one voxel at a time, these techniques are largely reserved for small-featured structures.[74]

Just as the biomaterials field has successfully coopted multiphoton laser scanning from the photonics community for additive manufacturing, light sheet microscopy is now also poised for adoption to rapidly generate relatively high-resolution structures. In light sheet microscopy, the sample is illuminated through one axis while its fluorescence signal is detected through one perpendicular, enabling very fast scanning of large volumes that would be prohibitive to image by conventional laser scanning microscopy.[75] One early and very recent adaptation of this technique for additive manufacturing – xolography – polymerizes structures by illuminating the resin with a projected image and an intersecting light sheet at different wavelengths and along orthogonal axes.[76] Photopolymerization is achieved in a dual-color photoinitiator (DCPI) system, in which the activity of a benzophenone type II photoinitiator is optically regulated with a ultraviolet light-responsive spiropyran photoswitch. This approach improves upon the resolution of stereolithography by an order of magnitude, while generating large-scale objects at four-to-five orders of magnitude faster than multiphoton lithography. For biological applications, we anticipate that the first-generation DCPI will be limited by cytotoxicity and carcinogenicity similar to its benzophenone precursor, but look forward to the development of biocompatible initiators for this uniquely enabling additive manufacturing technique.[77]

Subtractive Manufacturing of Engineered Tissues

Subtractive manufacturing, whereby patterned removal of a subset of bulk starting material, has also found utility for tissue fabrication; micron-scale resolution over complex void volumes have made these the strategy of choice for creating well-defined microvascular networks. Utilizing high-intensity femtosecond-pulsed lasers to induce nonspecific photoablation of hydrogel materials (e.g., PEG, collagen), early efforts demonstrated the feasibility of laser-based subtractive manufacturing as a technique to generate perfusable microvascular arrays within a biomaterial.[78,79] Though nonspecific photoablation can be used to fabricate vessels down to the size of a human capillary,[80] the process is slow and requires high illumination intensities which are generally not cytocompatible. This process can be sped up dramatically through employment of photolabile moieties within the gel backbone, enabling material degradation and capillary-sized vessel patterning in the presence of living cells.[30,81] Further improvements have been made by employing small molecule photosensitizers in conjunction with degradable gels.[82] Despite these successes, there remains substantial room for improvements on the speed and throughput of subtractive tissue engineering, calling for the same ingenuity which catalyzed the additive manufacturing improvements reviewed above.

Engineered Control of Tissue Anisotropy

The microstructural alignment, or anisotropy, of an engineered biomaterial can impact a variety of tissue and cellular responses, including neurite outgrowth along aligned surfaces, fibroblast activation following myocardial infarction, and the coordinated contraction of muscle for force generation.[83–85] Anisotropic biomaterials are traditionally fabricated through electrospinning or by directional freezing prior to lyophilization.[86,87] Alternatively, as collagen in solution has negative diamagnetic anisotropy, aligned collagen hydrogels can be formed under a supermagnetic field.[83] Until recently, however, the magnetic alignment of collagen gels could only be accomplished in bulk and was limited by the specialized instrumentation required.[88]

Even more recently, local spatial control of material anisotropy has been achieved on a bioprinter with a regular magnet by embedding streptavidin-conjugated iron nanoparticles into a collagen/agarose bioink such that magnetic field-induced particle movement during gelation promoted collagen fiber alignment.[89] By equipping the 3D bioprinter with a magnet, anisotropy could be induced upon ink deposition through pulsed magnetic fields.[89]

In another powerful approach complementary to directed material alterations, tissue anisotropy can be introduced directly through acoustic patterning of cells.[90] In this technique, cells are forced into the pressure nodes of a standing ultrasound wave as a hydrogel is polymerized around them, thus generating repeating lines or points of cells within the gel as the basis for microstructural anisotropy. This approach has been successfully employed to pattern myoblasts into aligned engineered muscle.

Cell-Guided Construction of Engineered Tissues

All of the above fabrication techniques offer a high degree of user control over guiding biological structure. Yet, complex biological structures may also be fabricated by offering control over self-assembly to encapsulated cells and providing minimal cues to guide self-emergent tissue architecture. This approach to tissue construction comes from the philosophy of organoid biology, which harnesses stem-cell aggregation and self-guided differentiation into complex biological structures.[91] To take organoid biology beyond the millimeter scale, the same cells or cellular aggregates used to form organoids can also be extruded through a syringe at high density, and allowed to self-aggregate in a workflow termed bioprinting-assisted tissue emergence (BATE).[92] This approach derives all complexity from organoid self-assembly, and as a result requires no specialized equipment for the syringe extrusion printing. The future of bioprinting holds larger and even more complex structures than ever, yet these advances may very well be delivered in simple and streamlined workflows.

Engineering Cells as Mechanical and Biochemical Actuators of the Microenvironment

Genetic engineering and synthetic biological approaches have become more accessible than ever, leading to several recent studies that use engineered cells themselves to pattern phenotype and the microenvironment (Figure 4). Both biochemical and mechanical actuation

through synthetic gene circuits have been explored to spatially control tissue structure and function.

The most extensive suite of remote triggers for cells may be borrowed from the field of optogenetics, which focuses on the use of light to precisely direct gene regulation and cell function. Optogenetic triggers, which come in the form of proteins that may dimerize, conformationally change, aggregate, or open/close a channel, have been developed to manipulate nearly every level of biological signaling.[93] Though we have already introduced studies which borrow optogenetic proteins to impart dynamic biomaterial properties, this rich toolkit can also affords control over cell adhesion to the matrix and neighboring cells,[94,95] migration,[96] protein expression,[97–99] and ion flux,[100] all of which could be used to control the extracellular microenvironment of an engineered tissue from the inside out. Paralleling development of orthogonal biomaterial triggers, orthogonal optogenetic triggers have also been developed and enable multiplexed control over cell function.[101,102] Optogenetic control over cell migration can be combined with light-sensitive material chemistries.[103] For example, stem cells transfected with a photoactivatable Rac kinase were rendered susceptible to migration in the direction of a 458 nm light pulse, while hydrogel channels could be dynamically ablated through cleavage of an *ortho*-nitrobenzyl moiety as a PEG hydrogel crosslink.[103] Simultaneously controlling cell behavior from inside-out and outside-in offers unique opportunities to engineer the dynamic reciprocity between cell and environment that can be ubiquitously found throughout development and disease.

Using the human heat shock promoter HSPA7, several past studies have successfully hijacked the heat stress response to induce transgene expression in response to mild heating.[104,105] Recent work adopts this strategy for tissue engineering, through the incorporation of perfusable channels through which hot fluid may be pumped, thus creating well-defined temperature profiles throughout the material that governs patterned gene expression of encapsulated cells.[106] This method was then used to induce spatial expression of liver enzymes through patterned expression of a Wnt signaling regulator.[106]

Most biological tissues are not uniform slabs, and instead exhibit complex, curved geometries that spontaneously emerge throughout development and dictate tissue-specific functions. Just as bulk mechanical properties of a tissue can influence cell state, local topological and mechanical cues also dictate the behavior of tissues and the cells within them.[107,108] Both as a means of replicating developmental emergence of tissue shapes and as a strategy for creating appropriately shaped tissues for regenerative therapies, the actuation of cell contraction has been harnessed as a mechanism for generating curved structures from biomaterials.[109,110] This can be accomplished by either patterning a contractile cell type or modulating that cell's ability to compact zones of a material. DNA-programmed assembly of cells has been used to localize seed mesenchymal cells onto collagen hydrogels and rationally induce folding of these gels into a user-specified shape by cellular contraction.[109] Through entirely synthetic cell patterning, this technique was used to recapitulate the incredibly complex tessellated curvature of embryonic chick gut lumen. During the dynamic folding process, actively contractile mesenchymal cells were found able to guide the migration of "passenger" vascular endothelial cells into the nascent folds. Alternatively, by spatially controlling the incorporation of an peptide which inhibits cell contraction in

an evenly seeded gel, flat hydrogels can be made curved by inducing regions of high contraction by cells in the material.[110] These curved gels were fabricated to recreate the geometry of the human cornea, and were able to guide the differentiation pattern of human epithelial stem cells into corneal epithelium. Though in this case contraction was indirectly induced by providing fetal bovine serum in the medium, logical "AND" gate crosslinkers susceptible to one exogenous stimulus and one cell-secreted factor could be harnessed for tighter control over cell remodeling and contraction of a material.[111]

In the future, we envision that instead of producing a protein in bulk and biochemically patterning it within gels, proteins may instead be produced *in situ* by encapsulated cells under tight 4D transcriptional control by optogenetic or thermogenetic means. This concept has only recently been preliminarily explored, whereby bacteria transformed with an optogenetic protein plasmid (pDawn) and encapsulated within a gel expressed and secreted Red Fluorescent Protein in response to blue light.[112] With several strategies to genetically install chemical groups which facilitate gel-protein conjugation, secreted proteins could be sequestered by the hydrogel in a manner mimicking the role of the ECM.

Engineering Simplicity

The options available to a researcher wishing to engineer a biological microenvironment – material platforms, conjugation chemistries, mechanical and biochemical factors to consider – are nearly limitless. Contrary as it may sound, simplicity is also a key factor to consider when engineering complex microenvironments and is a crucial to the utility of any tool towards the study of disease. Many life-sciences labs are not equipped with the instrumentation or personnel for organic synthesis of the precursors and reagents used in many dynamic biomaterials. It is for this reason that Matrigel remains the one of the most widely-used 3D matrices, despite limitations in batch variability and a lack of tunability.[113] Conversely, biomaterials labs depend on expert collaborators to provide impactful applications for the uniquely enabling materials that they develop. In many ways, biomaterials development to study the microenvironment have outpaced the utilization of such materials for studying biology.

Many systems highlighted herein gain utility from simplicity. SLATE bioprinting uses Food and Drug Administration (FDA)-approved food additive dyes as photoabsorbers.[69] Recent studies pushing forward the culture of organoids in synthetic matrices have used hydrolysis as a trigger for the dynamic substrate softening required to support organoid formation.[34] BATE relies upon biology to provide emergent complexity in printed organoids, but only requires simple extrusion printing using a syringe and a manually controlled microscope stage.[92] These types of systems are easy for a non-engineer to adopt and exploit to catalyze impactful and translatable findings with respect to any disease of interest.

Our group and others have made a turn towards genetically encoded approaches for hydrogel formation and modulation, circumventing many of the insurmountable barriers that synthetic organic chemistry have imposed on biology labs interest in using biomaterial tools.[53,114–116] By co-expressing pre-existing enzymes for site-specific protein modification (e.g., NMT, sortase) alongside a protein of interest, bioorthogonal handles can be installed *in situ*

for direct incorporation into a hydrogel network with no post-synthetic modification.[53] Spontaneous protein-protein binding (e.g., SpyCatcher/Tag ligation) can be exploited for hydrogel crosslinking with no synthetic elements or catalysts, distilling the field of synthetic hydrogel matrices into a format accessible to biologists.[116,117]

Future Directions

As the field of dynamic biomaterials develops increasingly modular biomaterials platforms and flexible bioconjugation chemistries, it is now possible to take many popular biomaterials platforms off the shelf and simultaneously specify an expansive set of biochemical and physical properties in tandem. Future work will certainly continue to push the limits of stimulus responsiveness towards improved multiplexing, utilization of triggers with *in vivo* relevance, scaffolding elements which integrate biological signals and generate feedback, and full spatiotemporal regulation that matches all biological scales. With such expanded levels of customization, it will be increasingly important to seek out the simplest mechanisms of control required for any given experimental question.

We believe that the field still has much to borrow from the emergent and neighboring spheres of optogenetics and protein engineering. Current approaches typically use a naturally derived protein or peptide as is to impart a biomaterial with a biological function. Yet, with modern-day protein engineering tools, it is possible to optimize proteins for sustained bioactivity and tunable release from a biomaterial scaffold.[118] Furthermore, as *de novo* protein design continues to reach maturity, we envision a future in which protein elements may be rationally designed from grounds up as desired components of biomaterials.[119] Already there is an ever-expanding toolkit of structural components[120–122] and protein logic gates[123–125] which – in combination with site-specific modification strategies to incorporate these elements into a material – provide ripe grounds for exploration.

Lastly, while the philosophy of engineering complexity from the outside in has yielded unprecedented control over the biological microenvironment, approaches that harness self-emergent complexity such as BATE and the broader field of organoid biology have demonstrated that engineered tissues may also be constructed from naïve cell types; as complex 3D folds may be generated from relatively simple 2D cell patterns, these strategies promise to yield relatively mature engineered tissues with very few exogenous cues in a largely unsupervised manner. Such emergent strategies may prove more effective for some tissue engineering applications than traditional approaches involving patterned materials. Certainly, both philosophies should be considered by biologists, biomaterial scientists, and tissue engineers alike, and we turn towards many exciting future studies to identify which approach is best suited for a given biological application.

Funding Sources

ABBREVIATIONS

3D	three-dimensional
4D	four-dimensional
BATE	bioprinting-assisted tissue emergence
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DCPI	dual-color photoinitiator
dsDNA	Double-stranded deoxyribonucleic acid
ECM	extracellular matrix
FDA	United States Food and Drug Administration
FRESH	freeform reversible embedding of suspended hydrogels
MAPK	mitogen-activated protein kinase
MSC	mesenchymal stem cell
NMT	N-myristoyltransferase
PEG	Poly(ethylene glycol)
PDGF-BB	platelet-derived growth factor-BB
SLATE	stereolithographic apparatus for tissue engineering
ssDNA	single-stranded deoxyribonucleic acid
TrAP	traction-activated payload
YAP	yes-associated protein

REFERENCES

(1). Muncie JM; Weaver VMThe Physical and Biochemical Properties of the Extracellular Matrix Regulate Cell Fate. In Current Topics in Developmental Biology; Academic Press Inc., 2018; Vol. 130, pp 1–37. 10.1016/bs.ctdb.2018.02.002. [PubMed: 29853174]

(2). Wang Y; Hu G; Hill RC; Dzieciatkowska M; Hansen KC; Zhang XB; Yan Z; Pei MMatrix Reverses Immortalization-Mediated Stem Cell Fate Determination. Biomaterials2021, 265, 120387. 10.1016/j.biomaterials.2020.120387. [PubMed: 32987274]

(3). Lampi MC; Reinhart-King CATargeting Extracellular Matrix Stiffness to Attenuate Disease: From Molecular Mechanisms to Clinical Trials. Science Translational Medicine. American Association for the Advancement of Science13, 2018, p 475. 10.1126/scitranslmed.aao0475.

(4). Dalby MJ; García AJ; Salmeron-Sanchez MReceptor Control in Mesenchymal Stem Cell Engineering. Nature Reviews Materials. Nature Publishing Group131, 2018, pp 1–14. 10.1038/natrevmats.2017.91.

(5). Engler AJ; Sen S; Sweeney HL; Discher DEMatrix Elasticity Directs Stem Cell Lineage Specification. Cell2006, 126 (4), 677–689. 10.1016/j.cell.2006.06.044. [PubMed: 16923388]

(6). Sewanan LR; Schwan J; Kluger J; Park J; Jacoby DL; Qyang Y; Campbell SGExtracellular Matrix From Hypertrophic Myocardium Provokes Impaired Twitch Dynamics in Healthy Cardiomyocytes. JACC Basic to Transl. Sci2019, 4 (4), 495–505. 10.1016/j.jacbts.2019.03.004.

(7). Xu R; Boudreau A; Bissell MJTissue Architecture and Function: Dynamic Reciprocity via Extra- and Intra-Cellular Matrices. Cancer and Metastasis Reviews. NIH Public Access2009, pp 167–176. 10.1007/s10555-008-9178-z. [PubMed: 19160017]

(8). Chaudhuri O; Cooper-White J; Janmey PA; Mooney DJ; Shenoy VBEffects of Extracellular Matrix Viscoelasticity on Cellular Behaviour. Nature. Nature Research827, 2020, pp 535–546. 10.1038/s41586-020-2612-2.

(9). Sun Z; Guo SS; Fässler RIntegrin-Mediated Mechanotransduction. Journal of Cell Biology. Rockefeller University Press1121, 2016, pp 445–456. 10.1083/jcb.201609037.

(10). Elosegui-Artola A; Trepat X; Roca-Cusachs PControl of Mechanotransduction by Molecular Clutch Dynamics. Trends in Cell Biology. Elsevier Ltd51, 2018, pp 356–367. 10.1016/j.tcb.2018.01.008. [PubMed: 29496292]

(11). Caporizzo MA; Chen CY; Bedi K; Margulies KB; Prosser BLMicrotubules Increase Diastolic Stiffness in Failing Human Cardiomyocytes and Myocardium. Circulation2020, 141 (11), 902–915. 10.1161/CIRCULATIONAHA.119.043930. [PubMed: 31941365]

(12). A Simple Indentation Device for Measuring Micrometer-Scale Tissue Stiffness. 2010. 10.1088/0953-8984/22/19/194120.

(13). Charrier EE; Pogoda K; Wells RG; Janmey PAControl of Cell Morphology and Differentiation by Substrates with Independently Tunable Elasticity and Viscous Dissipation. Nat. Commun2018, 9 (1), 1–13. 10.1038/s41467-018-02906-9. [PubMed: 29317637]

(14). Chaudhuri O; Gu L; Darnell M; Klumpers D; Bencherif SA; Weaver JC; Huebsch N; Mooney DJSubstrate Stress Relaxation Regulates Cell Spreading. Nat. Commun2015, 6 (1), 6365. 10.1038/ncomms7365.

(15). Loebel C; Mauck RL; Burdick JALocal Nascent Protein Deposition and Remodelling Guide Mesenchymal Stromal Cell Mechanosensing and Fate in Three-Dimensional Hydrogels. Nat. Mater2019, 18 (8), 883–891. 10.1038/s41563-019-0307-6. [PubMed: 30886401]

(16). Nam S; Lee J; Brownfield DG; Chaudhuri OViscoplasticity Enables Mechanical Remodeling of Matrix by Cells. 2016. 10.1016/j.bpj.2016.10.002.

(17). Wisdom KM; Adebowale K; Chang J; Lee JY; Nam S; Desai R; Rossen NS; Rafat M; West RB; Hodgson L; Chaudhuri OMatrix Mechanical Plasticity Regulates Cancer Cell Migration through Confining Microenvironments. Nat. Commun2018, 9 (1), 1–13. 10.1038/s41467-018-06641-z. [PubMed: 29317637]

(18). Grolman JM; Weinand P; Mooney DJExtracellular Matrix Plasticity as a Driver of Cell Spreading. Proc. Natl. Acad. Sci2020, 202008801. 10.1073/pnas.2008801117.

(19). van Oosten ASG; Chen X; Chin LK; Cruz K; Patteson AE; Pogoda K; Shenoy VB; Janmey PAEmergence of Tissue-like Mechanics from Fibrous Networks Confined by Close-Packed Cells. Nature2019, 573 (7772), 96–101. 10.1038/s41586-019-1516-5. [PubMed: 31462779]

(20). Bordeleau F; Mason BN; Lollis EM; Mazzola M; Zanotelli MR; Somasegar S; Califano JP; Montague C; LaValley DJ; Huynh J; Mencia-Trinchant N; Abril YLN; Hassane DC; Bonassar LJ; Butcher JT; Weiss RS; Reinhart-King CAMatrix Stiffening Promotes a Tumor Vasculature Phenotype. Proc. Natl. Acad. Sci. U. S. A. 2017, 114 (3), 492–497. 10.1073/pnas.1613855114. [PubMed: 28034921]

(21). Matera DL; DiLillo KM; Smith MR; Davidson CD; Parikh R; Said M; Wilke CA; Lombaert IM; Arnold KB; Moore BB; Baker BMMicroengineered 3D Pulmonary Interstitial Mimetics Highlight a Critical Role for Matrix Degradation in Myofibroblast Differentiation. Sci. Adv2020, 6 (37), eabb5069. 10.1126/sciadv.abb5069. [PubMed: 32917680]

(22). Madl CM; Katz LM; Heilshorn SCTuning Bulk Hydrogel Degradation by Simultaneous Control of Proteolytic Cleavage Kinetics and Hydrogel Network Architecture. ACS Macro Lett. 2018, 7 (11), 1302–1307. 10.1021/acsmacrolett.8b00664. [PubMed: 32523799]

(23). Stowers RS; Allen SC; Suggs LJ; Anseth KSDynamic Photofuning of 3D Hydrogel Stiffness. Proc. Natl. Acad. Sci. U. S. A. 2015, 112 (7), 1953–1958. 10.1073/pnas.1421897112. [PubMed: 25646417]

(24). Young JL; Engler AJHydrogels with Time-Dependent Material Properties Enhance Cardiomyocyte Differentiation in Vitro. Biomaterials2011, 32 (4), 1002–1009. 10.1016/j.biomaterials.2010.10.020. [PubMed: 21071078]

(25). Guvendiren M; Burdick JAStiffening Hydrogels to Probe Short- and Long-Term Cellular Responses to Dynamic Mechanics. Nat. Commun2012, 3 (1), 1–9. 10.1038/ncomms1792.

(26). Mabry KM; Lawrence RL; Anseth KSDynamic Stiffening of Poly(Ethylene Glycol)-Based Hydrogels to Direct Valvular Interstitial Cell Phenotype in a Three-Dimensional Environment. Biomaterials2015, 49, 47–56. 10.1016/J.BIOMATERIALS.2015.01.047. [PubMed: 25725554]

(27). Liu H-Y; Greene T; Lin T-Y; Dawes CS; Korc M; Lin C-CEnzyme-Mediated Stiffening Hydrogels for Probing Activation of Pancreatic Stellate Cells. Acta Biomater. 2017, 48, 258–269. 10.1016/j.actbio.2016.10.027. [PubMed: 27769941]

(28). Stoppel WLWL; Gao AEAE; Greaney AMAMAM; Partlow BPBPBP; Bretherton RCRC; Kaplan DLDLDL; Black LDLDElastic, Silk-Cardiac Extracellular Matrix Hydrogels Exhibit Time-Dependent Stiffening That Modulates Cardiac Fibroblast Response. J. Biomed. Mater. Res. - Part A2016, 104 (12), 3058–3072. 10.1002/jbm.a.35850.

(29). Günay KA; Ceccato TL; Silver JS; Bannister KL; Bednarski OJ; Leinwand LA; Anseth KSPEG–Anthracene Hydrogels as an On-Demand Stiffening Matrix To Study Mechanobiology. Angew. Chemie - Int. Ed2019, 58 (29), 9912–9916. 10.1002/anie.201901989.

(30). Kloxin AM; Kasko AM; Salinas CN; Anseth KSPhotodegradable Hydrogels for Dynamic Tuning of Physical and Chemical Properties. Science2009, 324 (5923), 59–63. 10.1126/science.1169494. [PubMed: 19342581]

(31). DeForest CA; Anseth KSCytocompatible Click-Based Hydrogels with Dynamically Tunable Properties through Orthogonal Photoconjugation and Photocleavage Reactions. Nat. Chem2011, 3 (12), 925–931. 10.1038/nchem.1174. [PubMed: 22109271]

(32). Brown TE; Marozas IA; Anseth KSAmplified Photodegradation of Cell-Laden Hydrogels via an Addition-Fragmentation Chain Transfer Reaction. Adv. Mater2017, 29 (11), 1605001. 10.1002/adma.201605001.

(33). Rapp TL; Highley CB; Manor BC; Burdick JA; Dmochowski IJRuthenium-Crosslinked Hydrogels with Rapid, Visible-Light Degradation. Chem. - A Eur. J2018, 24 (10), 2328–2333. 10.1002/chem.201704580.

(34). Gjorevski N; Sachs N; Manfrin A; Giger S; Bragina ME; Ordóñez-Morán P; Clevers H; Lutolf MPDesigner Matrices for Intestinal Stem Cell and Organoid Culture. Nature2016, 539 (7630), 560–564. 10.1038/nature20168. [PubMed: 27851739]

(35). Valdez J; Cook CD; Ahrens CC; Wang AJ; Brown A; Kumar M; Stockdale L; Rothenberg D; Renggli K; Gordon E; Lauffenburger D; White F; Griffith LOn-Demand Dissolution of Modular, Synthetic Extracellular Matrix Reveals Local Epithelial-Stromal Communication Networks. Biomaterials2017, 130, 90–103. 10.1016/J.BIOMATERIALS.2017.03.030. [PubMed: 28371736]

(36). Rosales AM; Anseth KSThe Design of Reversible Hydrogels to Capture Extracellular Matrix Dynamics. Nat. Rev. Mater2016, 1. 10.1038/natrevmats.2015.12.

(37). Rosales AM; Vega SL; DelRio FW; Burdick JA; Anseth KSHydrogels with Reversible Mechanics to Probe Dynamic Cell Microenvironments. Angew. Chemie - Int. Ed2017, 56 (40), 12132–12136. 10.1002/anie.201705684.

(38). Rosales AM; Mabry KM; Nehls EM; Anseth KSPhotoresponsive Elastic Properties of Azobenzene-Containing Poly(Ethylene-Glycol)-Based Hydrogels. Biomacromolecules2015, 16 (3), 798–806. 10.1021/bm501710e. [PubMed: 25629423]

(39). Liu L; Shadish JA; Arakawa CK; Shi K; Davis J; DeForest CACyclic Stiffness Modulation of Cell☐Laden Protein–Polymer Hydrogels in Response to User☐Specified Stimuli Including Light. Adv. Biosyst2018, 2 (12), 1800240. 10.1002/adbi.201800240. [PubMed: 34316509]

(40). Hörner M; Raute K; Hummel B; Madl J; Creusen G; Thomas OS; Christen EH; Hotz N; Gübeli RJ; Engesser R; Rebmann B; Lauer J; Rolauffs B; Timmer J; Schamel WWA; Pruszak J; Römer W; Zurbriggen MD; Friedrich C; Walther A; Minguet S; Sawarkar R; Weber WPhytochrome☐Based Extracellular Matrix with Reversibly Tunable Mechanical Properties. Adv. Mater2019, 31 (12), 1806727. 10.1002/adma.201806727.

(41). Shadish JA; DeForest CASite-Selective Protein Modification: From Functionalized Proteins to Functional Biomaterials. Matter. Cell Press18, 2020, pp 50–77. 10.1016/j.matt.2019.11.011.

(42). Guimaraes CP; Witte MD; Theile CS; Bozkurt G; Kundrat L; Blom AEM; Ploegh HLSite-Specific C-Terminal and Internal Loop Labeling of Proteins Using Sortase-Mediated Reactions. Nat. Protoc2013, 8 (9), 1787–1799. 10.1038/nprot.2013.101. [PubMed: 23989673]

(43). Heal WP; Wright MH; Thinon E; Tate EWMultifunctional Protein Labeling via Enzymatic N-Terminal Tagging and Elaboration by Click Chemistry. Nat. Protoc2012, 7 (1), 105–117. 10.1038/nprot.2011.425.

(44). Zakeri B; Fierer JO; Celik E; Chittock EC; Schwarz-Linek U; Moy VT; Howarth MPeptide Tag Forming a Rapid Covalent Bond to a Protein, through Engineering a Bacterial Adhesin. Proc. Natl. Acad. Sci. U. S. A2012, 109 (12), E690–E697. 10.1073/pnas.1115485109. [PubMed: 22366317]

(45). Krall N; Da Cruz FP; Boutureira O; Bernardes GJLSite-Selective Protein-Modification Chemistry for Basic Biology and Drug Development. Nature Chemistry. Nature Publishing Group21, 2016, pp 103–113. 10.1038/nchem.2393.

(46). Hermanson GTBioconjugate Techniques: Third Edition; Elsevier Inc., 2013. 10.1016/C2009-0-64240-9.

(47). Hahn MS; Miller JS; West JLThree-Dimensional Biochemical and Biomechanical Patterning of Hydrogels for Guiding Cell Behavior. Adv. Mater2006, 18 (20), 2679–2684. 10.1002/adma.200600647.

(48). Luo Y; Shoichet MSA Photolabile Hydrogel for Guided Three-Dimensional Cell Growth and Migration. Nat. Mater2004, 3 (4), 249–254. 10.1038/nmat1092. [PubMed: 15034559]

(49). DeForest CA; Polizzotti BD; Anseth KSSequential Click Reactions for Synthesizing and Patterning Three-Dimensional Cell Microenvironments. Nat. Mater2009, 8 (8), 659–664. 10.1038/nmat2473. [PubMed: 19543279]

(50). DeForest CA; Tirrell DAA Photoreversible Protein-Patterning Approach for Guiding Stem Cell Fate in Three-Dimensional Gels. Nat. Mater2015, 14 (5), 523–531. 10.1038/nmat4219. [PubMed: 25707020]

(51). Broguiere N; Lüchtefeld I; Trachsel L; Mazunin D; Rizzo R; Bode JW; Lutolf MP; Zenobi□Wong MMorphogenesis Guided by 3D Patterning of Growth Factors in Biological Matrices. Adv. Mater2020, 32 (25), 1908299. 10.1002/adma.201908299.

(52). Azagarsamy MA; Anseth KSWavelength-Controlled Photocleavage for the Orthogonal and Sequential Release of Multiple Proteins. Angew. Chemie - Int. Ed2013, 52 (51), 13803–13807. 10.1002/anie.201308174.

(53). Shadish JA; Strange AC; DeForest CAGenetically Encoded Photocleavable Linkers for Patterned Protein Release from Biomaterials. J. Am. Chem. Soc2019, 141 (39), 15619–15625. 10.1021/jacs.9b07239. [PubMed: 31525979]

(54). Shadish JA; Benuska GM; DeForest CABioactive Site-Specifically Modified Proteins for 4D Patterning of Gel Biomaterials. Nat. Mater2019, 1. 10.1038/s41563-019-0367-7. [PubMed: 30542100]

(55). DeForest CA; Anseth KSPhotoreversible Patterning of Biomolecules within Click-Based Hydrogels. Angew. Chemie - Int. Ed2012, 51 (8), 1816–1819. 10.1002/anie.201106463.

(56). Grim JC; Brown TE; Aguado BA; Chapnick DA; Viert AL; Liu X; Anseth KSA Reversible and Repeatable Thiol–Ene Bioconjugation for Dynamic Patterning of Signaling Proteins in Hydrogels. ACS Cent. Sci2018, 4 (7), 909–916. 10.1021/acscentsci.8b00325. [PubMed: 30062120]

(57). Hammer JA; Ruta A; West JLUsing Tools from Optogenetics to Create Light-Responsive Biomaterials: LOVTRAP-PEG Hydrogels for Dynamic Peptide Immobilization. Ann. Biomed. Eng2020, 48 (7), 1885–1894. 10.1007/s10439-019-02407-w. [PubMed: 31720906]

(58). Teixeira SPB; Domingues RMA; Shevchuk M; Gomes ME; Peppas NA; Reis RLBiomaterials for Sequestration of Growth Factors and Modulation of Cell Behavior. Adv. Funct. Mater2020, 1909011. 10.1002/adfm.201909011.

(59). Belair DG; Le NN; Murphy WLDesign of Growth Factor Sequestering Biomaterials. Chem. Commun2014, 50 (99), 15651–15668. 10.1039/c4cc04317k.

(60). Hinz BThe Extracellular Matrix and Transforming Growth Factor-B1: Tale of a Strained Relationship. Matrix Biol. 2015, 47, 54–65. 10.1016/j.matbio.2015.05.006. [PubMed: 25960420]

(61). Sterner-Kock A; Thorey IS; Koli K; Wempe F; Otte J; Bangsow T; Kuhlmeier K; Kirchner T; Jin S; Keski-Oja J; Von Melchner HDisruption of the Gene Encoding the Latent Transforming Growth Factor-β Binding Protein 4 (LTBP-4) Causes Abnormal Lung Development, Cardiomyopathy, and Colorectal Cancer. Genes Dev. 2002, 16 (17), 2264–2273. 10.1101/gad.229102. [PubMed: 12208849]

(62). Stejskalová A; Oliva N; England FJ; Almquist BDBiologically Inspired, Cell-Selective Release of Aptamer-Trapped Growth Factors by Traction Forces. Adv. Mater2019, 31 (7), 1806380. 10.1002/adma.201806380.

(63). Badeau BA; DeForest CAProgramming Stimuli-Responsive Behavior into Biomaterials. Annu. Rev. Biomed. Eng2019, 21 (1), 241–265. 10.1146/annurev-bioeng-060418-052324. [PubMed: 30857392]

(64). Armstrong JPK; Stevens MMUsing Remote Fields for Complex Tissue Engineering. Trends in Biotechnology. Elsevier Ltd31, 2020, pp 254–263. 10.1016/j.tibtech.2019.07.005.

(65). English MA; Soenksen LR; Gayet RV; de Puig H; Angenent-Mari NM; Mao AS; Nguyen PQ; Collins JJProgrammable CRISPR-Responsive Smart Materials. Science2019, 365 (6455), 780–785. 10.1126/science.aaw5122. [PubMed: 31439791]

(66). Lee A; Hudson AR; Shiwarski DJ; Tashman JW; Hinton TJ; Yerneni S; Bliley JM; Campbell PG; Feinberg AW3D Bioprinting of Collagen to Rebuild Components of the Human Heart. Science2019, 365 (6452), 482–487. 10.1126/science.aav9051. [PubMed: 31371612]

(67). Mirdamadi E; Tashman JW; Shiwarski DJ; Palchesko RN; Feinberg AWFRESH 3D Bioprinting a Full-Size Model of the Human Heart. ACS Biomater. Sci. Eng2020, acsbiomaterials.0c01133.10.1021/acsbiomaterials.0c01133.

(68). Mondschein RJ; Kanitkar A; Williams CB; Verbridge SS; Long TEPolymer Structure-Property Requirements for Stereolithographic 3D Printing of Soft Tissue Engineering Scaffolds. Biomaterials. Elsevier Ltd91, 2017, pp 170–188. 10.1016/j.biomaterials.2017.06.005.

(69). Grigoryan B; Paulsen SJ; Corbett DC; Sazer DW; Fortin CL; Zaita AJ; Greenfield PT; Calafat NJ; Gounley JP; Ta AH; Johansson F; Randles A; Rosenkrantz JE; Louis-Rosenberg JD; Galie PA; Stevens KR; Miller JSMultivascular Networks and Functional Intravascular Topologies within Biocompatible Hydrogels. Science2019, 364 (6439), 458–464. 10.1126/science.aav9750. [PubMed: 31048486]

(70). Skylar-Scott MA; Mueller J; Visser CW; Lewis JAVoxelated Soft Matter via Multimaterial Multinozzle 3D Printing. Nature2019, 575 (7782), 330–335. 10.1038/s41586-019-1736-8. [PubMed: 31723289]

(71). Lee UN; Day JH; Haack AJ; Bretherton RC; Lu W; DeForest CA; Theberge AB; Berthier ELayer-by-Layer Fabrication of 3D Hydrogel Structures Using Open Microfluidics. Lab Chip2020, 20 (3), 525–536. 10.1039/c9lc00621d. [PubMed: 31915779]

(72). Ligon SC; Liska R; Stampfl J; Gurr M; Mülhaupt RPolymers for 3D Printing and Customized Additive Manufacturing. Chemical Reviews. American Chemical Society89, 2017, pp 10212–10290. 10.1021/acs.chemrev.7b00074. [PubMed: 28756658]

(73). Ovsianikov A; Mironov V; Stampf J; Liska REngineering 3D Cell-Culture Matrices: Multiphoton Processing Technologies for Biological and Tissue Engineering Applications. Expert Review of Medical Devices. 119, 2012, pp 613–633. 10.1586/erd.12.48.

(74). Saha SK; Wang D; Nguyen VH; Chang Y; Oakdale JS; Chen SCScalable Submicrometer Additive Manufacturing. Science2019, 366 (6461), 105–109. 10.1126/science.aax8760. [PubMed: 31604310]

(75). Reynaud EG; Peychl J; Huisken J; Tomancak PGuide to Light-Sheet Microscopy for Adventurous Biologists; 2014. 10.1038/nmeth.3222.

(76). Regehly M; Garmshausen Y; Reuter M; König NF; Israel E; Kelly DP; Chou CY; Koch K; Asfari B; Hecht SXolography for Linear Volumetric 3D Printing. Nature2020, 588 (7839), 620–624. 10.1038/s41586-020-3029-7. [PubMed: 33361791]

(77). Taschner R; Gauss P; Knaack P; Liska RBiocompatible Photoinitiators Based on Poly□α□ketoesters. J. Polym. Sci2020, 58 (2), 242–253. 10.1002/pol.20199929.

(78). Brandenberg N; Lutolf MPIn Situ Patterning of Microfluidic Networks in 3D Cell-Laden Hydrogels. Adv. Mater2016, 28 (34), 7450–7456. 10.1002/adma.201601099. [PubMed: 27334545]

(79). Heintz KA; Bregenzer ME; Mantle JL; Lee KH; West JL; Slater JHFabrication of 3D Biomimetic Microfluidic Networks in Hydrogels. Adv. Healthc. Mater2016, 5 (17), 2153–2160. 10.1002/adhm.201600351. [PubMed: 27239785]

(80). Arakawa C; Gunnarsson C; Howard C; Bernabeu M; Phong K; Yang E; DeForest CA; Smith JD; Zheng YBiophysical and Biomolecular Interactions of Malaria-Infected Erythrocytes in Engineered Human Capillaries. Sci. Adv2020, 6 (3), eaay7243. 10.1126/sciadv.aay7243. [PubMed: 32010773]

(81). Arakawa CK; Badeau BA; Zheng Y; DeForest CAMulticellular Vascularized Engineered Tissues through User□Programmable Biomaterial Photodegradation. Adv. Mater2017, 29 (37), 1703156. 10.1002/adma.201703156.

(82). Lunzer M; Shi L; Andriotis OG; Gruber P; Markovic M; Thurner PJ; Ossipov D; Liska R; Ovsianikov AA Modular Approach to Sensitized Two□Photon Patterning of Photodegradable Hydrogels. Angew. Chemie Int. Ed2018, 57 (46), 15122–15127. 10.1002/anie.201808908.

(83). Morgan JR; Yarmush ML; Girton TS; Dubey N; Tranquillo RTMagnetic-Induced Alignment of Collagen Fibrils in Tissue Equivalents. In Tissue Engineering; Humana Press, 2003; Vol. 18, pp 67–74. 10.1385/0-89603-516-6:67.

(84). Bugg D; Bretherton RC; Kim P; Olszewski E; Nagle A; Schumacher AE; Chu N; Gunaje J; DeForest CA; Stevens K; Kim D-H; Davis JMInfarct Collagen Topography Regulates Fibroblast Fate via P38-Yes-Associated Protein Transcriptional Enhanced Associate Domain Signals. Circ. Res2020, CIRCRESAHA.119.316162.10.1161/CIRCRESAHA.119.316162.

(85). Jana S; Levengood SKL; Zhang MAnisotropic Materials for Skeletal-Muscle-Tissue Engineering. Advanced Materials. Wiley-VCH Verlag1228, 2016, pp 10588–10612. 10.1002/adma.201600240. [PubMed: 27865007]

(86). Wegst UGK; Schecter M; Donius AE; Hunger PMBiomaterials by Freeze Casting. Philos. Trans. R. Soc. A Math. Phys. Eng. Sci2010, 368 (1917), 2099–2121. 10.1098/rsta.2010.0014.

(87). Sill TJ; von Recum HAElectrospinning: Applications in Drug Delivery and Tissue Engineering. Biomaterials. Elsevier51, 2008, pp 1989–2006. 10.1016/j.biomaterials.2008.01.011. [PubMed: 18281090]

(88). Eguchi Y; Ohtori S; Sekino M; Ueno SEffectiveness of Magnetically Aligned Collagen for Neural Regeneration in Vitro and in Vivo. Bioelectromagnetics2015, 36 (3), 233–243. 10.1002/bem.21896. [PubMed: 25728875]

(89). Betsch M; Cristian C; Lin Y-Y; Blaeser A; Schöneberg J; Vogt M; Buhl EM; Fischer H; Duarte Campos DFIncorporating 4D into Bioprinting: Real-Time Magnetically Directed Collagen Fiber Alignment for Generating Complex Multilayered Tissues. Adv. Healthc. Mater2018, 7 (21), 1800894. 10.1002/adhm.201800894.

(90). Armstrong JPK; Puetzer JL; Serio A; Guex AG; Kapnisi M; Breant A; Zong Y; Assal V; Skaalure SC; King O; Murty T; Meinert C; Franklin AC; Bassindale PG; Nichols MK; Terracciano CM; Hutmacher DW; Drinkwater BW; Klein TJ; Perriman AW; Stevens MMEngineering Anisotropic Muscle Tissue Using Acoustic Cell Patterning. Adv. Mater2018, 30 (43), 1802649. 10.1002/adma.201802649.

(91). Simian M; Bissell MJOrganoids: A Historical Perspective of Thinking in Three Dimensions. J. Cell Biol2017, 216 (1), 31–40. 10.1083/jcb.201610056. [PubMed: 28031422]

(92). Brassard JA; Nikolaev M; Hübscher T; Hofer M; Lutolf MPRecapitulating Macro-Scale Tissue Self-Organization through Organoid Bioprinting. Nat. Mater2020, 1–8. 10.1038/s41563-020-00803-5. [PubMed: 31853035]

(93). Mumford TR; Roth L; Bugaj LJReverse and Forward Engineering Multicellular Structures with Optogenetics. Curr. Opin. Biomed. Eng2020, 100250. 10.1016/j.cobme.2020.100250.

(94). Ollech D; Pflästerer T; Shellard A; Zambarda C; Spatz JP; Marcq P; Mayor R; Wombacher R; Cavalcanti-Adam EAAn Optochemical Tool for Light-Induced Dissociation of Adherens Junctions to Control Mechanical Coupling between Cells. Nat. Commun2020, 11 (1), 1–13. 10.1038/s41467-020-14390-1. [PubMed: 31911652]

(95). Baaske J; Mühlhäuser WWD; Yousefi OS; Zanner S; Radziwill G; Hörner M; Schamel WWA; Weber WOptogenetic Control of Integrin-Matrix Interaction. Commun. Biol2019, 2 (1), 1–8. 10.1038/s42003-018-0264-7. [PubMed: 30740537]

(96). Zhang J; Luo Y; Poh CLBlue Light-Directed Cell Migration, Aggregation, and Patterning. J. Mol. Biol2020, 432 (10), 3137–3148. 10.1016/j.jmb.2020.03.029. [PubMed: 32247761]

(97). Kim NY; Lee S; Yu J; Kim N; Won SS; Park H; Heo WDo. Optogenetic Control of MRNA Localization and Translation in Live Cells. Nat. Cell Biol2020, 22 (3), 341–352. 10.1038/s41556-020-0468-1. [PubMed: 32066905]

(98). Konermann S; Brigham MD; Trevino AE; Hsu PD; Heidenreich M; Cong L; Platt RJ; Scott DA; Church GM; Zhang FOptical Control of Mammalian Endogenous Transcription and Epigenetic States. Nature2013, 500 (7463), 472–476. 10.1038/nature12466. [PubMed: 23877069]

(99). Nguyen NT; He L; Martinez-Moczygemba M; Huang Y; Zhou YRewiring Calcium Signaling for Precise Transcriptional Reprogramming. ACS Synth. Biol2018, 7 (3), 814–821. 10.1021/acssynbio.7b00467. [PubMed: 29489336]

(100). Deisseroth K; Hegemann PThe Form and Function of Channelrhodopsin. Science. American Association for the Advancement of Science915, 2017. 10.1126/science.aan5544.

(101). Kramer MM; Mühlhäuser WWD; Weber W; Radziwill GMultichromatic Control of Signaling Pathways in Mammalian Cells. Adv. Biosyst2020, 2000196. 10.1002/adbi.202000196.

(102). Rasoulinejad S; Mueller M; Nzigou Mombo B; Wegner SVOrthogonal Blue and Red Light Controlled Cell-Cell Adhesions Enable Sorting-out in Multicellular Structures. ACS Synth. Biol2020, 9 (8), 2076–2086. 10.1021/acssynbio.0c00150. [PubMed: 32610009]

(103). Guo Q; Wang X; Tibbitt MW; Anseth KS; Montell DJ; Elisseeff JHLight Activated Cell Migration in Synthetic Extracellular Matrices. Biomaterials2012, 33 (32), 8040–8046. 10.1016/j.biomaterials.2012.07.013. [PubMed: 22889487]

(104). Smith RC; Machluf M; Bromley P; Atala A; Walsh KSpatial and Temporal Control of Transgene Expression through Ultrasound-Mediated Induction of the Heat Shock Protein 70B Promoter in Vivo. Hum. Gene Ther2002, 13 (6), 697–706. 10.1089/104303402317322267. [PubMed: 11936969]

(105). Miller IC; Gamboa Castro M; Maenza J; Weis JP; Kwong GARemote Control of Mammalian Cells with Heat-Triggered Gene Switches and Photothermal Pulse Trains. ACS Synth. Biol2018, 7 (4), 1167–1173. 10.1021/acssynbio.7b00455. [PubMed: 29579381]

(106). Corbett DC; Fabyan WB; Grigoryan B; O'Connor CE; Johansson F; Batalov I; Regier MC; DeForest CA; Miller JS; Stevens KRThermofluidic Heat Exchangers for Actuation of Transcription in Artificial Tissues. Sci. Adv2020, 6 (40), eabb9062. 10.1126/sciadv.abb9062. [PubMed: 32998880]

(107). Iskratsch T; Wolfenson H; Sheetz MPAppreciating Force and Shape-the Rise of Mechanotransduction in Cell Biology. Nature Reviews Molecular Cell Biology. Nature Publishing Group1211, 2014, pp 825–833. 10.1038/nrm3903.

(108). Callens SJP; Uyttendaele RJC; Fratila-Apachitei LE; Zadpoor AASubstrate Curvature as a Cue to Guide Spatiotemporal Cell and Tissue Organization. Biomaterials. Elsevier Ltd21, 2020, p 119739. 10.1016/j.biomaterials.2019.119739. [PubMed: 31911284]

(109). Hughes AJ; Miyazaki H; Coyle MC; Zhang J; Laurie MT; Chu D; Vavrušová Z; Schneider RA; Klein OD; Gartner ZJEngineered Tissue Folding by Mechanical Compaction of the Mesenchyme. Dev. Cell2018, 44 (2), 165–178.e6. 10.1016/j.devcel.2017.12.004. [PubMed: 29290586]

(110). Miotto M; Gouveia RM; Ionescu AM; Figueiredo F; Hamley IW; Connon CJ4D Corneal Tissue Engineering: Achieving Time-Dependent Tissue Self-Curvature through Localized Control of Cell Actuators. Adv. Funct. Mater2019, 29 (8), 1807334. 10.1002/adfm.201807334.

(111). Badeau BA; Comerford MP; Arakawa CK; Shadish JA; DeForest CAEngineered Modular Biomaterial Logic Gates for Environmentally Triggered Therapeutic Delivery. Nat. Chem2018, 10 (3), 251–258. 10.1038/nchem.2917. [PubMed: 29461528]

(112). Sankaran S; del Campo AOptoregulated Protein Release from an Engineered Living Material. Adv. Biosyst2018, 3 (2), 1800312. 10.1002/adbi.201800312.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

(113). Aisenbrey EA; Murphy WLSynthetic Alternatives to Matrigel. Nature Reviews Materials. Nature Research71, 2020, pp 539–551. 10.1038/s41578-020-0199-8.

(114). Xiang D; Wu X; Cao W; Xue B; Qin M; Cao Y; Wang WHydrogels With Tunable Mechanical Properties Based on Photocleavable Proteins. Front. Chem2020, 8, 7. 10.3389/fchem.2020.00007. [PubMed: 32047736]

(115). Yang Z; Yang Y; Wang M; Deng X; Zhang W-B; Sun F; Wang T; Kiu Francis Fok H; Jiang B; Xiao W; Kou S; Guo Y; Yan YDynamically Tunable, Macroscopic Molecular Networks Enabled by Cellular Synthesis of 4-Arm Star-like Proteins HIGHLIGHTS The Integration of Protein Topology Engineering and Materials Science Cellular Synthesis of 4-Arm Star-like Proteins Enabled by Split. Cell Matter2019. 10.1016/j.matt.2019.09.013.

(116). Sun F; Zhang W. Bin; Mahdavi A; Arnold FH; Tirrell DASynthesis of Bioactive Protein Hydrogels by Genetically Encoded SpyTag-SpyCatcher Chemistry. Proc. Natl. Acad. Sci. U. S. A2014, 111 (31), 11269–11274. 10.1073/pnas.1401291111. [PubMed: 25049400]

(117). Yang Z; Yang Y; Wang M; Wang T; Fok HKF; Jiang B; Xiao W; Kou S; Guo Y; Yan Y; Deng X; Zhang W-B; Sun FDynamically Tunable, Macroscopic Molecular Networks Enabled by Cellular Synthesis of 4-Arm Star-like Proteins. Matter2019, 0 (0). 10.1016/j.matt.2019.09.013.

(118). Hettiaratchi MH; O'meara MJ; O'meara TR; Pickering AJ; Letko-Khait N; Shoichet MSReengineering Biocatalysts: Computational Redesign of Chondroitinase ABC Improves Efficacy and Stability; 2020; Vol. 6.

(119). Huang P-S; Boyken SE; Baker DThe Coming of Age of de Novo Protein Design. 10.1038/nature19946.

(120). Hsia Y; Mout R; Sheffler W; Edman NI; Vulovic I; Park Y-J; Redler RL; Bick MJ; Bera AK; Courbet A; Kang A; Brunette TJ; Nattermann U; Tsai E; Saleem A; Chow CM; Ekiert D; Bhabha G; Veesler D; Baker DHierarchical Design of Multi-Scale Protein Complexes by Combinatorial Assembly of Oligomeric Helical Bundle and Repeat Protein Building Blocks. bioRxiv2020, 2020.07.27.221333.10.1101/2020.07.27.221333.

(121). hsia Y; Bale JB; Gonen S; Shi D; Sheffler W; Fong KK; nattermann U; Xu C; huang P-S; Ravichandran R; Yi S; Davis trisha; Gonen tamir; King neil P.; Baker DDesign of a Hyperstable 60-Subunit Protein Icosahedron. Nature2016. 10.1038/nature18010.

(122). Bale JB; Gonen S; Liu Y; Sheffler W; Ellis D; Thomas C; Cascio D; Yeates TO; Gonen T; King NP; Baker DAccurate Design of Megadalton-Scale Two-Component Icosahedral Protein Complexes. Science2016, 353 (6297), 389–394. 10.1126/science.aaf8818. [PubMed: 27463675]

(123). Foight GW; Wang Z; Wei CT; Greisen P; Warner KM; Cunningham-Bryant D; Park K; Brunette TJ; Sheffler W; Baker D; Maly DJMulti-Input Chemical Control of Protein Dimerization for Programming Graded Cellular Responses. Nat. Biotechnol2019, 37 (10), 1209–1216. 10.1038/s41587-019-0242-8. [PubMed: 31501561]

(124). Chen Z; Kibler RD; Hunt A; Busch F; Pearl J; Jia M; VanAernum ZL; Wicky BIM; Dods G; Liao H; Wilken MS; Ciarlo C; Green S; El-Samad H; Stamatoyannopoulos J; Wysocki VH; Jewett MC; Boyken SE; Baker DDe Novo Design of Protein Logic Gates. Science2020, 368 (6486), 78–84. 10.1126/science.aay2790. [PubMed: 32241946]

(125). Lajoie MJ; Boyken SE; Salter AI; Bruffey J; Rajan A; Langan RA; Olshefsky A; Muhunthan V; Bick MJ; Gewe M; Quijano-Rubio A; Johnson J; Lenz G; Nguyen A; Pun S; Correnti CE; Riddell SR; Baker DDesigned Protein Logic to Target Cells with Precise Combinations of Surface Antigens. Science2020, 369 (6511), eaba6527. 10.1126/science.aba6527.
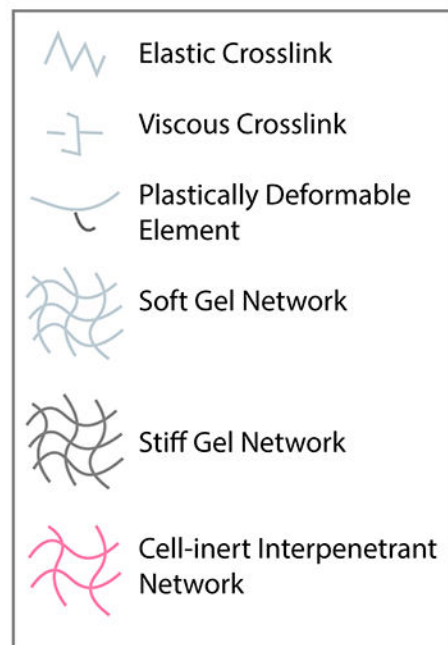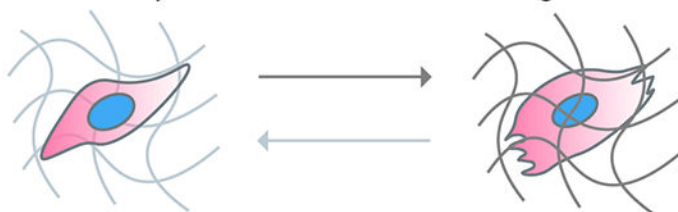
Figure 1 – .

Mimicking complex mechanical aspects of microenvironmental signaling. With the use of modular and synthetic biomaterials, researchers have begun to elucidate the mechanical effects of ECM on cell function beyond its modulus. These properties include the time-dependent components of viscoelasticity and viscoplasticity, as well as dynamic changes to the modulus. Though material modulus and crosslink density are often used interchangeably in the biomaterials community, current efforts seek to decouple material crosslinking, stiffness, degradability, and fibral architecture so as to elucidate their independent effects on cell function.
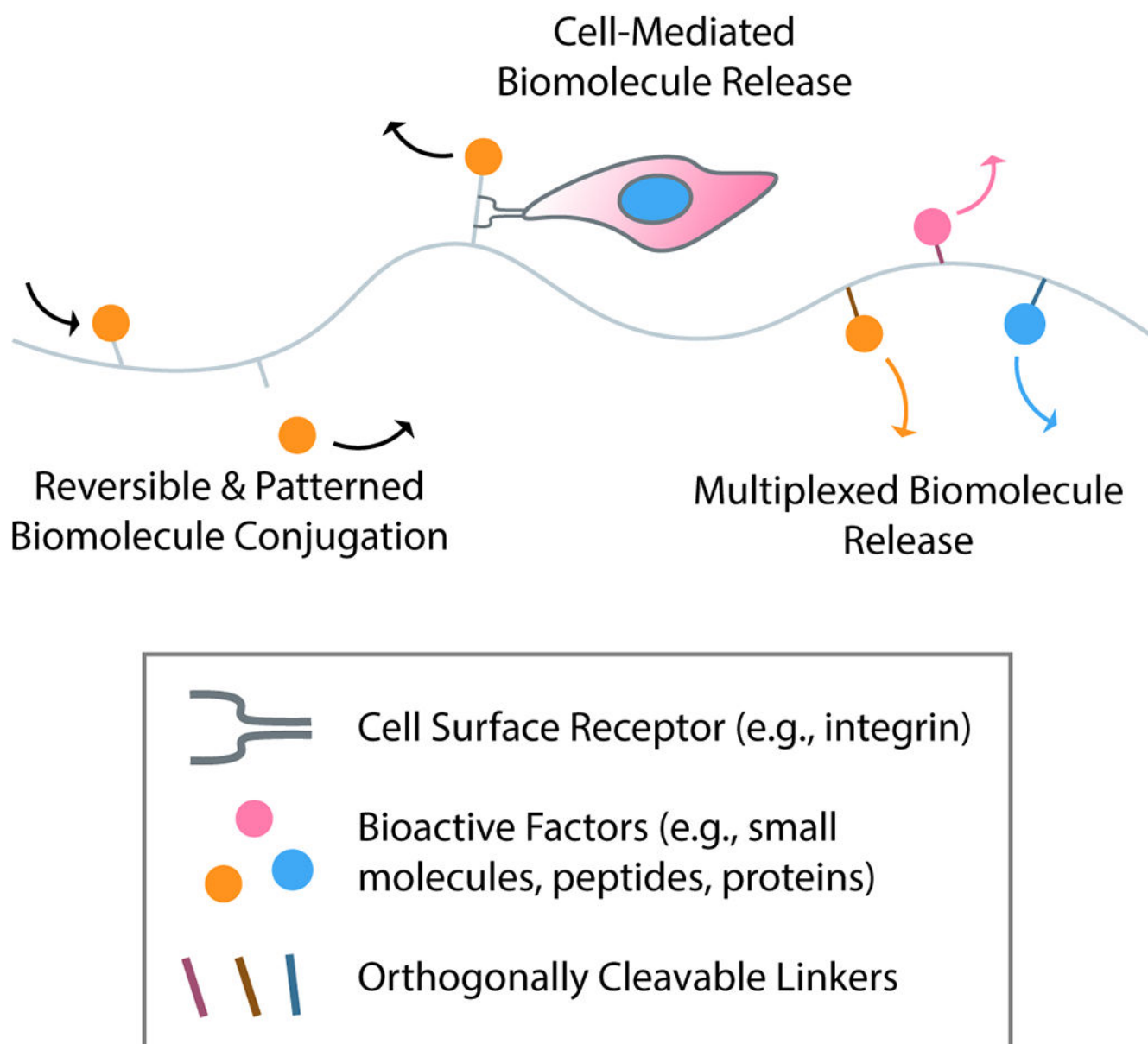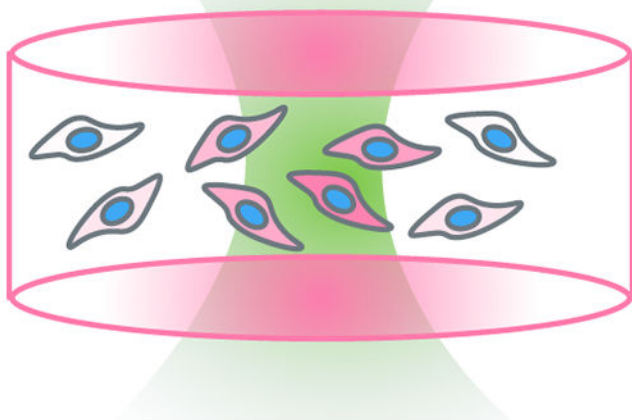
Figure 2 – .
Recapitulating complex biochemical aspects of microenvironmental signaling. Exploiting bioorthogonal chemistries to immobilize or release biomolecules (e.g., small molecules, peptides, proteins) from biomaterials, reversible patterning of synthetic matrices may be achieved. New technologies such as traction-activated payloads (TrAPs) facilitate cell-mediated release of biomolecules from materials. With an ever-increasing suite of triggers for biomolecule release, the field is progressing towards systems capable of highly multiplexed triggers for on-demand and independent biomolecule release.

Faster & Higher Resolution Bioprinting
Native, Unmodified Bioinks
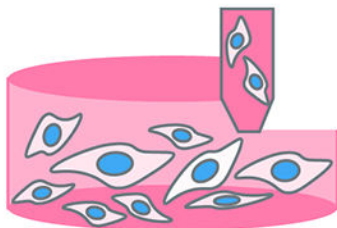
Cytocompatible Subtractive
Tissue Engineering
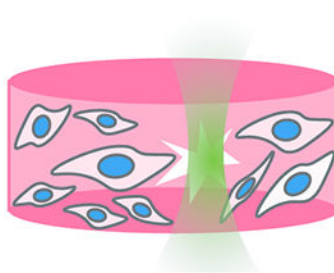
Printable Anisotropy

Figure 3 – .

Advances in engineering complex tissue structures. Recent efforts in bioprinting has boosted resolution and speed, yielding faster, parallelizable, and more generalizable techniques for additive tissue construction. Photolabile hydrogel crosslinks now permit creation of intricate voids and microvascular structures through subtractive engineering. Bioprinting dynamic materials with switchable anisotropy also has opened the door to local control over material microstructure.

Opto- and Thermogenetic Tissue Patterning

Cell Contraction-Guided Tissue Morphogenesis
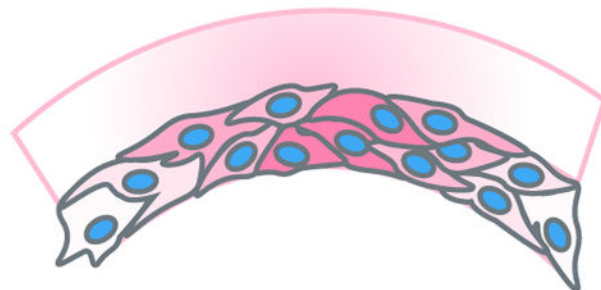
Figure 4 – .
Employing cells as direct biochemical and mechanical actuators of the microenvironment. Borrowing tools from optogenetics, genetic engineering, and synthetic biology, an expanded toolbox exists to direct cell function within engineered materials. Opto- and thermogenetic cell patterning approaches provide a powerful route to directly modulate cell state within a material. Cells may also be used to guide macroscale tissue structure through controlled contraction.