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# Advancing Synthetic Hydrogels through Nature-Inspired Materials Chemistry

*Bram G. Soliman, Ashley K. Nguyen, J. Justin Gooding, and Kristopher A. Kilian**

**Synthetic extracellular matrix (ECM) mimics that can recapitulate the complex biochemical and mechanical nature of native tissues are needed for advanced models of development and disease. Biomedical research has heavily relied on the use of animal-derived biomaterials, which is now impeding their translational potential and convoluting the biological insights gleaned from in vitro tissue models. Natural hydrogels have long served as a convenient and effective cell culture tool, but advances in materials chemistry and fabrication techniques now present promising new avenues for creating xenogenic-free ECM substitutes appropriate for organotypic models and microphysiological systems. However, significant challenges remain in creating synthetic matrices that can approximate the structural sophistication, biochemical complexity, and dynamic functionality of native tissues. This review summarizes key properties of the native ECM, and discusses recent approaches used to systematically decouple and tune these properties in synthetic matrices. The importance of dynamic ECM mechanics, such as viscoelasticity and matrix plasticity, is also discussed, particularly within the context of organoid and engineered tissue matrices. Emerging design strategies to mimic these dynamic mechanical properties are reviewed, such as multi-network hydrogels, supramolecular chemistry, and hydrogels assembled from biological monomers.**

## 1. Introduction

The native extracellular matrix (ECM) provides crucial biochemical and biophysical instruction to resident cells, which ultimately go on to direct the morphogenesis, maturation, and homeostasis of tissues and organs. Cells sense ECM mechanics by exerting traction forces against their surroundings and interpreting the resistance received through specialized mechanoreceptors on the cell surface. This can activate mechanosensitive signaling pathways (e.g., Rho-ROCK, YAP-TAZ, and other pathways), which induce downstream changes in gene and protein expression in a process known as mechanotransduction. Changes in ECM mechanics are detected by cells within seconds to minutes, and regulate inter-/intracellular tension, which governs geometry, polarization, and spatial organization within tissue. At longer time scales, these same signaling networks can have lasting impact on physiological outcomes like regeneration and pathological outcomes like cancer progression, by regulating the chromatin state underlying gene expression[1] (known as epigenetic changes). Overall, the biophysical and biochemical attributes of the ECM that drive cell behavior are central to normal and pathological processes.

Successful fabrication of ECM mimics in the laboratory requires an understanding of the chemical properties that give rise to the physical attributes. ECM mimics are often composed of cell-laden hydrogels as this class of material mimics the highly hydrated nature of native tissues. These native tissues are formed through assembly of polysaccharide and protein-based biopolymers, into architectures with defined biochemical and mechanical properties that are cell instructive.[2–4] These natural biopolymers are composed of intricate hierarchical structures and a complex chemical makeup, which contribute to the dynamic mechanical nature of native tissues. The mechanics of native tissues is characterized by complex viscoelastic behavior that is dependent on both time and the rate of mechanical loading. When deformed, viscoelastic materials show both an instantaneous elastic response (characteristic of a solid), followed by a time-dependent energy dissipation (characteristic of viscous fluids). There is now a growing body of literature that has demonstrated that viscoelasticity has a profound impact on both cell- and tissue-level behavior, such as stem cell fate,[5] neural maturation,[6] immune cell

B. G. Soliman, A. K. Nguyen, J. J. Gooding, K. A. Kilian
School of Chemistry
University of New South Wales
Sydney, NSW 2052, Australia
E-mail: k.kilian@unsw.edu.au
B. G. Soliman, A. K. Nguyen, J. J. Gooding, K. A. Kilian
Australian Centre for NanoMedicine
University of New South Wales Sydney
Sydney, NSW 2052, Australia
K. A. Kilian
School of Materials Science and Engineering
University of New South Wales
Sydney, NSW 2052, Australia

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specification,[7] and vascular morphogenesis,[8] as well as pathological processes such as cancer progression.[9] On the other hand, the inherent chemical complexity of biological polymers results in a poorly defined matrix that is challenging to unravel and fully comprehend. Thus, it is necessary to develop synthetic matrices that can recapitulate the dynamic, time-dependent mechanics inherent to biological ECMs.

Traditionally, many synthetic hydrogel systems have featured linearly elastic networks of high molecular-weight polymers, which allow a high degree of engineering control over the cell microenvironment, but do not bear or distribute cell-generated stresses in a physiological way. Decellularized animal-secreted ECMs mimic the complex mechanical responses of tissues but drastically restrict experimental control and reproducibility. Mimicry of biopolymeric materials with tissue-like dynamic behavior has been challenging with our current synthetic toolbox but recent progress in biomimetic materials chemistry and biofabrication design has begun to narrow this gap. The use of viscoelastic materials can more realistically capture how the transmission of exogenous forces throughout the matrix impacts cell and tissue behavior, as well as how endogenous cellular forces can permanently remodel the local ECM. This is particularly relevant due to the rise of macro-scale multicellular 3D cultures such as organoids and biofabricated tissue constructs, which can exert significant strain on their surroundings and require matrices that can be readily remodeled in order to proliferate and mature appropriately in vitro.[10–12] Improved understanding of how to replicate the dynamic mechanical nature of the ECM within synthetic matrices will allow researchers to recapture the intricacies of in vivo tissue patterning, morphogenesis, and maturation in in vitro settings. This is essential to building engineered tissues that may one day meet the physiological complexity of native tissues.

This review summarizes the current synthetic approaches available for designing dynamic hydrogels, with a focus on strategies to decouple and tune key characteristics of the matrix, for use in biofabrication and microphysiological systems. An overview of the biological ECM is provided, and an assortment of mechanical and structural properties of engineered matrices are discussed. Biofabrication techniques to recreate macroscale tissue architecture are explored. Methods to generate synthetic hydrogels with dynamic matrix mechanics that can be tuned using supramolecular chemistry approaches are examined. Recent design strategies to optimize viscoelastic scaffolds for organoid and organotypic culture are reviewed and discussed. Finally, design considerations for synthetic hydrogels in the context of downstream omics analyses, commercial viability, and clinical translation are briefly discussed.

## 2. The Native ECM

The design criteria for cell-laden synthetic matrices are based inherently on the ECM within native tissues in homeostasis, regeneration, and disease. This section aims to briefly introduce the key noncellular components within these environments that require mimicry in synthetic matrices used for tissue engineering and regenerative medicine purposes. The ECM is composed of a network of interplaying nonfibrillar and fibrillar components (**Figure 1**).

The nonfibrillar components, mainly consisting of glycosaminoglycans (GAGs) and proteoglycans, form a nanoporous hydrogel. Proteoglycans are large proteins that bind polysaccharide (e.g., GAG) side chains. These side chains are negatively charged, which serves a dual function: the charges play an important role in maintaining the osmotic balance of the overall tissue and these charges facilitate sequestering of growth factors that are released from cells.[13] Due to the tensile stresses originating from the osmotic pressure generated by GAGs, as well as the nanoporous nature of this component, proteoglycans contribute to the elastic properties of the ECM such as stiffness, which in turn is defined as the resistance of the hydrogel's polymer network to an applied strain.[14] Furthermore, GAGs interact with fibrillar components of the ECM through noncovalent interactions, thereby contributing to the viscoelastic response of the ECM (i.e., time-dependent energy dissipation).[15] Additionally, the attraction of water into the network by GAGs and proteoglycans contribute to the viscous component of the ECM.[16] Fibrillar components such as collagens, fibronectin, and elastin, as well as nonfibrillar collagens, are responsible for tissue strength, toughness, and deformability in response to external or cell-induced strains.[17] Of the native fibrillar components, collagen is relatively stiff when compared to fibronectin and elastin, while elastin is more compliant and fibronectin demonstrates high tensile flexibility. Altogether, the composition and concentration of these components in the ECM defines the overall tissue mechanics, which vary several orders of magnitude across all tissues.[14] The supramolecular nature of the fibrillar component ensures that the overall ECM is dynamic in nature. The presence of fibrils provides the native ECM with viscoelastic properties. Downstream effectors of these changes include stress relaxation (i.e., change in stress upon constant strain applied to the polymer network) and creep (i.e., change in strain upon constant stress applied to the polymer network). These viscoelastic components undergo reversible changes when strains are applied that lie within the linear viscoelastic region. When strains are applied that exceed this region, or when small strains are applied over a prolonged period, irreversible changes to the polymer network may occur. Several recent studies evidence the emerging interest in this aspect of viscoelasticity (referred to as viscoplasticity or matrix plasticity).[18,19]

The orientation of the fibrillar structures is also of importance. To exemplify, the heterogeneous orientation of collagen fibers in cartilage provides the overall tissue with the required toughness to withstand external loads, whilst shielding the underlying bone from these loads.[20] Collagen turnover is common in tissues due to the continuous production of new collagens and the simultaneous degradation of existing collagens as these proteins are enzymatically digested by stromal cells.[21] Cells can also interplay with these matrices due to adhesive sequences that are present on collagens, laminin and fibronectin. This interplay is crucial as cells sense and respond to biochemical as well as biophysical changes in their direct microenvironment. The interpenetrating network of fibrillar and nanoporous components that make up the noncellular part of the ECM can be disturbed in response to tissue damage and in pathophysiological conditions. In one example, damaged cartilage is replaced by fibrous cartilage with inferior mechanical properties due to the misalignment of new

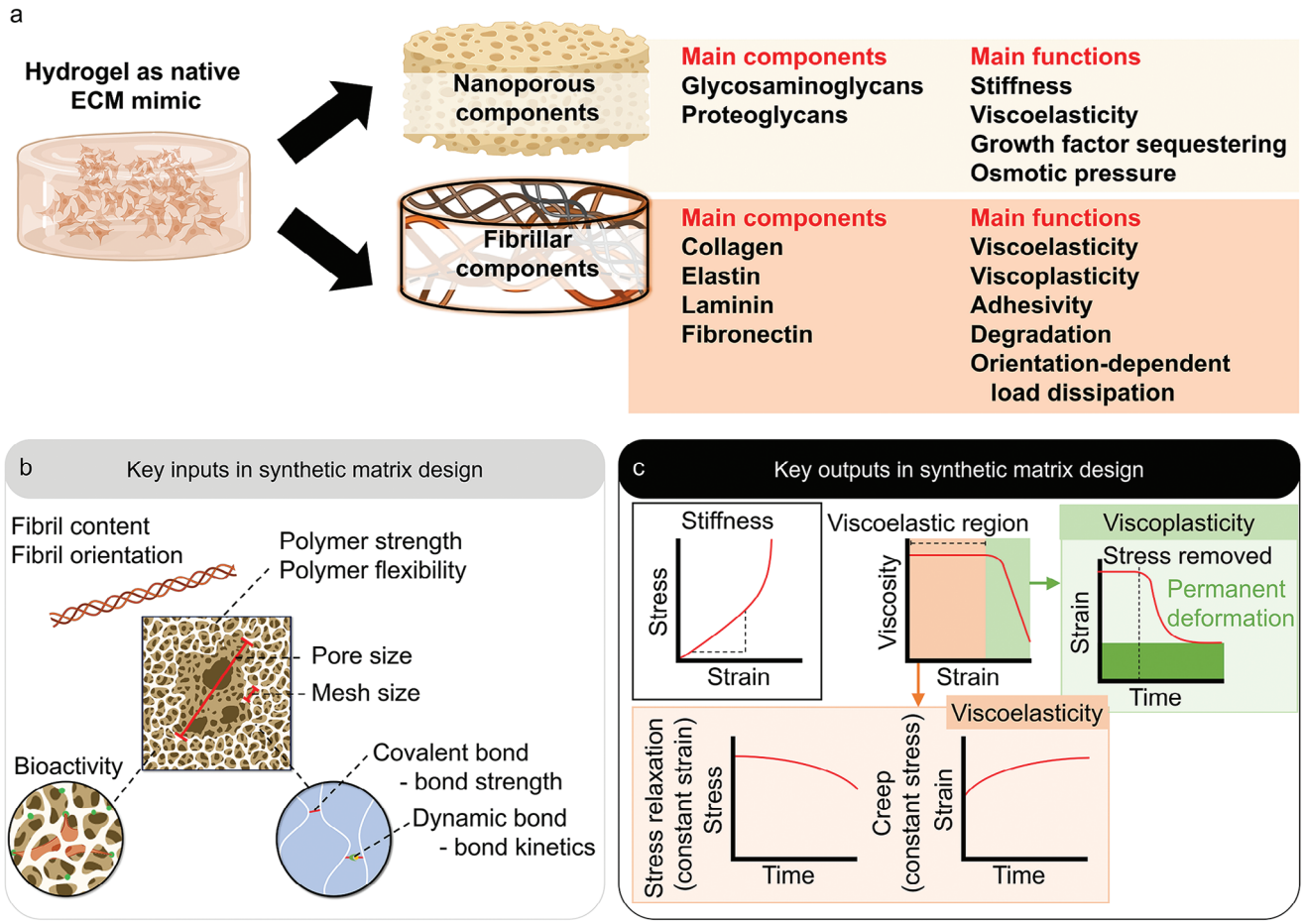


**Figure 1.** Key parameters of the native extracellular matrix informing synthetic hydrogel matrix design. a) Synthetic hydrogel design aims to replicate the nanoporous and fibrillar components of the native extracellular matrix. The nanoporous component primarily consists of glycosaminoglycans (e.g., hyaluronic acid, heparin, chondroitin sulfate), either as a soluble component or bound to side chains of a core protein, forming proteoglycans. The nanoporous component plays a key role in the providing strength and elasticity to the matrix as the negatively charged glycosaminoglycans attract water. Additionally, glycosaminoglycans can sequester proteins for additional biofunctionality (e.g., growth factors, adhesive sequences). Collagen, elastin, laminin and fibronectin fibrils are interpenetrated within the nanoporous network. These fibrils provide the native extracellular matrix with viscoelastic and plastic properties. Furthermore, fibrils contain bioactive sites that provide the matrix with degradability and adhesivity. b) Hydrogel matrices are defined by the mesh size and the pore size of the polymer network. The concentration and orientation of fibrillar components also play a key role in defining the physical characteristics of the polymer network. Bioactive sequences and crosslinks between matrix components are present in the matrix. The kinetics of the latter plays an important role in the physical properties of the hydrogel; in contrast to covalent bonds, dynamic bonds are reversible, and the kinetics of their association and dissociation has an effect on the overall viscoelastic behavior of the hydrogel. c. Whilst stiffness is typically considered in defining the synthetic matrix, interest is emerging in other physical properties of the synthetic matrix, including biomimicry of the matrix's viscoelastic and viscoplastic properties. Elements in panel a and b created with BioRender.com.

collagen fibrils. During cancer progression, the matrix stiffens due to increased deposits of ECM components.[22,23]

Evidently, ECM composition can vary considerably across different tissue types of the human body, and even across different points in time and space within the same tissue, understanding its overall architecture and how matrix mechanics influences (and is influenced by) development, homeostasis, injury, and disease, can highlight important design criteria when creating tissue-mimetic biomaterials. This complex, dynamic nature of the ECM provides a particularly engaging challenge for material scientists: to what extent should synthetic matrices mimic the various components of the ECM and how can complex mechanical behavior be encoded within the synthetic matrix design?

## 3. The Chemical Toolkit for Engineering Hydrogels as ECM Mimics

Engineered hydrogels can be designed to precisely match the biochemical composition, mechanical properties, and structural characteristics of specific tissues in a reproducible and tunable manner. An understanding of the chemical toolkit available in engineering hydrogels is crucial to appreciate matrix design and the downstream effects that the choice of material, mode of crosslinking, and the type of crosslinks have in defining the cellular microenvironment. This section aims to provide a brief overview of the main elements of hydrogel design for engineering ECM mimics.

Generally, ECM-mimicking hydrogels are fabricated from either natural biopolymers, synthetic polymers, or hybrid material composed of both components. Natural hydrogels are typically formed from fibrillar proteins and other ECM components purified from animal tissue such as collagen, fibrin, and hyaluronan, but also include those of plant and insect origin such as alginate, cellulose, and silk fibroin. The most prevalent natural hydrogels are decellularized basement-membrane extracts, typically sourced from Engelbreth–Holm–Swarm mouse tumor cells. These matrices have been commercialized for decades under brand names such as Matrigel or Cultrex, and currently dominate the in vitro cell culture research space, particularly in applications involving organoid or patient tissue cultures. Although highly effective at supporting native cell and tissue processes, they are poorly defined and often composed of a heterogenous mixture of different proteins and protein-related factors. Combined with issues of batch-to-batch variability, these factors make tuning and decoupling material properties such as mechanics and biochemical presentation difficult. Furthermore, since animal-derived materials risk immunogenic reactions and pathogen contamination, chemically defined and fully synthetic hydrogels are more far more sought after for tissue engineering applications slated for clinical translation.

Synthetic hydrogels are typically formed from radical-initiated polymerization of nonnatural monomers. Poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA) are popular polymer backbone choices due to their hydrophilicity, ease-of-handling, cytocompatibility, as well as the antifouling properties inherent to PVA.[24] Synthetic polymers typically require chemical modifications, such as conjugation of integrin-binding motifs, or incorporation of hydrolytically labile or enzyme-cleavable linkages, to transform them from inert physical supports into bioactive matrices that can engage with encapsulated cells.[25] Functional groups can be grafted onto the hydroxyl groups on PEG and PVA to provide handles for crosslinking to enable hydrogel formation. Hydrolysable hydrogels are also commonly utilized to introduce degradability. *N*-isopropylacrylamide and poly(hydroxyethyl methacrylate), and hydrogels composed of poly(lactic acid), and poly(glycolic acid) building blocks, are popular synthetic choices for degradable hydrogels.

Most natural biomaterials support native tissue processes such as cellular assembly and morphogenesis because they show similar nonlinearly elastic mechanical properties to those observed in native tissues, such as stress-relaxation, matrix plasticity, and strain-stiffening behavior. These properties arise from the noncovalent, physical crosslinking of their networks, such as the hydrophobically driven assembly of collagen triple helices or the $Ca^{2+}$ ion-dependent crosslinking of alginate hydrogels, which can reversibly associate and dissociate in response to the accumulation of cell-generated forces. In real tissues, the rupture of weak sacrificial crosslinks allows for appropriate distribution of stress within collagen fibrils,[26] a phenomenon that can be replicated in engineered matrices by incorporating weak but dynamic physical crosslinks.[27] On the other hand, synthetic hydrogels have typically been fabricated using covalent crosslinking strategies, such as the polymerization of vinyl groups (e.g., acrylates and methacrylates),[28] Michael-type additions,[29,30] thiol-ene click chemistry,[31] and condensation reactions.[32] While these irreversible covalent bonds confer strength to the material, this results in static hydrogels that obstruct native tissue assembly processes due to the network's inability to relax and dissipate built-up stresses. Importantly, natural animal-derived biomaterials also support mammalian cells exceptionally well because they are innately bioactive due to their inbuilt display of receptor-binding ligands and their sensitivity to proteolytic degradation. The incorporation of cell-adhesion binding motifs or enzyme-sensitive cleavage sites is critical to consider in synthetic matrix design, as these user-defined cell-matrix interactions can also be used to influence downstream cellular behavior. Further discussion of these topics can be found elsewhere.[14,33,34]

Hybrid materials made using both natural and synthetic components have become a popular method of combining the individual advantages of natural and synthetic hydrogels, while mitigating some of their drawbacks. Design strategies can be as simple as physical blending of the natural and synthetic components, or more complicated orthogonal crosslinking of natural and synthetic polymers into double network or interpenetrating network hydrogels. Biological polymers can also be chemically modified to present reactive functional groups such as amines, thiols, maleimides, methacrylates, norbornenes, among others. In this approach, materials such as gelatin methacrylate (GelMA) hydrogels,[35,36] thiolated polysaccharide hydrogels,[37,38] and hydrogels incorporating hyaluronic acid modified with norbornene (NorHA),[39] methacrylate (MeHA),[40,41] and maleimide (MHA)[42] have been fabricated. Although not considered true hybrid materials since they can be used to form hydrogels standalone,[43] or in combination with other chemically reactive biological molecules,[44] they are also commonly used for crosslinking synthetic polymers bearing complementary functional groups.[45] In this way, viscoelasticity can be engineered into hybrid hydrogels through the grafting of viscoelastic biopolymers (e.g., alginate or collagen) to a PEG backbone. Yet, hybrid hydrogels still suffer from the inherent limitations of natural hydrogels.

Beyond relying on the addition of inherently viscoelastic natural biopolymers to imbue engineered matrices with stress-relaxation behavior, supramolecular chemistry approaches such as dynamic covalent or adaptable covalent crosslinking have also been explored. These include the use of hydrazone bonds,[46] disulfide bonds,[47] imine bonds,[48] thioester exchange,[49] allyl sulfides,[50] and boronate bonds[51] within polymeric networks such as PEG. Host–guest interactions using molecules such as $\beta$-cyclodextrin[52] and cucurbiturils[53] are another approach. The supramolecular assembly of small synthetic molecules like ureidopyrimidinone (UPy),[54] benzene-1,3,5-tricarboxamide (BTA),[55,56] and squaramide monomers[57,58] have also been explored as a method for creating dynamic hydrogels. Oftentimes, hydrogel design features a combination of permanent covalent crosslinks, dynamic covalent crosslinks, and physical or supramolecular crosslinks to build matrices that can access a large range of stiffnesses, but still display stress-relaxation behavior similar to that of native ECMs (**Figure 2**).

## 4. Advances in the Use of Biological Monomers in Synthetic Hydrogel Design

Dynamic synthetic ECMs can also be made using the supramolecular assembly of biopolymers of naturally occurring

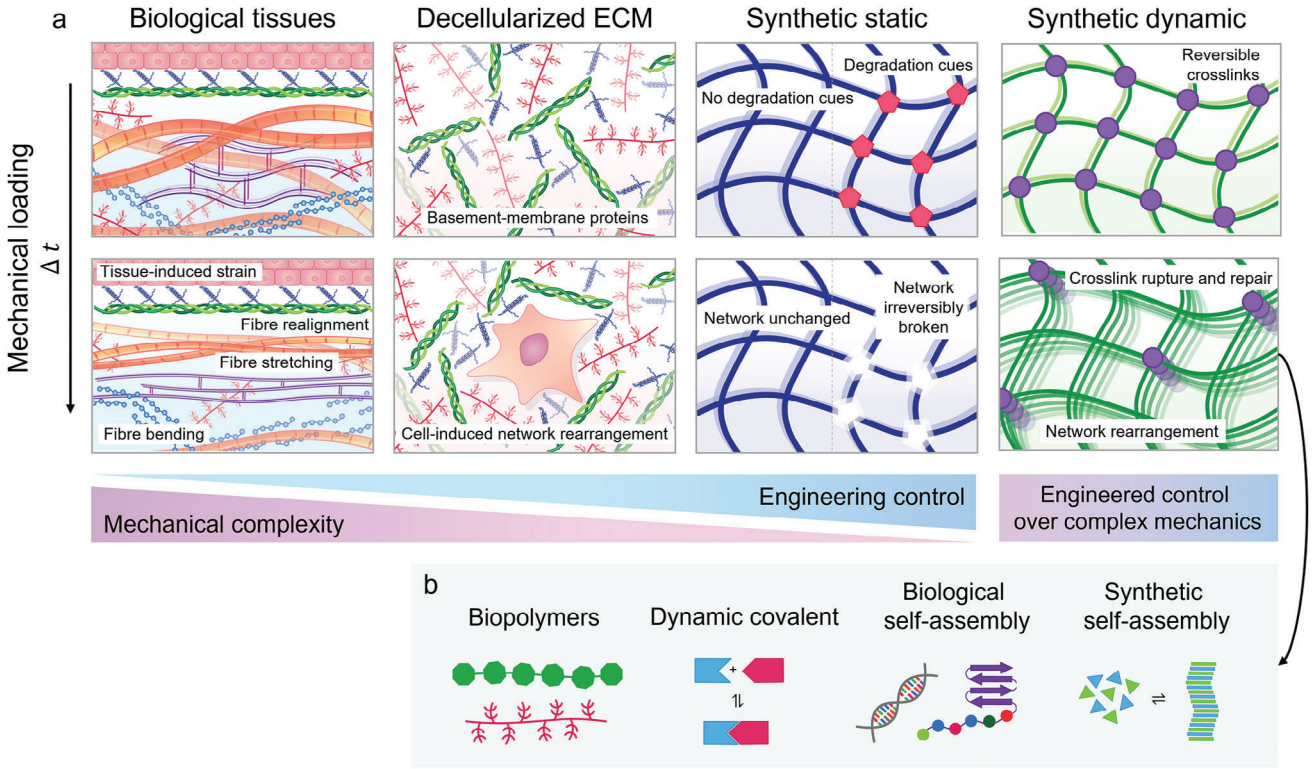


**Figure 2.** A comparison of the biological ECM, decellularized basement membrane extract (BME) matrices, covalent synthetic hydrogels, and dynamic synthetic hydrogels. a) The hydrogel networks of these four groups behave drastically differently in the way they respond to mechanical loads, with biological tissues exhibiting complicated mechanical properties that arise from the composition of the local ECM and the proximity of other cells and tissues. Decellularized BME networks are composed of basement membrane proteins that can dynamically respond to cellular forces but lack the structural integrity of fibrillar ECM proteins. Synthetic covalent hydrogels are elastic, and the network does not change unless degradation cues are engineered into the matrix. Synthetic dynamic hydrogels are formed through weak reversible crosslinks that can dissociate and reassociate to rearrange the network in response to mechanical forces. b) The main design strategies used for the engineering of dynamic synthetic hydrogels, such as the use of inherently viscoelastic biopolymers like alginate, dynamic covalent chemistries, and the self-assembly of either biological or nonbiological monomers.

monomers such as amino acids and nucleic acids. Biological systems have long relied on the self-assembly of proteins, peptides, and DNA to create functional and dynamic materials within living organisms. Now that these same biological polymers can be manufactured with high yield and purity using recombinant protein expression and solid phase synthesis techniques, they have become a powerful building block for the assembly of chemically defined and dynamic hydrogels.[59,60] Guided by the same design principles that govern biological self-assembly pathways, researchers can now rationally design bioinspired synthetic hydrogels with material properties encoded on the molecular level through changes in amino acid or nucleotide sequence. Considering the current trends around this class of materials, and its promise to synthetic matrix design, this section expands on the chemical toolkit described prior and provides a detailed overview of the design and application of biological monomers in synthetic hydrogel design.

Engineered protein hydrogels reminiscent of the native ECM can be made using polypeptide or protein constructs with discrete bioactive and chemically reactive regions tailored using recombinant protein technology. Genes corresponding to the structural and functional segments found in ECM components can be cloned and synthesized in a microbial host, such as *E. coli*. These recombinant proteins can then be crosslinked into a hydrogel network by reaction with functionalized hydrophilic polymers or other chemical crosslinkers, as well as through self-assembly pathways akin to that of the full-length protein (e.g., collagen fibrillogenesis, elastin coacervation). Using this approach, structural units derived from full-length ECM components such as collagen-like proteins,[61] elastin-like proteins,[62] and fibronectin–laminin protein fragments[63] have been used to fabricate ECM-mimetic hydrogels (**Figure 3a**). Ligand–receptor pairs not naturally found in ECM proteins can also be used as a dynamic crosslinker, where hydrogel mechanics can be modulated based on the binding affinity of the protein pair.[64,65]

More recently, recombinant protein hydrogel design has begun to rely less on the exact duplication of naturally occurring protein domains. As more is learnt about biophysical phenomena such as protein–protein interactions, protein folding/unfolding, and sequence–structure relationships, researchers have focused on devising artificial proteins that are chemically and physically distinct from any native counterpart.[66,67] For instance, Hu et al. designed synthetic collagen fibrils exhibiting non-natural hierarchical assembly, which supported osteogenic differentiation in mesenchymal stromal cells to the same extent as natural collagen, but allowed for nanometer-scale changes in fiber dimension

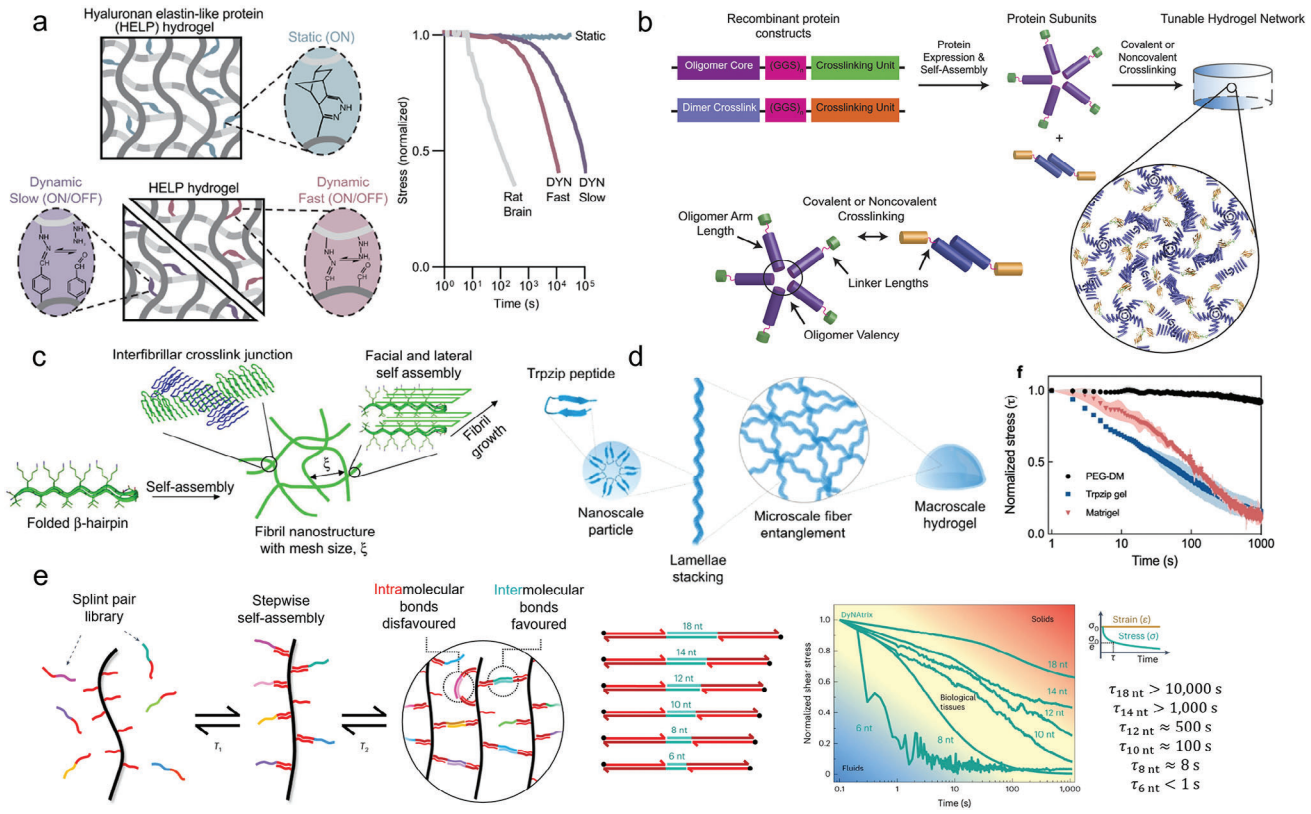


**Figure 3.** Dynamic hydrogel designed using biological building blocks. a) Recombinant elastin-like protein and hyaluronan crosslinked with dynamic covalent hydrazone bonds to form viscoelastic matrices. Reproduced from Roth et al. with permission.[6] 2023, AAAS. b) Recombinant proteins of de novo design, where the modular approach allows hydrogel properties to tuned through various parameters including oligomer valency, oligomer arm length, crosslinker length, and reversible nature of protein–peptide crosslink. Reproduced from Mout et al. with permission.[70] 2024, NAS. c) Beta hairpin peptide hydrogels known as MAX peptides, where self-assembly of the peptide monomers into $\beta$-sheet rich fibrils results in hydrogel formation. Reproduced from Branco et al. with permission.[187] 2009, Elsevier. d) Self-assembling and stress-relaxing hydrogels based on the assembly of the tryptophan zipper peptide motif. Reproduced from Nguyen et al. with permission.[87] 2023, Nature Portfolio. e) Dynamic hydrogel networks formed through the hybridization of DNA crosslinker strands. Increasing or decreasing the length of the hybridized region can be used to control stress-relaxation half-times of the DNA hydrogel. Reproduced from Peng et al. with permission.[91] 2023, Nature Portfolio.

and morphology based on changes in amino acid sequence.[68] The ability to tailor the design of protein hydrogels from the bottom-up provides a high level of control over the resulting matrix properties. For instance, Dranseike et al. demonstrated that fiber morphology and bulk hydrogel mechanics can be tuned by varying the sequence of two modular protein domains, yielding matrices spanning 0.1–10 kPa.[69] Hydrogel systems featuring de novo designed proteins have been demonstrated. Mout et al. reported programmable viscoelasticity of a protein hydrogel based on changes in oligomer valency, arm length, and linker flexibility[70] (Figure 3b). The emergence of machine learning models such as AlphaFold[71] and RFdiffusion[72] now provides unparalleled insight into protein structure prediction and in silico protein design. Powered by these computational frameworks, it is expected that engineered protein-based biomaterials will no longer focus on mere mimicry of native ECM proteins and shift toward the use of human-designed protein structures not observed in nature.

Although peptide chains of several hundred amino acids are typically needed for protein assembly, significantly shorter peptides can also display highly sophisticated self-assembly behavior. Known as self-assembling peptides, these short sequences (generally <30 residues) can partially recapitulate the structure-function relationship of full-length fibrillar proteins, while still being easily manufactured using solid phase synthesis techniques.[73,74] Through careful design of the amino acid sequence, these peptides can be programmed to form a variety of supramolecular nanostructures in a highly predictable and reproducible manner. Many self-assembling peptides are peptide "hydrogelators" – sequences which spontaneously assemble into nanofibers in response to environmental triggers (e.g., temperature, pH, ion concentration). These peptide nanofibers eventually reach a critical concentration whereby they physically entangle enough to form hydrogel networks. To date, many peptide hydrogels have been successfully used as cell culture scaffolds, capable of supporting osteocytes,[75] chondrocytes,[76] pluripotent stem cells,[77] neuronal differentiation,[78,79] and angiogenesis.[80]

Like early recombinant protein hydrogels, most original self-assembling peptide hydrogelators were derived from sequences found in nature. For example, the first self-assembling peptide to be reported, EAK16 (now more commonly known as RADA16), was found in the zuotin protein of the yeast

*Saccharomyces cerevisiae*,[81] and the popular diphenylalanine (FF) motif was first identified in the Alzheimer's $\beta$-amyloid plaques.[82] Elementary protein motifs such as the $\alpha$-helix and the $\beta$-sheet have also heavily inspired the synthetic design of analogous $\alpha$-helical,[83] $\beta$-sheet,[84] and $\beta$-hairpin[85] peptide hydrogelators. However, most of the peptide hydrogelators that lead the tissue engineering space today are the same sequences discovered more than 30 years ago, such as RADA16 (commercially known as PuraMatrix), octapeptides (commercially known as PeptiGels), and MAX peptides (Figure 3c), with most new self-assembling sequences arising as iterations of pre-existing gelator sequences.

The enormous design space centered around the twenty canonical amino acids means that classic trial-and-error experimentation of new self-assembling sequences is virtually inaccessible due to steep time and labor costs. To navigate the immense combinatorial sequence space more efficiently, efforts have now begun to focus more closely on the computational-driven discovery of peptide hydrogelators and accurate a priori prediction of hydrogel formation. Using molecular dynamics, Frederix et al. simulated the self-assembly of all 8000 possible tripeptides to screen for top nanofiber-forming sequences, which were then successfully shown to form hydrogels.[86] Nguyen et al. used a combination of rational design and molecular dynamics simulations to discover a new structural motif known as the tryptophan zipper peptide that can form viscoelastic, self-assembled hydrogels[87] (Figure 3d). To further accelerate peptide hydrogelator discovery, many are now applying machine-learning models and deep-learning algorithms to pinpoint new self-assembling sequences with increasing accuracy. For instance, Xu et al. screened $10^4$ tetrapeptide sequences, synthesized 160 of these, and used the gelation results as a machine-learning training dataset to improve the hit rate of accurate hydrogel formation.[88] It is anticipated that as data-driven design approaches and high-throughput computational discovery pipelines continue to improve, they will be instrumental in broadening the library of peptide and protein-based motifs useful for biomaterial design.

Dynamic hydrogels can also be made using the four nucleotides that comprise DNA (adenosine, cytosine, guanine, and thymine), where crosslinking of the network can be tuned using the Watson–Crick base-pairing rules. Hydrogels crosslinked solely based on the hybridization of branched DNA molecules have been developed and used for 3D cell culture,[89] showing some promise in tissue engineering applications. For example, work by Athanasiadou et al. recently demonstrated pure DNA hydrogels promoted osteogenic regeneration in vivo due to the ability of DNA to promote hydroxyapatite and calcium phosphate mineralization.[90] Another popular design strategy involves covalently tethering single-stranded DNA (ssDNA) to a high-molecular weight polymeric backbone.[91] In this approach, ssDNA oligos mediate reversible crosslinking through hybridization with their complementary oligonucleotide, while the large backbone confers strength and stability to the bulk hydrogel. Because of the highly precise sequence selectivity of DNA, these designs enable very fine control over hydrogel properties. Peng et al. demonstrated how deliberate nucleotide mismatches in the DNA crosslinker strands could be used to shift the self-assembly pathway toward favoring intermolecular crosslinks over intramolecular interactions, consequently influencing crosslinking efficiency and the final hydrogel stiffness (Figure 3e). Additionally, simply increasing the length of the hybridized DNA region from 6 to 18 nucleotides can modulate the stress relaxation half-time of their matrices from seconds to hours.[91] Exogenous DNA designed to interact with an already formed DNA hydrogel is also possible, offering stimuli-responsive systems. For instance, by leveraging toe-hold displacement reactions, Deshpande et al. showed that addition of invading DNA strands to a preformed hydrogel can be used destabilize existing double-stranded DNA crosslinks and soften the matrix on-demand.[92]

## 5. Synthetic Routes to Decouple Biophysical and Biochemical Matrix Parameters

The hydrogel matrix can be deconvoluted into a range of physical properties including stiffness, porosity, confinement, viscoelasticity (i.e., stress relaxation, strain stiffening, and viscoplasticity) and degradability. In this section, we discuss strategies to gain a mechanistic understanding of the synthetic hydrogel matrix through isolating the contribution of individual biochemical and biophysical properties in directing cell behavior.

Stiffness is traditionally recognized as a key factor in driving cell decision making across a range of healthy and pathological cell lines and primary cells. Hydrogel stiffness plays for instance a role in cell clustering. This process is driven by biochemical signals (e.g., adhesivity, degradation), but the extent and rate of cell clustering is highly dependent on the space available to the cells to migrate, which in turn is dependent on stiffness.[93] Limiting cell clustering can have important implications for various biological systems; mammary epithelial cells form acini when cultured in matrices of physiological stiffness, whilst these cells form disordered structures resembling a malignant phenotype in increased stiffness found in cancerous breast tissue,[94] driven through chromatin reorganization at the epigenetic level due to constricting mammary cell nuclei.[95] Porosity is also traditionally considered as a biophysical factor within hydrogel design, thought to primarily dictate cell motility and diffusion kinetics of nutrients and oxygen. Well-defined synthetic matrices have recently allowed decoupling of pore size and stiffness, providing insights into the separate roles of these parameters. Hydrolysable porogens can for instance be used to generate size-controllable pores within hydrogels of defined stiffness during cultivation.[96] Alternatively, the use of multiarm linkers and double networks can aid in decoupling pore size from stiffness.[97] Application of these tools will enable further insights into the isolated role of stiffness and pore size on the behavior of cell encapsulated within hydrogels, and integrated approaches investigating these factors in conjunction with other factors such as viscoelasticity will be crucial in understanding the extent to which these factors interplay in guiding cell behavior within synthetic matrices.

The importance of considering the viscoelasticity of the hydrogel matrix has become apparent toward improving biomimicry of these hydrogels. This insight has originated primarily from studies that utilize natural biomaterials that possess inherent viscoelasticity. The viscoelasticity of alginate can for instance be tailored through adjusting the molecular weight and polymer concentration.[98] Moreover, the viscoelastic properties of these hydrogels can be decoupled from their stiffness by simultaneously adjusting crosslinker calcium chloride concentration, changing polymer chain motility for faster stress relaxation

whilst keeping the overall crosslinking density constant.[99] Hybrid matrices composed of alginate and PEG spacers have been used to improve the control over viscoelasticity.[100] PEG chains provide steric hindrance to alginate polymers in this hybrid system, effectively reducing their mobility and inhibiting the rate of stress relaxation. Nam et al. demonstrated how adjusting the total PEG mass amount within hybrid alginate-PEG hydrogels affected cell fate of encapsulated mesenchymal stromal cells; increasing the length and/or concentration of PEG spacers led to an increase in stress relaxation that in turn affected cell proliferation, adhesion and resulted in stiffness-independent osteogenesis.[100]

Hybrid and fully synthetic hydrogels can incorporate ionic or dynamic bonds to facilitate viscoelasticity[101] such as bonds between hydrazine and aldehydes (i.e., hydrazone bonds),[102–104] as well as guest–host interactions (e.g., cyclodextrin and adamantane)[105,106] and peptide-based hydrogels that provide viscoelasticity through reversible physical interactions between amino acids.[107] Several studies have aimed to delineate the effects of dynamic bond kinetics on stress relaxation and gain an understanding of the key design features of dynamically crosslinked hydrogels in modulating cell behavior. Whilst mainly utilizing hybrid hydrogel systems, insights provided by these studies can still drive design of synthetic hydrogels. Lou et al.[104] used catalyst aminomethyl benzimidazole to increase the dynamic bond exchange rates in hybrid hydrazone-linked networks of PEG and hyaluronic acid, systematically investigating the contribution of these rates, polymer density and crosslinking density. The authors observed that stress relaxation was predominantly dependent on the exchange rates of the bonds rather than the polymer chains themselves, identifying the bond exchange rates as the rate-limiting step. Consequently, polymer concentration could be altered to change overall stiffness without affecting the rate of stress relaxation. In line with this report, Yang et al.[105] found that changing the guest molecule in a guest–host dynamic hydrogel could effectively alter the bond exchange rates, thus altering the viscoelastic properties of these hydrogels (**Figure 4**). At the same time, the crosslink and polymer densities were kept constant, ensuring effective decoupling of stiffness and viscoelasticity. Within this model, the authors found how dynamic bond kinetics play a role in determining the speed at which cells can spread and move within a matrix. Whilst cell traction forces were not sufficient to break formed bonds, these small forces (< 100 pN) were found to be able to push dissociated guest–host bonds away from each other, leaving space for cells to move and/or spread. More rapid bond exchange rates reduced the dynamic bond lifetime, providing cells with these openings more often. Effectively, the change in bond lifetime had a drastic effect on the rate of cell spreading, which occurred as early as after one day of culture in hydrogels with faster bond exchanges as compared to seven days in the hydrogels with slower bond exchange kinetics. These examples highlight how dynamic bonds can be used to steer cell behavior through modulating stress relaxation properties of the hydrogels, albeit a thorough characterization of the dynamic bond kinetics within fully synthetic hydrogels will be crucial moving forward.

Whilst the aforementioned mode of cell migration was dependent on transient changes in the dynamic microenvironment of viscoelastic hydrogels, permanent deformations of this network (i.e., viscoplastic matrices) can also initiate cell migration. Wisdom et al.[109] developed a hydrogel system in which the viscoplasticity could be tailored (**Figure 5**), independent of the bulk hydrogel stiffness. Through this model, the authors found an underlying mechanism in which cells squeeze into pores, causing nuclear deformations that in turn activate mechanosensitive ion channels that increase intracellular pressures sufficiently to cause permanent widening of the pores.[99,110] This protease-independent migration process was more rapid in hydrogels with increased viscoplasticity. This mode of cell migration was furthermore found to only occur in viscoelastic matrices in contrast to elastic matrices,[111] thus providing a clear link between viscoplasticity and cell motility. The importance of viscoplasticity in hydrogel design is becoming increasingly apparent. With that in mind, it should be noted that there is an opportunity to investigate viscoplasticity, as well as viscoelasticity, in the context of fully synthetic matrices as most of the studies involving viscoplasticity have been performed using natural matrices.

A disadvantage of dynamic crosslinks is the low fracture strength, limiting the ability to generate matrices with well-defined viscoelastic properties for tissue applications requiring mechanically robust and stiff matrices. A trade-off may be made by combining nonreversible covalent bonds and dynamic crosslinks within a singular matrix. In one example, vinylsulfone-containing PEG (to allow Michael addition in the presence of thiolated crosslinkers) and was combined with cytosine-containing PEG (as a nucleobase for the formation of hydrogen bonds),[12] allowing the formation of hydrogel with stiffnesses ranging from < 1 to 3 kPa that remained stable for long-term culture. Still, hybrid matrices inherently require balancing between the two different crosslinking strategies and often do not yield the optimal biological outcomes, spurring the need for fully dynamic hydrogel matrices. A recent report showed feasibility to design stiffer matrices based on a quinoline-cucurbituril guest–host interaction. Grafting hexanoate groups on the quinoline enabled independent tailoring of the viscoelasticity of the polymer network through reversible pH-dependent (de)protonation of the hexanoate's carboxylic acid residue.[106] Whilst the pH-dependency of this system limits the compatibility of this particular system with cell encapsulation, similar systems that operate in a physiologically relevant window are expected to emerge that may further drive the application of dynamic synthetic matrices in in vitro tissue models. In a key report, Liu et al. demonstrated how sliding cyclodextrin groups could be connected to PEG chains, enabling stress relaxation through crowding of the cyclodextrin groups which in turn allowed the PEG chains higher degrees of freedom to orient in the direction of strain. This freedom in polymer chain orientation in response to external stress resulted in a significant increase in hydrogel toughness.[112]

Fibrillar proteins such as collagen, fibrin and elastin make up a large portion of the ECM. It is thus not surprising that this class of proteins has been adopted to fabricate hydrogel matrices, or incorporated as a component of double-[113] or interpenetrating networks,[27,114] to investigate how fibrillar components of the ECM affect encapsulated cells. Fibrillar hydrogel matrices inherently possess rapid stress relaxation characteristics as the fibrillar components are associated with higher association constants than dynamic crosslinks,[27] as well as viscoplasticity/mechanical plasticity.[18,115] Whilst introducing adhesive sequences to the polymer backbone is sufficient to enable cell

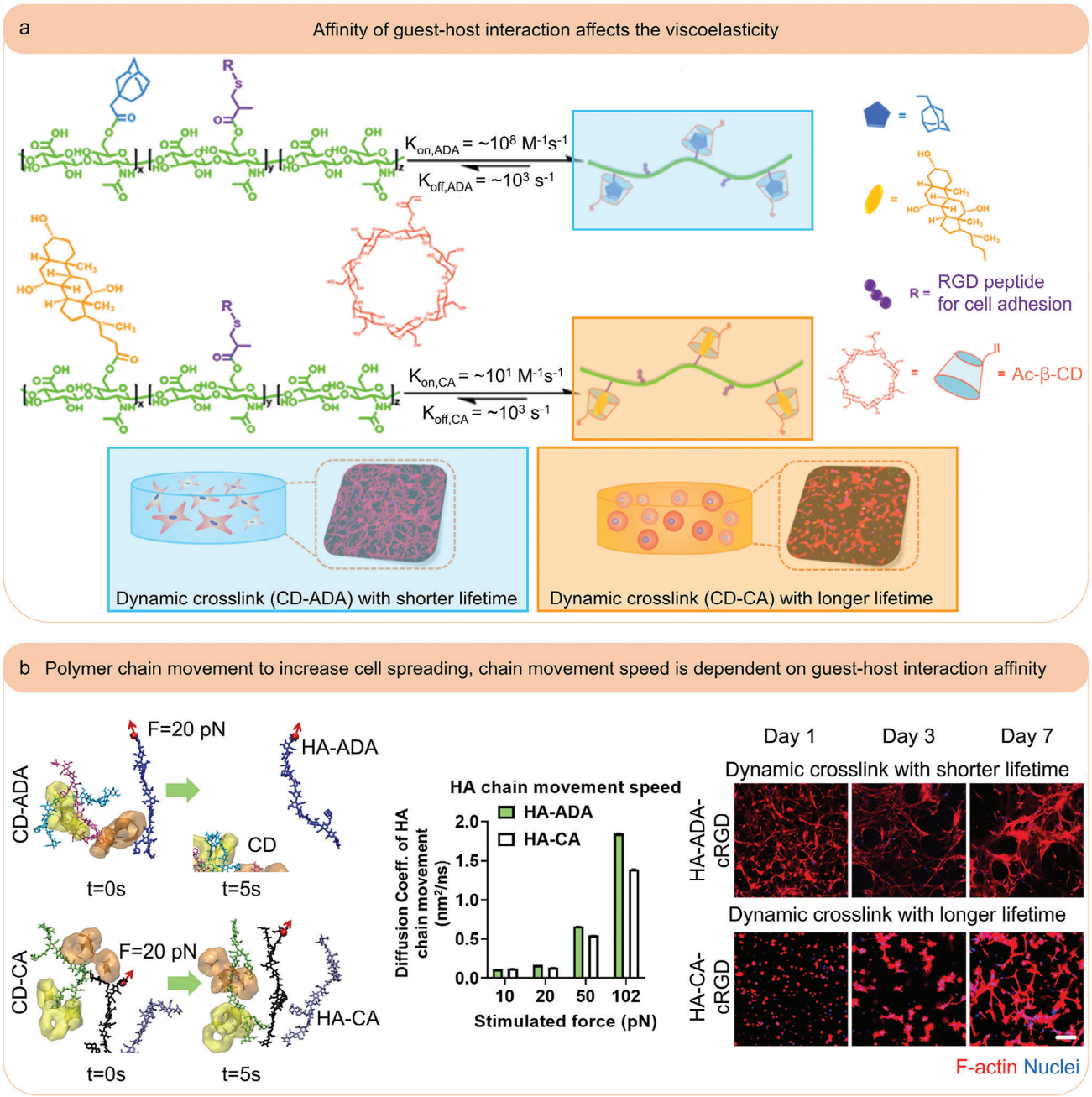


**Figure 4.** Dynamic bonds that dissociate more rapidly enable more rapid cell movement in 3D matrices. a) Stiffness and viscoelasticity was successfully decoupled by changing the host molecule in hydrogel matrices crosslinked through guest–host interactions. Guest adamantane (ADA) demonstrated fast association and dissociation kinetics (leading to crosslinks with a shorter lifetime), whilst guest cholic acid (CA) demonstrated slower association and dissociation kinetics. b) Cell traction forces were found to be sufficient to push dissociated the host mono-acryloyl cyclodextrin (CD) and guest away from each other. The rate at which this separation occurred was faster in hydrogels with more rapidly dissociating guest–host interactions, which in turn drove faster cell spreading and movement. Reproduced under the terms of the Creative Commons CCBY 4.0 License.[108]

attachment, fibrillar components seem furthermore crucial in inducing cell alignment and for attached cells to form mature focal adhesions.[27,116]

Electrospinning can be used to fabricate fibrous variations of known biomaterials such as collagen, silk and hyaluronic acid.[117] The majority of electrospinning platforms have utilized the fibrous meshes as substrates rather than matrices due to the challenge of incorporating spun polymer meshes within a 3D matrix. In one example, Davidson et al. reported a method to incorporate electrospun norbornene-functionalized hyaluronic acid meshes into PEG-thiol hydrogel matrices by straightforward fragmentation of the meshes through a small-gauge (< 0.8 mm)

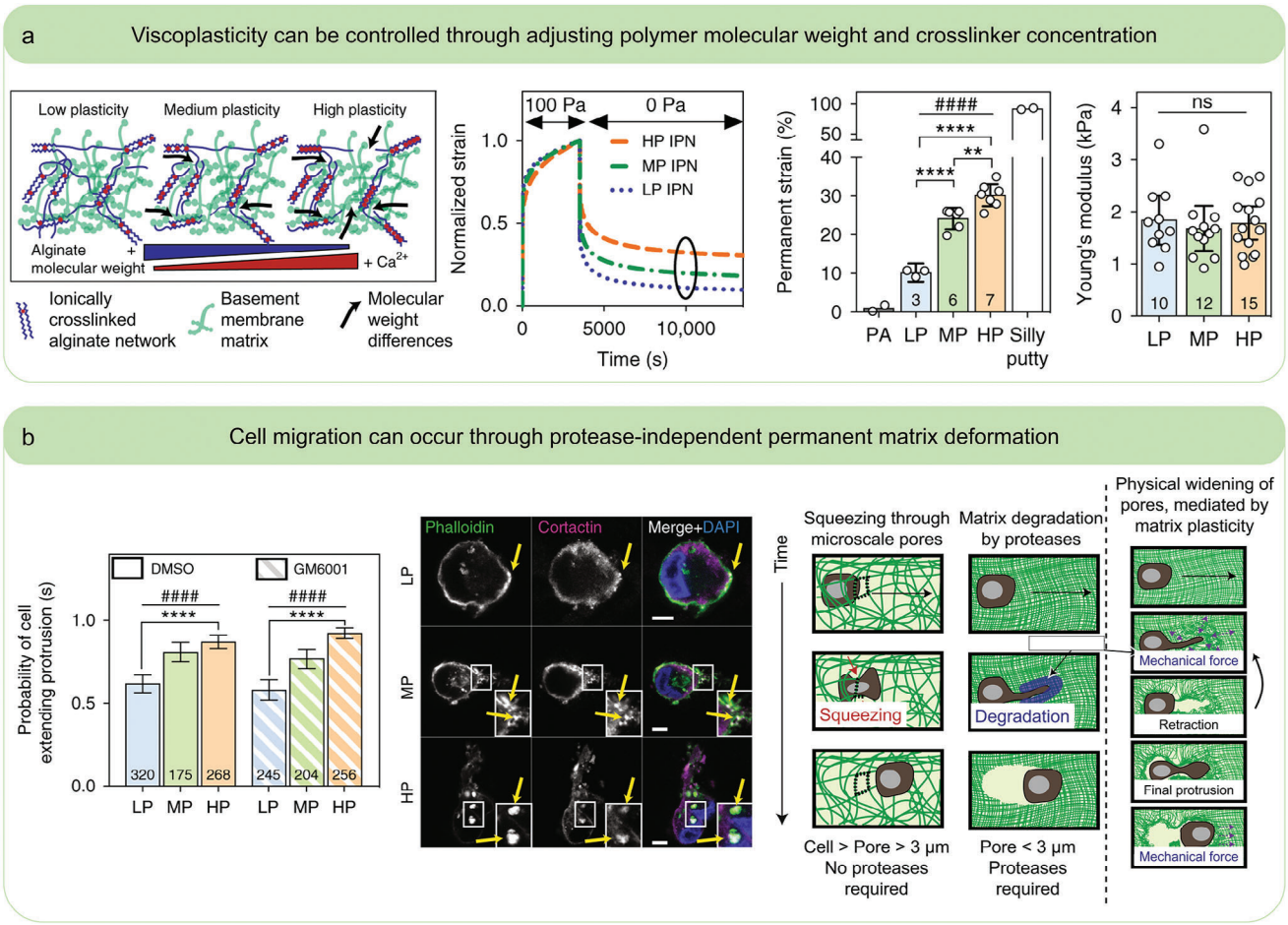


**Figure 5.** Cell protrusions occur within hydrogel matrices with high viscoplasticity. a) Alginate of different molecular weights was combined with reconstituted basement membrane to form an interpenetrating network (IPN). The alginate molecular weight was balanced against the crosslinker ($Ca^{2+}$) concentration to keep the overall stiffness constant but adjust the degree of freedom of the alginate polymer network, which in turn affected the hydrogel viscoplasticity. Hydrogels with low (LP), medium (MP) and high (HP) degrees of viscoplasticity were prepared in this way. b) Cells were found to be able to increasingly form protrusions in hydrogels with higher viscoplasticity. Knocking down protease-dependent migration through protease inhibitor GM6001 demonstrated that the protrusion formation was dependent on the physical properties of the hydrogels rather than protease-mediated migration. The proposed method of migration involved the permanent breakdown of the polymer network by invadopodia. Reproduced under the terms of the Creative Commons CC-BY 4.0 License.[109]

needle and subsequent photo-crosslinking of the meshes and PEG through thiol-ene click reaction. When encapsulated, mesenchymal stromal cells were demonstrated to align to the fibers, showing proof-of-principle of this technique.[118] Alternatively, self-assembling fibrous components can be generated within hydrogel matrices. Biologically-derived biomaterials such as gelatin can for instance be combined with synthetic additives to drive orientation of gelatin polymers into fibers, thereby generating fibrillar structures akin to collagen.[119]

Approaches that enable fibrillar hydrogel formation from purely synthetic materials are thus still desired. A promising design strategy for such matrices involves the use of amphipathic monomers (e.g., peptide amphiphiles,[120] block copolymers) and self-assembling peptides as these components can be fully defined and synthetically prepared. Farsheed et al. designed self-assembling peptides with either cationic or anionic charges that naturally form $\beta$-sheets that orientate into fibrils upon mixing of the two components.[121] Nguyen et al. developed a peptide sequence that forms multiscale fibrillar structures purely through interactions with itself.[87] Lovett et al. found that two amphiphilic block-*co*-polymers formed entanglements that self-assembled into fibrillar structures upon temperature-induced crosslinking into hydrogels.[122] Pardo et al. used magnetic nanoparticles to drive fibril formation of a decellularized extracellular matrix.[123] In addition to requiring further synthetic design strategies to incorporate fibrillar components within synthetic matrices, future research should focus on developing methods that enable controlled orientation of both nonfibrillar and fibrillar components to more accurately mimic tissues such as heart muscle and cartilage, wherein tissue mechanics are heavily dependent on the orientation of these components. One potential strategy could be the involvement of sliding bonds, as these bonds have been shown to allow alignment of nonfibrillar polymers.[112] The next generation of synthetic matrices will likely include approaches that enable tailorable orientation of fibrillar components.

Whilst physical properties of synthetic matrices are crucial in guiding cellular response within these matrices, the incorporation of bioactive sequences within these matrices is of equal importance. Synthetic matrices are often functionalized with peptide sequences or full-length proteins to ensure cell adhesion and drive cell migration or differentiation through incorporation of tissue- or matrix-specific sequences. These hydrogels can be functionalized with scrambled sequences of otherwise bioactive peptides, enabling a sophisticated investigation into the effect of particular bioactive sequences.[93] It should be noted that the many synthetic matrices utilize the same functional groups on the polymer backbone for crosslinking and grafting bioactive sequences, effectively linking the biophysical and biochemical parameters of these hydrogels. Within this context, the incorporation of charged glycosaminoglycans such as hyaluronic acid and heparin, or mimics thereof, to drive growth factor sequestering and regulate osmotic pressures can be considered as a means to decouple the bioactive ligand density from the physical properties of the hydrogels.[124,125] More elegantly, smart macromolecular design can be exploited to design hydrogels that decouple these parameters. In one example, supramolecular hydrogels were made based on oligo(ethylene glycol) that were functionalized with ureido-pyrimidinone groups that could undergo hydrogen bonding to form crosslinks. Crucially, identical chains were prepared with or without adhesive peptide sequences, and the ratios of these two components could be systematically varied to generate hydrogels with similar mechanical properties albeit with varying amounts of randomly spaced bioactive sequences.[54] The authors found a synergistic effect between stiffness and ligand concentration in driving epithelial polarity (**Figure 6**). At stiffnesses below the physiological condition, cell-matrix interactions were insufficiently strong to maintain epithelial polarity, causing lumen collapse. In contrast, hydrogels matching the physiological stiffness provided sufficient rigidity to allow formation of epithelial cysts with accurate polarity. The formation of such structures was however conditional on the presence of sufficient adhesive ligands. Models such as this hydrogel system thus clearly allow investigation of the roles of individual ligands, as well as their interplay with physical parameters of the hydrogels.

Introducing degradability within the hydrogel matrix is especially important to mimic some of the dynamics of cell motility and migration.[127] Cruz-Acuña et al.[93] for instance found that degradability of a PEG-based synthetic matrix was required to preserve renal epithelial cell viability during culture and Madl et al.[128] observed that plasticity of neural progenitor cells was mainly dependent on the degradability of the synthetic matrix as opposed to its stiffness. Furthermore, challenges remain in characterizing the mechanical evolution of synthetic hydrogels following extensive periods of cell culture. Most rheological measurements of hydrogels report their mechanics at one point in time (typically prior to cell encapsulation) or gelation kinetics in the absence of cells. Mechanical characterization of synthetic matrices that have been remodeled or degraded by encapsulated cells has still been largely unexplored. Techniques to investigate hydrogel mechanics in situ or in vivo will be instrumental in optimizing synthetic matrix design for engineered tissues. In addition to understanding the roles of biochemical signals, the importance of distribution of biochemical signals on cell adhesion,[129,130] differentiation[131] and migration[132] has been underlined in 2D models. Translation of these principles in 3D models will be important to study how biochemical signal distribution plays a role in defining the 3D cellular microenvironment, although methods enabling this level of control remain unexplored.

Overall, stiffness, pore size, viscoelasticity, viscoplasticity, presentation of fibrillar components and biochemical cues, as well as degradability, have been shown to be individually relevant to cell behavior, differentiation, and morphogenesis within synthetic matrices. The extent to which these factors should be encoded into the synthetic matrix in order to drive dynamic tissue processes remains, however, an outstanding question. The unprecedented control over these individual properties, as well as the controllable synergism of multiple matrix cues, that can now be achieved in designing synthetic matrices is sure to provide the means to probe this query effectively within the scientific community.

## 6. From Cell Assays to Functioning Tissue: Engineering Dynamic Synthetic Matrices for 3D Organoid Cultures and Microphysiological Systems

The application of synthetic matrices to organoids and other microphysiological systems is especially of interest. Organoids are self-organizing 3D in vitro cultures that display some of the structural and functional characteristics observed in native tissues. As such, organoids are far more physiologically relevant in vitro models compared to traditional monolayer methods that are comprised of a homogenous population of cells with no 3D organization. Compared to traditional 2D monolayer cultures, as well as simple 3D cellular aggregates (i.e., spheroids), organoids more closely resemble their native organ counterpart in terms of cellular heterogeneity, gene and protein expression patterns, metabolic function, and micro- to macroscale tissue architecture. Importantly, human organoids help to narrow the divide between rudimentary in vitro cell culture models and in vivo animal studies, which are plagued by high rates of failure once translated across species. Moreover, the ability to generate patient-specific organoids from tissue biopsies or from induced pluripotent stem cells (iPSCs), allows them to be used in personalized drug screens that could improve the success rate of translational therapies. In addition, organoids represent a potentially scalable source of patient-derived cells, which could also be used to generate engineered tissues for transplantation. Considering the high potential impact that successful organoid culture could make, this section focuses on this tissue model with the aim to summarize the advances in strategies toward matrix design, and how these strategies may be applied to synthetic matrices, for accelerating progress within organoid tissue engineering.

Major roadblocks need to be addressed before organoids can truly move toward clinical translation, primarily their reliance on animal-derived biomaterials. Additionally, although some xenogenic hydrogels like bovine collagen or gelatin are already routinely used in clinical applications today, the inability to tune and decouple hydrogel mechanics and biochemical presentation with these materials make them less than ideal for organoid

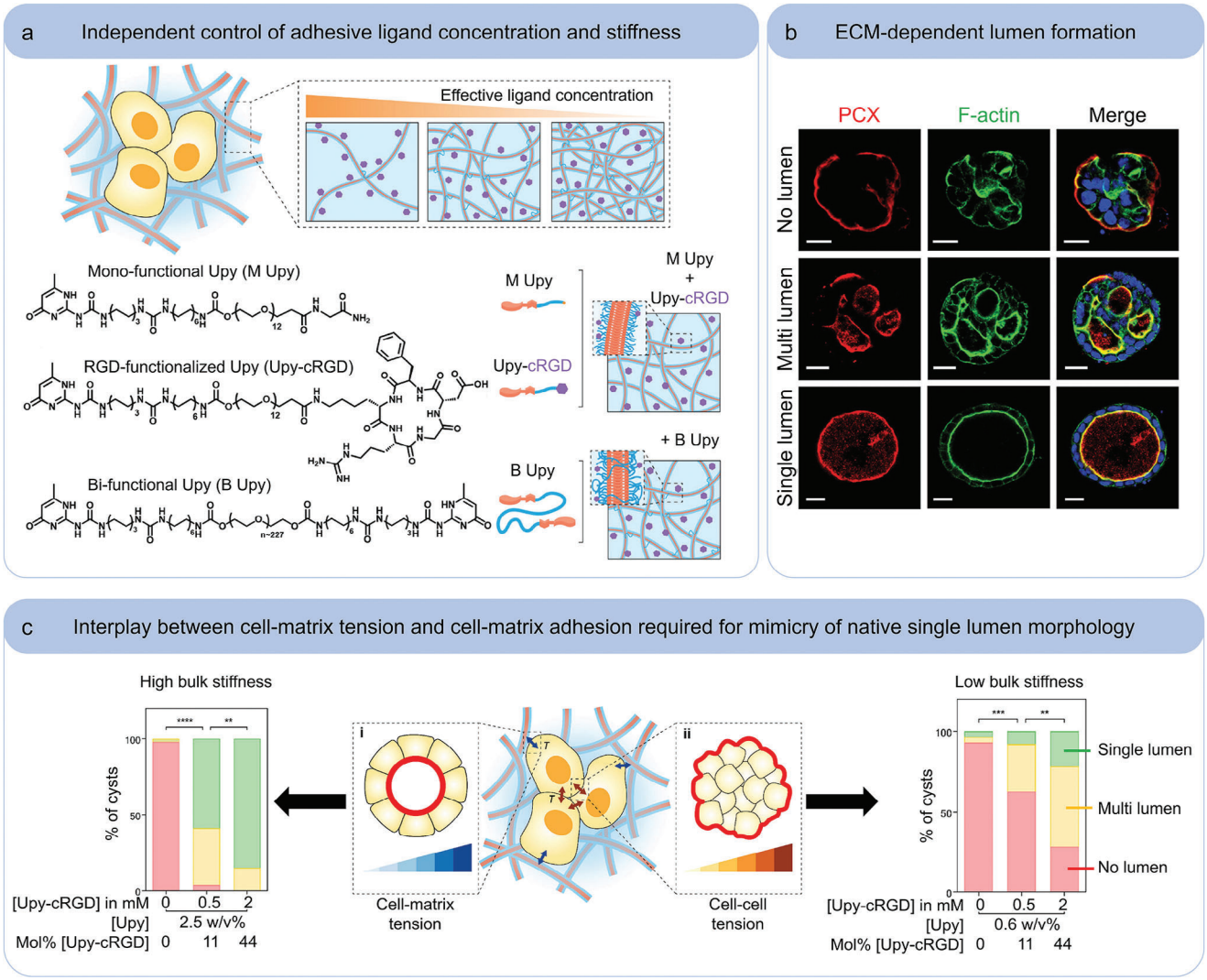


**Figure 6.** Synergistic effect of ligand density and stiffness in determining epithelial cell polarity. a) Supramolecular hydrogels were fabricated in which stiffness and ligand density could be controlled independently through adjusting the ratio between three individual components. These components consisted mono- or bi-functional ureido-pyrimidinone (UPy) building blocks functionalized with urea oligo(ethylene glycol) linkers, either with or without adhesive peptides grafted onto them. b) Epithelial cells formed three types of structures within these hydrogels, consisting of incorrect morphologies such as collapsed structures without lumen as well as structures with multiple lumen, and the correct native morphology with a singular lumen and basal-out polarity. c) Near physiological tissue stiffness (i.e., "high bulk stiffness"), sufficient cell-matrix tension existed to maintain lumen. In softer environments (i.e., "low bulk stiffness"), cell–cell tension dominated, resulting in collapse of epithelial structures. Within the physiological range, sufficient ligand density was required to ensure accurate epithelial polarity. Reproduced under the terms of the Creative Commons Non-Commercial CC-BY 4.0 License.[126]

engineering. For organoids that require scaffold-guided growth (e.g., epithelial organoids), Matrigel is almost exclusively the matrix used for cellular assembly and morphogenesis. Early designs of synthetic matrices to replace Matrigel primarily featured PEG networks functionalized with adhesive peptide ligands.[97,133,134] To relieve the compressive forces generated during organoid growth and morphogenesis, softening of these elastic networks was achieved through the use of protease-cleavable sequences[135] or hydrolytic degradation of the polymer backbone.[10] However, these methods result in permanent degradation of the matrix, restricting their utility for long-term organoid culture. Importantly, while these degradable motifs mimic native biological processes, nature rebuilds the matrix in ways that remain challenging to recreate in synthetic materials.

The development of Matrigel replacements for organoid culture in recent years have focused on the ability to tailor matrix properties to match those seen in biological tissues (e.g., brain tissue is soft and fast relaxing, while cartilage is orders of magnitude stiffer and less viscoelastic).[136] Morphogenetic processes such as symmetry breaking, which is the process where otherwise uniform cellular aggregates are forced into arrangements that presuppose functional tissues,[137] are found to be regulated through matrix properties such as viscoelasticity. For example, Chrisnandy et al. formulated PEG hydrogels crosslinked through

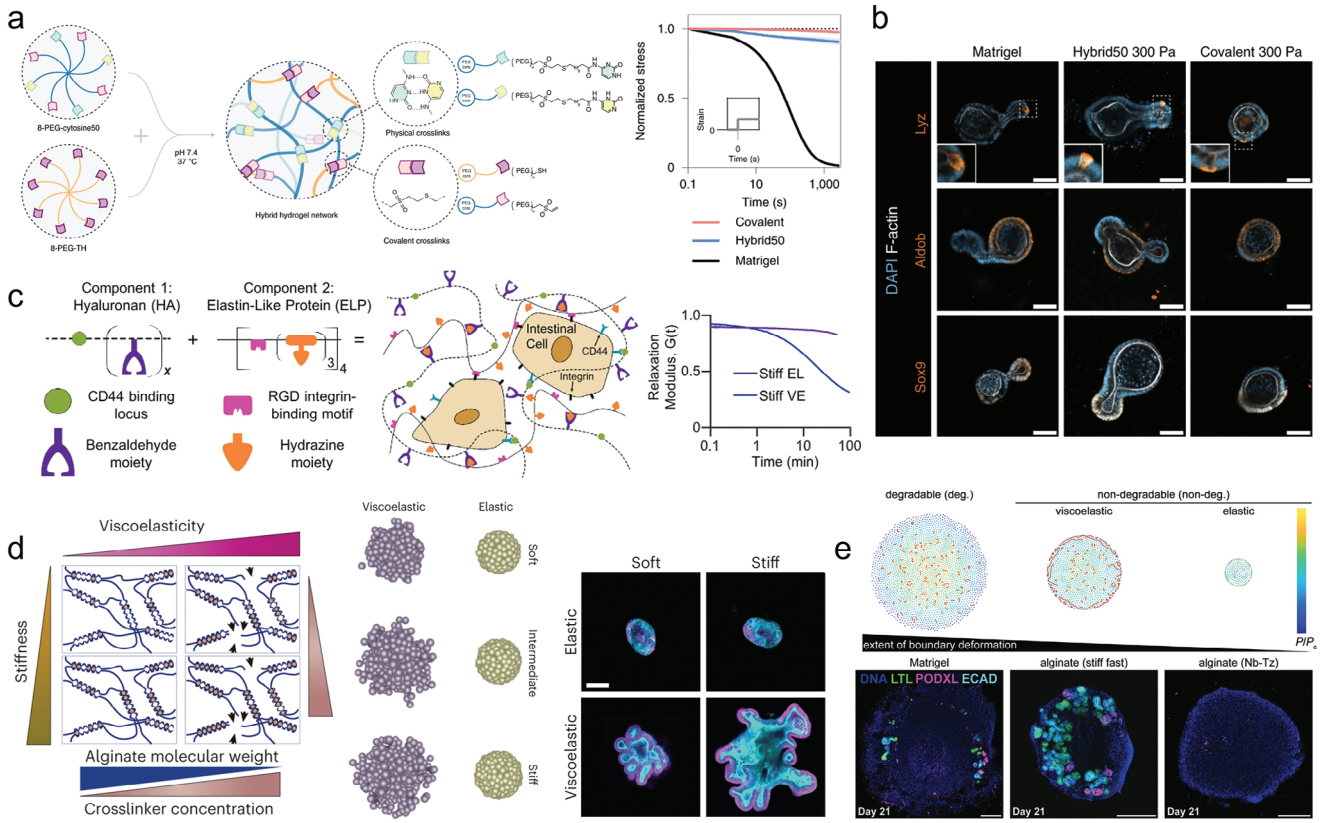


**Figure 7.** Synthetic viscoelastic matrices for organoid growth and morphogenesis. a) Stress-relaxing PEG matrices mediated by hydrogen bonding between cytosine molecules. Reproduced from Chrisnandy et al. with permission.[12] 2022, Nature Portfolio. b) Intestinal organoid morphogenesis in Matrigel, viscoelastic PEG hydrogels ("Hybrid50"), and covalent PEG hydrogels, demonstrating that stress-relaxation is required for organoid symmetry breaking. Reproduced from Chrisnandy et al. with permission.[12] 2022, Nature Portfolio. c) Recombinant hyaluronan elastin-like protein hydrogels, where viscoelasticity is tuned through the formation of dynamic hydrazone crosslinks. Reproduced from Hunt et al. with permission.[138] 2021, Wiley-VCH. d) Alginate-PEG hydrogels where polymer weight and $Ca^{2+}$ ion concentration is used to control matrix stiffness and viscoelasticity. Computational modelling of multicellular growth was used to predict the matrix properties most conducive to organoid morphogenesis. Reproduced from Eloseguí-Artola et al. with permission.[143] 2023, Nature Portfolio. e) Computational modelling of kidney organoid development was used to determine matrix properties necessary for enhanced nephrogenesis in organoid cultures. Reproduced from Nerger et al. with permission.[144] 2024, Wiley-VCH.

both covalent Michael-type addition between vinyl sulfone and thiol groups, as well as through hydrogen bonding mediated by the nucleobase cytosine[12] (**Figure 7a**). The degree of stress-relaxation could then be tuned based on the number of cytosine groups in the network, and the resulting viscoelastic matrices were shown to enable intestinal organoid morphogenesis without requiring programmed degradation of the hydrogel network (Figure 7b). Recombinant protein hydrogels such as hyaluronan elastin-like protein matrices have also been used to successfully culture intestinal organoids, as shown by Hunt et al.,[138] The network is crosslinked using dynamic covalent hydrazone linkages, and by varying the ratio of benzaldehyde–hydrazine versus aldehyde–hydrazine bonds, the level of matrix viscoelasticity could be controlled (Figure 7c). Polysaccharide-based materials have also been used for organoid culture, such as alginate[139] and nanocellulose hydrogels.[140] For instance, Ruiter et al. used oxidized alginate (bearing aldehyde groups) crosslinked with either oxime or hydrazone bonds to engineer matrices with rapid stress relaxation, demonstrating that the increase in stress relaxation speed induces enhanced kidney organoid. Future research is expected to focus on formulating fully synthetic matrices that provide greater control over viscoelasticity and stiffness to drive morphogenesis. To exemplify, ureido-pyrimidinone hydrogels have been used for the culture of pancreatic organoids,[141] and the culture of kidney organoids was possible within synthetic peptide hydrogels (commercially known as Alpha4 and Alpha5).[142]

Rational matrix design through computational studies of tissue morphogenesis, where the effect of matrix mechanics can be more quickly assessed than in empirical assays, has aided in the development of matrices for organoid culture. Eloseguí-Artola et al. ran computational simulations of the tissue–matrix interface to predict which cell- and matrix physical parameters would be most conducive to morphogenesis[143] (Figure 7d). The authors found that the theoretical predictions could be experimentally validated, observing that organoid morphogenesis was regulated by the combination of stiffer matrices that promote cell motility and stress-relaxing matrices that can promote symmetry breaking. In another recent example, Nerger et al. also used computational models to explore the influence of matrix properties on the growth and nephrogenesis of kidney organoids[144] (Figure 7e). The model predicted that non-degradable and faster-relaxing matrices would enhance nephron

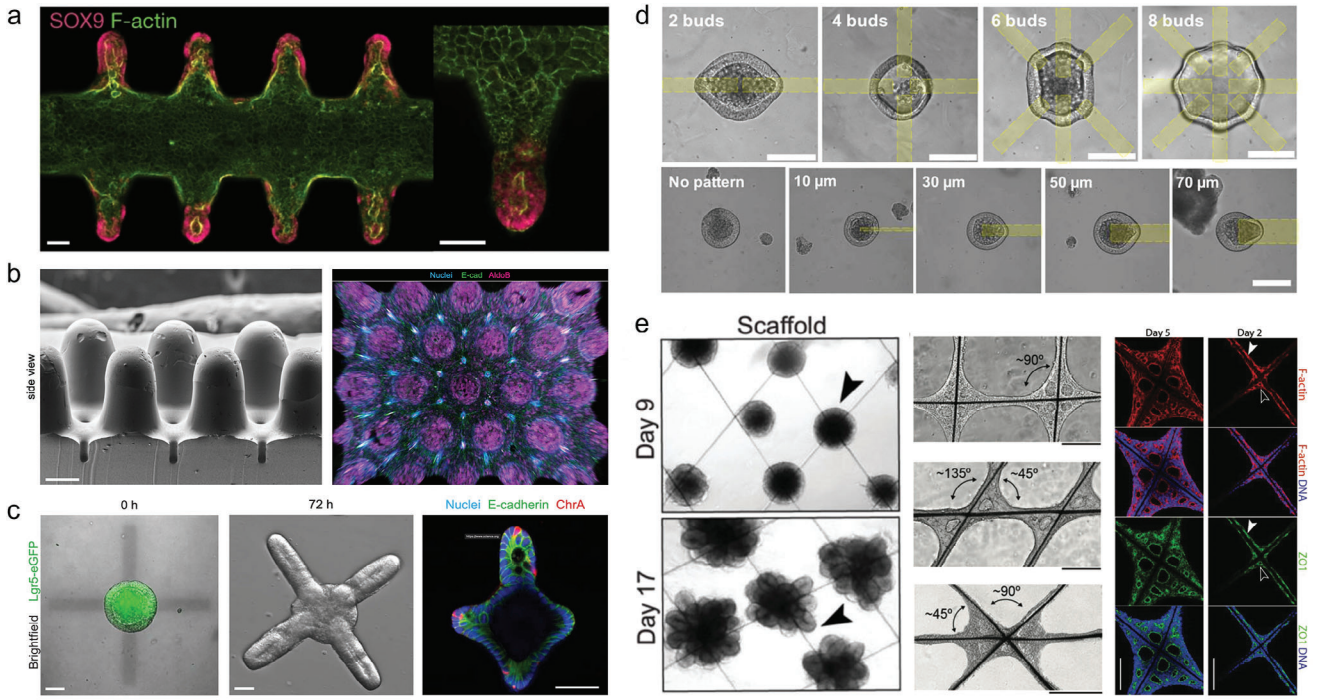


**Figure 8.** Engineering strategies to direct tissue-like symmetry breaking and patterning in organoid cultures. a) 2D recreation of native crypt-villus geometry in perfusable microfluidic devices directed appropriate intestinal stem cell localization. Reproduced from Nikolaev et al. with permission.[188] 2020, Nature Portfolio. b) Microfabricated PDMS substrates mimicking the 3D geometry of crypt-villus structures to fabricate intestinal tissue-like constructs. Reproduced from Gjorevski et al. with permission.[148] 2022, AAAS. c) Site-directed crypt formation in intestinal organoids using photoresponsive hydrogels designed to soften upon irradiation with 405 nm light. Reproduced from Gjorevski et al. with permission.[148] 2022, AAAS. d) Crypt formation of intestinal organoids controlled in situ through photoinduced viscoelasticity of user-defined regions within the hydrogel. Reproduced from Yavitt et al. with permission.[152] 2023, AAAS. e) Electrospun microfiber scaffolds for the high-throughput and reproducible culture of brain organoids. Modulating the scaffold geometry to yield varying angles between grid intersections enabled spatial control of lumen formation in embryoid bodies. Reproduced from Ritzau-Reid et al. with permission.[153] 2023, Wiley-VCH.

morphogenesis, which was subsequently experimentally validated using alginate-based matrices tuned to match the materials properties in the simulated matrix. Looking forward, the application of such computational strategies to synthetic matrices may be crucial in expediting the development of well-defined organoid and tissue models.

The complex shape, curvature, and topography of native tissues also plays a critical role in guiding symmetry breaking[145] and tissue patterning.[146,147] As a consequence, studies have demonstrated that these parameters play a role in controlling aspects of organoid growth such as cellular patterning and self-renewal,[148–150] pointing toward a need to define shape, curvature and topography in organoid matrix design. Microfabrication platforms that mimic the geometry and contours of native tissue environments can also aid in organoid culture.[151] In bulk hydrogel matrices, including Matrigel and synthetic substitutes, the random self-organization of organoids generally leads to heterogeneity and variability within cultures, which is a limitation that may be overcome with microfabrication platforms. For instance, native intestinal tissue architecture is characterized by finger-like protrusions known as villi, with a smaller stem-cell compartment between each protrusion known as the crypt. The geometry of the crypt–villus axis has been simulated in microfluidic chips (**Figure** 8a) and PDMS microwells (Figure 8b), with both successfully directing native cell localization and improving the maturation state of the organoid-tissue constructs. Photoresponsive hydrogels have also been used to spatially control the formation of crypts, with in situ matrix softening enabled through the use of photocleavable ortho-nitrobenzyl moieties[148] (Figure 8c), or light-induced rearrangement of allyl-sulfide crosslinks within PEG hydrogels[152] (Figure 8d). Microfibrous grid scaffolds fabricated using melt electrospinning writing techniques have also been used to culture brain organoids in a more high-throughput and reproducible fashion[153] (Figure 8e). Furthermore, the intersectional geometry of the grid scaffold can be manipulated, with the angle between the grid shown to control the position and size of developing lumens in pluripotent stem cell aggregates.

Designing synthetic matrices for the culture of organoids requires careful consideration of the tissue of origin, the species of origin, the cell origin (pluripotent or adult stem cell), and any variations in pathological phenotype (patient- or donor-specific differences). Many synthetic matrices are also still tested with non-human organoid models. For instance, murine intestinal organoids are frequently used because their growth is more robust and better captures the differentiated state of the mouse intestine, however their morphogenetic processes are still distinct from human organoids. In another example, human-derived organoids display less budding, depend on different niche factors for self-renewal, and contain epithelial cell types not found in mouse organoids. Caution should be used when extrapolating

optimal matrix properties from synthetic hydrogels used for cross-species organoid systems.

There are also substantial differences between human organoid lines. Today, organoids are routinely derived from both human adult stem cells (ASCs) and iPSCs. In general, ASC-derived organoids are better suited as models of homeostasis, regeneration, and adult-specific diseases, while iPSC-derived organoids are more useful models of tissue development and fetal disorders. iPSC-derived organoids are generally formed with a mesenchyme, and this has been shown to influence their growth in synthetic matrices. For example, because of the endogenous basement membrane produced by mesenchymal cells interspersed with iPSC-derived intestinal organoids, they were shown to be able to successfully grow in nonadhesive alginate hydrogels, while ASC-derived intestinal organoids could not.[139] This mesenchymal population is progressively diminished at each passage, with evidence showing the majority is lost by passage 10.[154] This suggests the passage number of organoid lines can drastically impact organoid matrix requirements and should be thoroughly considered in synthetic matrix design.

There are also differences in the regional identity of organoid, although umbrella terminology can mask this. For instance, intestinal tissue from the jejunum (i.e., small intestine) is vastly different to that of the colon (i.e., large intestine) in terms of morphology, cell populations, and matrix composition. This is reflected in their organoid counterparts, such as duodenal organoids show a higher stemness ex vivo compared to those from other regions.[155] Similarly, brain or cerebral organoids have specific regional identities such as forebrain, midbrain, and dorsal brain organoids.[156] These nuanced differences in tissue identity can have a profound impact on ECM requirements. Finally, there is natural variation inherent in donor tissue and among disease organoid models that should be considered too.

## 7. Macroscale Tissue Assembly Approaches

The majority of synthetic matrix design for tissue engineering, organotypic models and regenerative medicine involves cast hydrogels with little control over the spatial heterogeneity within these hydrogels and spatial distribution of distinct regions with varying synthetic matrix compositions. Moving forward, the mimicry of complex tissue environments will require spatial control of well-defined synthetic matrices that replicate key components of the ECM discussed in the previous sections. Biofabrication, the research field concerned with replicating the structural organization of living tissues through automated fabrication processes,[157] provides various platforms that in principle enable the spatial control desired of synthetic matrices. In this section, we aim to review the challenges that exist in adapting synthetic matrices to biofabrication platforms and review how technological advances in biofabrication may drive an expansion of the suite of synthetic matrices that can be processed through these platforms.

Biofabrication platforms can roughly be divided into droplet-based bioprinting,[158] lithography-based bioprinting and extrusion-based bioprinting. To fabricate living structures, each of these strategies requires the formulation of a cell-laden hydrogel precursors, termed bioinks, that need to meet specific requirements as dictated by the biofabrication platform. Droplet-based bioprinting involves the spatially controlled placement and subsequent fusion of droplets onto a collector plate, allowing layer-by-layer construct fabrication.[159] To ensure dispensing of these droplets, bioink need to possess low viscosity (<10 mPa.s),[160] limiting the application of droplet-based bioprinting to low polymer density inks that often yield soft hydrogel matrices.[97] Lithography-based bioprinting utilizes photopolymerizable bioink baths that are often referred to as bioresins. Through either spatially controlled laser exposure or light projections, a layer of the bioresin is cured after which noncrosslinked bioresin is removed and replaced with new bioresin. In this manner, constructs can be built in a layer-by-layer fashion. Still, lithography-based bioprinting demands relatively low viscosity (0.25–10 mPa s)[161] and is only compatible with photopolymerizable bioinks. Extrusion-based bioprinting is similar to droplet-based bioprinting but involves the dispensing of bioinks through shear-induced thinning of the bioink as it travels through the nozzle of a printer, essentially liquifying the ink. After extrusion, the bioink is required to rapidly crosslink to retain its shape, effectively allowing the fabrication of free-standing constructs in a layer-by-layer fashion. Whilst extrusion-based printing comparatively has the widest rheological window ($10^2$–$10^6$ mPa.s),[162] bioinks need to possess inherent shear-thinning properties as well as the ability to rapidly crosslink the bioink post extrusion for shape fidelity. Altogether, it is clear that the bioink design criteria are heavily dependent on the demands set by each of the main biofabrication platforms. As a result, advancements made in material design do not necessarily translate to the use of synthetic matrices in the field of biofabrication, limiting the spatial control that can be achieved with these matrices.

Most iPSC and organoid bioprinting approaches rely on the use of animal-derived materials such as Matrigel and collagen. Brassard et al. developed a microscope-based bioprinting system where organoids could be printed via extrusion into a Matrigel-collagen precursor solution (**Figure 9a**).[163] The architecture and fidelity of printed constructs was controlled through manual manipulation of the microscope stage, with cellular density tuned by changes in nozzle size, flow rate, and printing speed. Alternatively, cell suspensions have been dispensed using extrusion-based bioprinting for high-throughput fabrication of organoid-based tissue constructs. Lawlor et al. found that kidney organoids printed in thinner conformations gave rise to more individual glomeruli structures compared to thicker printed constructs of the same cell density. Their work demonstrated that bioprinting not only increased the throughput of organoid culture, but the geometry of the printed organoids led to improved nephron yield (Figure 9b).[164] Ho et al. demonstrated that bioinks comprised solely of spherical iPSC aggregates possess the rheological properties of jammed granular materials, making it possible to use wholly cellular bioinks in extrusion bioprinting systems[165] (Figure 9c). These bioprinted iPSC constructs remained viable and pluripotent and could subsequently be differentiated into each of the three germ layers post-printing. Matrix-free bioinks comprised of single iPSCs have also been used to generate pluripotent bioprinted constructs (Figure 9d). Work by Skyler-Scott et al. demonstrated that subsequent endoderm and neural differentiation of these constructs directed the formation and organization of vascular networks and neuronal projections,

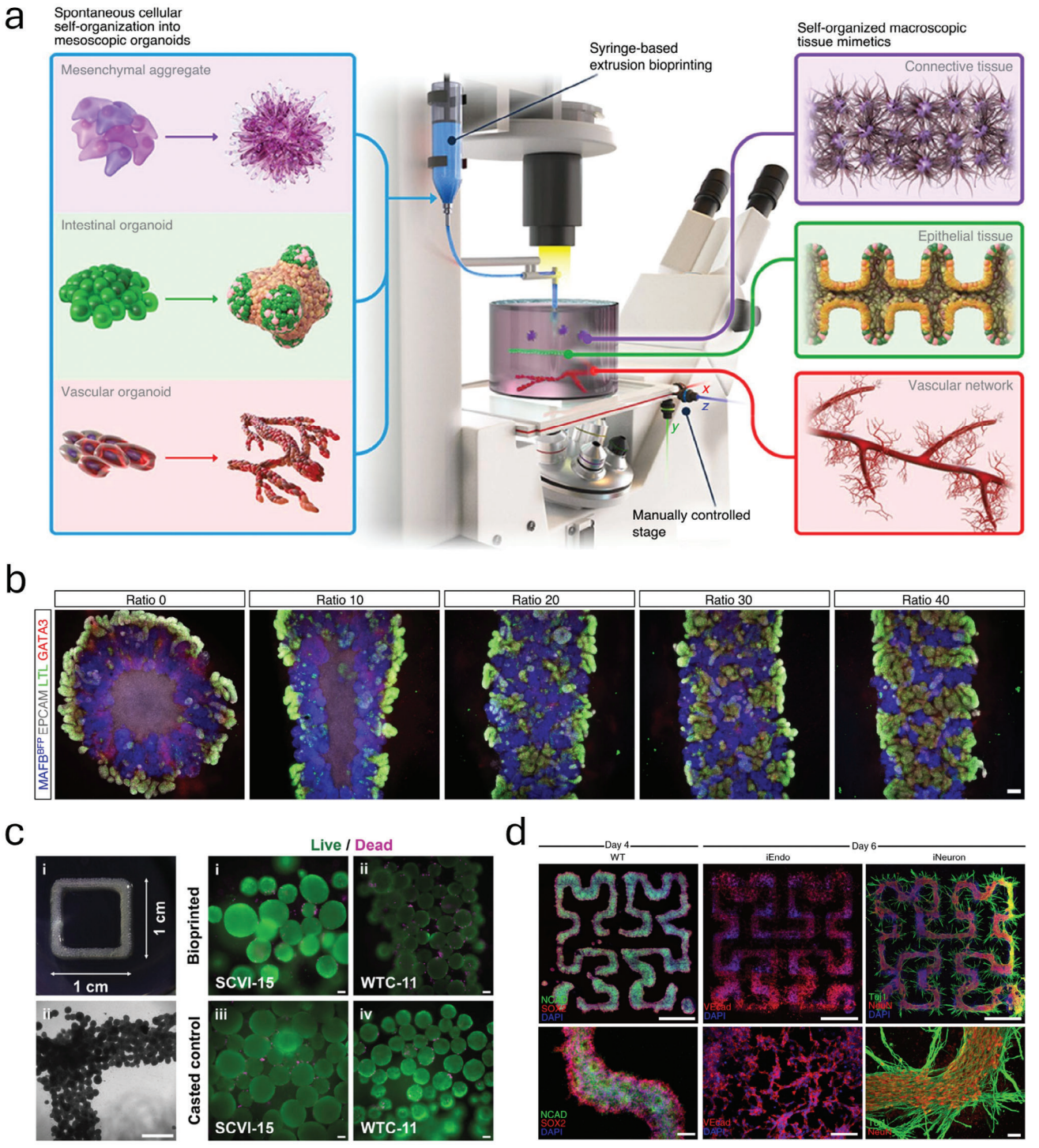


**Figure 9.** Organoid bioprinting systems used to control assembly, differentiation, and throughput fabrication of organoid-based tissues. a) Bioprinting of various organoid models using a manually controlled microscope bioprinting system. Reproduced from Brassard et al. with permission.[163] 2021, Nature Portfolio. b) Kidney organoids bioprinted into line constructs of varying heights, with decreased thickness and increased aspect ratio of the printed architecture enhancing glomeruli formation and morphogenesis. Reproduced from Lawlor et al. with permission.[164] 2021, Nature Portfolio. c) Bioprinting of a matrix-free, high-density iPSC aggregate-based ink that behaves like a jammed suspension to enable extrusion-based printing. Bioprinted constructs retained high levels of viability and pluripotency. Reproduced from Ho et al. with permission.[165] 2022, Wiley-VCH. d) Bioprinting of a matrix-free single-cell iPSC bioink, enabling geometric guidance of stem cell differentiation. Reproduced from Skyler-Scott et al. with permission.[166] 2022, Nature Portfolio.

respectively.[166] Furthermore, they demonstrated that tri-nozzle design could be used for the co-printing of three different cell types to fabricate multilayered tissue architectures.

To expand on the potential of organoid bioprinting, the umbrella of organoid-compatible bioinks that can be processed through biofabrication platforms needs to be expanded. Most of these efforts have centered around adjusting the bioink formulation to suit biofabrication platforms. One possibility is to combine materials such as collagen and Matrigel with synthetic biomaterials (i.e., hybrid bioinks). However, this approach is limited as the rheological properties of synthetic materials already primed for bioprinting may be perturbed by the inclusion of Matrigel or collagen, and the entire platform needs to cater to their strict gelation temperature regime. Alternatively, viscosity modulators can be added to bioinks to improve printability for extrusion-based bioprinting, but simultaneously will also affect the resultant hydrogel's properties.[167–169] Multistep crosslinking strategies can be applied to adjust the bioink's viscous properties in a stepwise fashion to meet the rheological demands during the stages of printing.[170,171] The application of this strategy has however only been demonstrated in a limited number of bioinks and the translatability to a wider range of bioinks may be challenging.

Recent years have seen several synthetic biomaterials being adopted as bioinks. Elastin-like peptides were for instance dispensed within a peptide amphiphile bath, forming spontaneous tubular filaments upon mixing, driven by hydrophobic interactions.[172] PEG-based bioinks were prepared with dynamic reversible bonds that provided the bioink with rapid shear-thinning and shape recovery post-printing required to process the bioink through extrusion-based bioprinting.[173] Hull et al. recently described the optimization of hyaluronan elastin-like protein matrices for use as an extrudable bioink by altering the hydrogel gelation kinetics through small molecule catalysts and inhibitors.[174] Overall, biomaterials with a range of stiffnesses have been successfully processed through biofabrication techniques. Similarly, viscoelasticity can be controlled through some extent within the context of biofabrication platforms. However, limitations imposed by current technological platforms may prevent the adoption of a wide enough range synthetic matrix formulations within biofabrication to cover the full range of biological tissues.

Rather than modifying the material properties to match platform technology, another approach to widen the range of available bioinks is driven by technological advances of the biofabrication platforms themselves. Filamented light biofabrication for instance enables a level of spatial control over the generation of filaments within bioinks, possibly replicating some of the fibrous components of the native ECM[175] (**Figure 10a**). This method exploits the inherent optical modulation instability of light to generate light beams that propagate through photoresin to generate aligned microfilaments. Excitingly, cells were able to align to these pores, emulating active organization overserved in many tissues. In another example, directional freezing of a silk solution, followed by freezing and subsequent lyophilization, resulted in the formation of directional pores onto which cells could align.[176] Whilst these techniques are currently limited to biologically derived matrices, it is reasonable to expect that this technique could be compatible with synthetic matrices as well.[175]

Granular hydrogels, which are composed of microgel building blocks that are held together through adhesive forces between the microgels, have recently gained attention. This type of hydrogel is attractive as the presence of macropores between the microgels that can be exploited by cells to increase cell motility. Granular inks have been processed within extrusion-based printing in two distinct ways, namely 1) the direct extrusion of granular inks composed of jammed microgels, wherein the adhesive forces between the microgels provide the inks with rheological properties well-suited for extrusion,[178] and 2) bioinks or aqueous solutions can be dispensed within baths composed of such microgels, in which microgels provide structural support to retain shape stability of the dispensed structures.[177] Romanazzo et al. exploited the latter to print calcium phosphate inks within microgel baths, wherein mesenchymal stromal cells could attach to the microgels and underwent osteogenic differentiation through the cues provided by the calcium phosphate inks[177] (Figure 10b). More recently, adjusting the microgel density within these baths was explored as another parameter to control the amount of interstitial space existent between the microgels.[179] The available interstitial space affected the extent of proliferation and migration observed by cells encapsulated within this space. Adjusting the interstitial space in conjunction with changing the microgel stiffness furthermore provided a strong enough cue to determine cell fate toward osteogenic and chondrogenic lineages and fabricate osteochondral interfaces. Alternatively, cellular spheroids can be used as modules to fill the interstitial space between microgels, where the ratio of the microgels and spheroids have been demonstrated to be a key parameter within bone and cartilage tissue engineering.[180] Moving forward, an exciting opportunity arises around adjusting the composition of microgels, cellular components as well as ink to include well-defined synthetic matrices.

Sacrificial inks have also been used to generate macro-porous interstitial spaces between or within hydrogels.[181] More recently, sacrificial inks were developed with tailorable dissolution kinetics for the spatiotemporal introduction of macropores within hydrogel matrices. Depending on the timing of dissolution, macroporosity increased bone and vascular tissue formation, as well as cell alignment. the development of sacrificial inks with controllable dissolution kinetics allowed temporal control over the presentation of macroporosity within hydrogels.[182] The temporal element of introducing macroporosity was found to affect cell differentiation in bone and vascular models.

Technological advances in biofabrication platforms have clearly widened the range of biomaterials that can be utilized within these platforms, enabling spatiotemporal control over the presentation of biochemical and biophysical cues within hydrogels. These rapid advances in biofabrication approaches have in many ways outpaced the development of biomimetic synthetic materials for 3D cell culture, which is readily apparent by the continued use of animal-derived matrices like Matrigel. Continuation of these technological advances may drive further widening of the biofabrication-compatible biomaterials to encompass a wide range of synthetic matrices in which biologically relevant

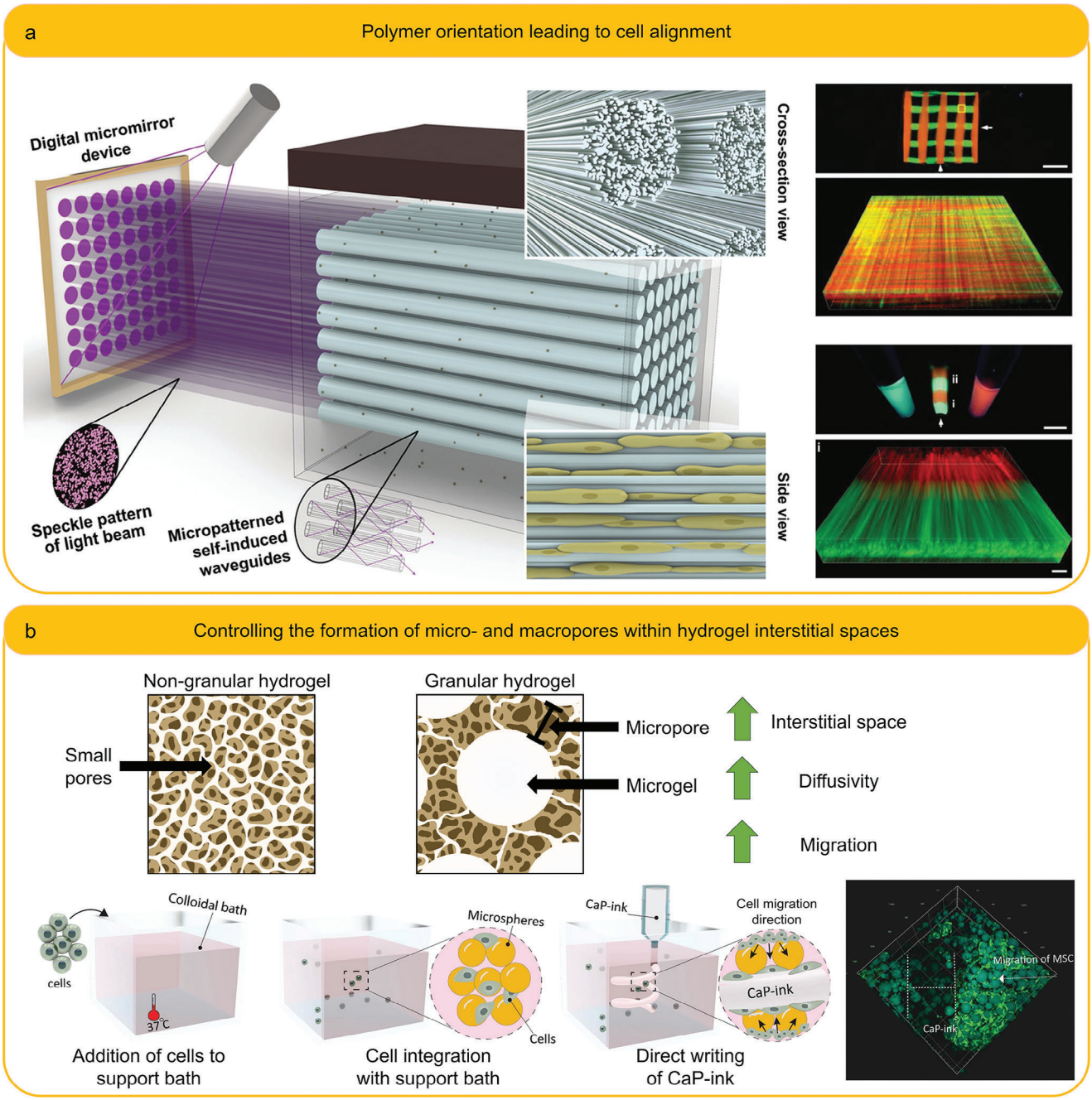


**Figure 10.** Examples of emerging biofabrication models for spatiotemporal control over hydrogel properties. a) Filamented light printing enabled the formation of filaments within a photoresin to form aligned homo- or heterogeneous structures. Cells aligned to these structures, enabling mimicry of native cellular orientation. Reproduced under the terms of the Creative Commons CC-BY 4.0 License.[175] b) Calcium phosphate ceramic ink was dispensed within a granular hydrogel bath. Due to the granularity, the inks could be printed with spatial control. Upon cultivation, mesenchymal stromal cells present within the interstitial spaces could exploit the increased open space to migrate to the calcium phosphate inks. Elements in panel b created with BioRender.com, remainder reproduced (adapted) with permission, Copyright 2021, WILEY-VCH Verlag GmbH & Co. KGaA, Weinham.[177]

parameters such as viscoelasticity, fibril content and orientation and bioactivity can be tightly controlled. These advances may eventually allow the replacement of animal-derived materials and should go hand in hand with advances in organoid technology to drive the next generation of spatiotemporally defined organoid cultures.

## 8. Alignment of Matrix Design with Usability, Commercial Accessibility, and Clinical Translation

The importance of implementing synthetic matrices in biology, tissue engineering, clinical applications and commercial use is becoming increasingly clear. With that in mind, it is crucial that

material scientists define the design criteria for the next generation of synthetic matrices based on the requirements from these intersecting research fields. For instance, systems biology is a promising field of research that combines bioinformatics with big data generated through proteomics and transcriptomics. To process tissue samples through proteomics and transcriptomics, cells need to be retrieved from tissues whilst limiting damage to tissues themselves. Tissue digestion without loss of relevant cells is not a trivial task. Similarly, removal of cells from biological and synthetic matrices in vitro is challenging. To alleviate this issue, the incorporation of cell-friendly methods for hydrogel degradation in synthetic matrix design should be considered. To exemplify, Nguyen et al. designed a peptide-based matrix that fluidizes under strain, which could be a potential way to retrieve cells from the matrix.[87] Photodegradable linkers could also be incorporated within synthetic matrices to retrieve cells from the matrix through an on-demand trigger.[152] Electrostatically-assembled hydrogel networks that can be degraded in the presence of NaCl have also been demonstrated as a method of harvesting embedded spheroids with minimal loss in viability.[183] Crucially, these modes of degradation should be independent of cell-mediated degradation and should not interfere cell-cell and cell-matrix interactions during cultivation.

Additionally, synthetic hydrogels intended for clinical use are subject to several design criteria, particularly for non-topical applications involving invasive delivery to internal tissue target sites. Synthetic materials should be confirmed as cytocompatible first, with the gelation mechanism and eventual degradation scheme designed to have no deleterious effect on cells. The mechanical stability of the hydrogel once deposited at a target tissue also needs to be considered, as strains imposed by bodily movements may perturb the scaffold over time.[184] Synthetic hydrogels should also have a bioabsorption and degradation rate that appropriately matches the kinetics of tissue regeneration, requiring matrices that are responsive to cellular remodeling and cell-mediated degradation. The manner and extent to which cells degrade and remodel synthetic matrices in the body is an important aspect of regenerative medicine, with the ideal outcome generally involving initial mechanical support over the short-term, and eventual dissolution and complete breakdown of the scaffold over the long-term once residual cells have repopulated the void area. Rheological properties of the material should also be considered. For instance, injectable hydrogels that can shear-thin upon extrusion through a syringe, and self-heal upon deposition, are highly desirable for the delivery of cells or other therapeutic cargo.[185] Finally, ascertaining if there is any possible inflammation response, the biodistribution of the material over time, and its clearance rate from the body, are important determining factors in the synthetic material's overall biocompatibility and true clinical utility.[186]

Often, matrices are formulated from synthetic materials that require complex chemical modifications. The infrastructure required to reproducibly produce these chemically modified matrices is not readily available to scientists of all backgrounds, establishing a dependency of the wider scientific community on commercially available synthetic matrices. In the coming years, it is projected that synthetic matrices that provide control over properties such as the viscoelasticity and fibrous nature of the materials will be made commercially available. The cost associated with purchase of these materials forms an obstacle for the accessibility of synthetic matrices to scientists from all backgrounds.

Compared to "gold standard" Matrigel, synthetic materials are cheaper; modified PEG hydrogels can for instance be produced at half the cost of Matrigel ($140 versus $405), but additives such as peptides and full-length proteins, as well as blended materials to ensure viscoelasticity or provide fibrous components are expected to drive up the overall costs of synthetic matrices. Reducing production costs of synthetic matrices is thus a key challenge that needs to be addressed by polymer and materials scientists. In such an effort, the introduction of microwave-assisted peptide synthesizers has improved peptide synthesis efficiency. The shift toward synthetic matrix alternatives is expected to increase peptide demand, which in turn will enable techniques such as microwave-assisted peptide synthesis to reduce overall production costs. Similar technological advances in the production of other components of the synthetic matrix may be an effective route to reduce the overall costs of the synthetic matrix.

Ensuring long-term product stability, for instance through lyophilization or product stability under repeated freeze–thaw cycles, will also be key to ensure ease-of-use. Consequently, testing compatibility with these sterilization processes should be of interest during the early stages of biomaterial development. Scaling up the manufacturing processes for hydrogels is an under-investigated research area that will require more attention in the years to come. The reaction kinetics during polymer synthesis may for instance be altered upon upscaling as mixing rate of reactants and reaction rate can cause in-batch variations as well as batch-to-batch variations if not tightly controlled. Bioreactors can be effective toward this goal, but their successful application is dependent on a thorough understanding of the reaction kinetics itself, which in turn should be a point of focus in the development of new synthetic matrices by materials scientists.

The sterilization strategy (e.g., autoclaving, filtration, ethylene oxide gas treatment, gamma irradiation, and UV sterilization) should also be considered. To exemplify, gas treatment was found to be compatible with GelMA, whilst autoclaving was observed to affect the physical properties of the resultant hydrogels. It is likely that the compatibility of sterilization methods may vary between synthetic biomaterials and this factor should thus be considered during product commercialization.

Looking forward, materials scientists play a crucial role in alleviating some of the pitfalls around biomaterial costs, accessibility, and scalability. Considering these pitfalls in early stages of material design will aid scaling up production for commercial use. For instance, aiming for the most straightforward, and least costly, chemical routes in novel materials toward desired products will reduce costs of commercial products that may arise from these materials. Similarly, reducing synthesis time, establishing protocols that limit the use of inaccessible specialized equipment, and protocols that focus on high yield and purity, will reduce the overall costs of commercial products.

## 9. Conclusions and Future Scope

The next generation of tissue models will likely be driven by the rational design of synthetic matrices that contain well-defined polymer networks, where the mechanical complexity observed in native tissues can be recreated and tuned to provide cells with

user-defined biophysical and biomechanical cues. Control over key traditional matrix parameters such as stiffness will need to be successfully paired with control over viscoelasticity, fibrous content and fiber orientation. Additionally, biochemical cues such as binding ligand composition, concentration and distribution will need to be presented in these synthetic matrices. Incorporating these biophysical and biochemical cues within synthetic matrices in a bioinspired spatiotemporal manner that reflects native tissue (patho)physiology to drive tissue assembly and morphogenesis provides a key challenge to scientists, but the tools to address this challenge are emerging.

Data-driven design strategies and high-throughput computational discovery will play an instrumental role in accelerating viscoelastic hydrogel engineering over the traditional trial-and-error syntheses, particularly when paired with the expansive matrix design space afforded by modular building blocks like amino acids, nucleic acids, or other synthetic small molecule hydrogelators. Ultimately, developing tissue-mimetic synthetic matrices that are 1) rheologically compatible with advances in biofabrication platforms and 2) biologically compatible with stem cell and organoid technologies will unlock the improved macroscale assembly and spatiotemporal control of engineered tissue models. Continued collaboration between bioengineers, chemists, material scientists and clinicians will be key to progressing toward the overall goal of one day engineering fully functional tissues.

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## Conflict of Interest

The authors declare no conflict of interest.

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[1] S. Nemec, K. A. Kilian, *Nat. Rev. Mater.* **2021**, *6*, 6983.
[2] M. Théry, A. Pépin, E. Dressaire, Y. Chen, M. Bornens, *Cell Motil. Cytoskeleton* **2006**, *63*, 341.
[3] M. Théry, V. Racine, A. Pépin, M. Piel, Y. Chen, J.-B. Sibarita, M. Bornens, *Nat. Cell Biol.* **2005**, *7*, 947.
[4] Q. Tseng, E. Duchemin-Pelletier, A. Deshiere, M. Balland, H. Guillou, O. Filhol, M. Théry, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 1506.
[5] O. Chaudhuri, L. Gu, D. Klumpers, M. Darnell, S. A. Bencherif, J. C. Weaver, N. Huebsch, H.-P. Lee, E. Lippens, G. N. Duda, D. J. Mooney, *Nat. Mater.* **2016**, *15*, 326.
[6] J. G. Roth, M. S. Huang, R. S. Navarro, J. T. Akram, B. L. LeSavage, S. C. Heilshorn, *Sci. Adv.* **2023**, *9*, eadh8313.
[7] K. Adu-Berchie, Y. Liu, D. K. Y. Zhang, B. R. Freedman, J. M. Brockman, K. H. Vining, B. A. Nerger, A. Garmilla, D. J. Mooney, *Nat. Biomed. Eng.* **2023**, 1374.
[8] Z. Wei, R. Schnellmann, H. C. Pruitt, S. Gerecht, *Cell Stem Cell* **2020**, *27*, 798.
[9] W. Fan, K. Adebowale, L. Váncza, Y. Li, M. F. Rabbi, K. Kunimoto, D. Chen, G. Mozes, D. K.-C. Chiu, Y. Li, J. Tao, Y. Wei, N. Adeniji, R. L. Brunsing, R. Dhanasekaran, A. Singhi, D. Geller, S. H. Lo, L. Hodgson, E. G. Engleman, G. W. Charville, V. Charu, S. P. Monga, T. Kim, R. G. Wells, O. Chaudhuri, N. J. Török, *Nature* **2024**, *626*, 635.
[10] N. Gjorevski, N. Sachs, A. Manfrin, S. Giger, M. E. Bragina, P. Ordez-Morán, H. Clevers, M. P. Lutolf, *Nature* **2016**, *539*, 560.
[11] E. A. Hushka, F. M. Yavitt, T. E. Brown, P. J. Dempsey, K. S. Anseth, *Adv. Healthcare Mater.* **2020**, *9*, 1901214.
[12] A. Chrisnandy, D. Blondel, S. Rezakhani, N. Broguiere, M. P. Lutolf, *Nat. Mater.* **2022**, *21*, 479.
[13] R. C. H. Gresham, C. S. Bahney, J. K. Leach, *Bioact. Mater.* **2021**, *6*, 1945.
[14] J. Lou, D. J. Mooney, *Nat. Rev. Chem.* **2022**, *6*, 726.
[15] A. B. Souza-Fernandes, P. Pelosi, P. R. Rocco, *Crit. Care* **2006**, *10*, 237.
[16] H. Sodhi, A. Panitch, *Glycosaminoglycans Tissue Eng.: Rev. Biomol.* **2021**, *11*, 29.
[17] B. Yue, *J. Glaucoma* **2014**, S20.
[18] Y. Jia, Y. Wang, L. Niu, H. Zhang, J. Tian, D. Gao, X. Zhang, T. J. Lu, J. Qian, G. Huang, F. Xu, *Adv. Healthcare Mater.* **2021**, *10*, 2001856.
[19] Z. Wei, M. Lei, Y. Wang, Y. Xie, X. Xie, D. Lan, Y. Jia, J. Liu, Y. Ma, B. Cheng, S. Gerecht, F. Xu, *Nat. Commun.* **2023**, *14*, 8307.
[20] A. J. Sophia Fox, A. Bedi, S. A. Rodeo, *Sports Health* **2009**, *1*, 461.
[21] B. Sun, *Cell Rep. Phys. Sci.* **2021**, *2*, 100515.
[22] M. J. Paszek, N. Zahir, K. R. Johnson, J. N. Lakins, G. I. Rozenberg, A. Gefen, C. A. Reinhart-King, S. S. Margulies, M. Dembo, D. Boettiger, D. A. Hammer, V. M. Weaver, *Cancer Cell* **2005**, *8*, 241.
[23] S. Mueller, L. Sandrin, *Hepat. Med.* **2010**, *2*, 49.
[24] G. Huang, F. Li, X. Zhao, Y. Ma, Y. Li, M. Lin, G. Jin, T. J. Lu, G. M. Genin, F. Xu, *Chem. Rev.* **2017**, *117*, 12764.
[25] M. &. Lutolf, J. A. Hubbel, *Nat. Biotechnol.* **2005**, *23*, 47.
[26] B. Rennekamp, C. Karfusehr, M. Kurth, A. Ünal, D. Monego, K. Riedmiller, G. Gryn'ova, D. M. Hudson, F. Gräter, *Nat. Commun.* **2023**, *14*, 2075.
[27] J. Lou, R. Stowers, S. Nam, Y. Xia, O. Chaudhuri, *Biomaterials* **2018**, *154*, 213.
[28] P. Martens, K. S. Anseth, *Polymer* **2000**, *41*, 7715.
[29] M. P. Lutolf, J. A. Hubbell, *Biomacromolecules* **2003**, *4*, 713.
[30] R. H. Utama, V. T. G. Tan, K. C. Tjandra, A. Sexton, D. H. T. Nguyen, A. P. O'Mahony, E. Y. Du, P. Tian, J. C. C. Ribeiro, M. Kavallaris, J. J. Gooding, *Macromol. Biosci.* **2021**, *21*, 2100125.
[31] C.-C. Lin, A. Raza, H. Shih, *Biomaterials* **2011**, *32*, 9685.
[32] X. Ding, Y. Yu, Y. Zu, *Biomed. Technol.* **2023**, *2*, 70.
[33] J. Nicolas, S. Magli, L. Rabbachin, S. Sampaolesi, F. Nicotra, L. Russo, *Biomacromolecules* **2020**, *21*, 1968.
[34] W. Xie, X. Wei, H. Kang, H. Jiang, Z. Chu, Y. Lin, Y. Hou, Q. Wei, *Adv. Sci.* **2023**, *10*, 2204594.
[35] A. I. Van Den Bulcke, B. Bogdanov, N. De Rooze, E. H. Schacht, M. Cornelissen, H. Berghmans, *Biomacromolecules* **2000**, *1*, 31.
[36] J. W. Nichol, S. T. Koshy, H. Bae, C. M. Hwang, S. Yamanlar, A. Khademhosseini, *Biomaterials* **2010**, *31*, 5536.
[37] L. Maleki, U. Edlund, A.-C. Albertsson, *Biomacromolecules* **2015**, *16*, 667.

[38] R. F. Pereira, C. C. Barrias, P. J. Bártolo, P. L. Granja, *Acta Biomater.* **2018**, *66*, 282.
[39] M. D. Davidson, M. E. Prendergast, E. Ban, K. L. Xu, G. Mickel, P. Mensah, A. Dhand, P. A. Janmey, V. B. Shenoy, J. A. Burdick, *Sci. Adv.* **2021**, *7*, eabi8157.
[40] M. Guvendiren, J. A. Burdick, *Nat. Commun.* **2012**, *3*, 792.
[41] S. Gerecht, J. A. Burdick, L. S. Ferreira, S. A. Townsend, R. Langer, G. Vunjak-Novakovic, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 11298.
[42] T. Wan, P. Fan, M. Zhang, K. Shi, X. Chen, H. Yang, X. Liu, W. Xu, Y. Zhou, *ACS Appl. Biol. Mater.* **2022**, *5*, 334.
[43] J. A. Burdick, C. Chung, X. Jia, M. A. Randolph, R. Langer, *Biomacromolecules* **2005**, *6*, 386.
[44] G. Camci-Unal, D. Cuttica, N. Annabi, D. Demarchi, A. Khademhosseini, *Biomacromolecules* **2013**, *14*, 1085.
[45] A. Ranga, M. P. Lutolf, J. Hilborn, D. A. Ossipov, *Biomacromolecules* **2016**, *17*, 1553.
[46] D. D. McKinnon, D. W. Domaille, T. E. Brown, K. A. Kyburz, E. Kiyotake, J. N. Cha, K. S. Anseth, *Soft Matter* **2014**, *10*, 9230.
[47] Y. Han, C. Liu, H. Xu, Y. Cao, *Chin. J. Chem.* **2022**, *40*, 1578.
[48] K.-H. Shen, Y.-Y. Yeh, T.-H. Chiu, R. Wang, Y.-C. Yeh, *ACS Biomater. Sci. Eng.* **2022**, *8*, 4249.
[49] T. E. Brown, B. J. Carberry, B. T. Worrell, O. Y. Dudaryeva, M. K. McBride, C. N. Bowman, K. S. Anseth, *Biomaterials* **2018**, *178*, 496.
[50] N. R. Gandavarapu, M. A. Azagarsamy, K. S. Anseth, *Adv. Mater.* **2014**, *26*, 2521.
[51] S. Tang, H. Ma, H.-C. Tu, H.-R. Wang, P.-C. Lin, K. S. Anseth, *Adv. Sci.* **2018**, *5*, 1800638.
[52] C. Loebel, A. Ayoub, J. H. Galarraga, O. Kossover, H. Simaan-Yameen, D. Seliktar, J. A. Burdick, *J. Mater. Chem. B* **2019**, *7*, 1753.
[53] A. C. Madl, C. M. Madl, D. Myung, *ACS Macro Lett.* **2020**, *9*, 619.
[54] L. Rijns, M. J. Hagelaars, J. J. B. van der Tol, S. Loerakker, C. V. C. Bouten, P. Y. W. Dankers, *Adv. Mater.* **2023**, 2300873.
[55] S. Hafeez, A. A. Aldana, H. Duimel, F. A. A. Ruiter, M. C. Decarli, V. Lapointe, C. van Blitterswijk, L. Moroni, M. B. Baker, *Adv. Mater.* **2023**, *35*, 2207053.
[56] S. Hafeez, F. R. Passanha, A. J. Feliciano, F. A. A. Ruiter, A. Malheiro, R. P. M. Lafleur, N. M. Matsumoto, C. van Blitterswijk, L. Moroni, P. Wieringa, V. L. S. LaPointe, M. B. Baker, *Biomater. Sci.* **2022**, *10*, 4740.
[57] T. Liu, L. van den Berk, J. A. J. Wondergem, C. Tong, M. C. Kwakernaak, B. T. Braak, D. Heinrich, B. van de Water, R. E. Kieltyka, *Adv. Healthcare Mater.* **2021**, *10*, 2001903.
[58] C. Tong, T. Liu, V. Saez Talens, W. E. M. Noteborn, T. H. Sharp, M. M. R. M. Hendrix, I. K. Voets, C. L. Mummery, V. V. Orlova, R. E. Kieltyka, *Biomacromolecules* **2018**, *19*, 1091.
[59] H. Yokoi, T. Kinoshita, S. Zhang, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 8414.
[60] S. C. Rizzi, M. Ehrbar, S. Halstenberg, G. P. Raeber, H. G. Schmoekel, H. Hagenmüller, R. Müller, F. E. Weber, J. A. Hubbell, *Biomacromolecules* **2006**, *7*, 3019.
[61] P. A. Parmar, L. W. Chow, J.-P. St-Pierre, C.-M. Horejs, Y. Y. Peng, J. A. Werkmeister, J. A. M. Ramshaw, M. M. Stevens, *Biomaterials* **2015**, *54*, 213.
[62] H. Wang, D. Zhu, A. Paul, L. Cai, A. Enejder, F. Yang, S. C. Heilshorn, *Adv. Funct. Mater.* **2017**, *27*, 1605609.
[63] M. T. Kozlowski, H. N. Zook, D. N. Chigumba, C. P. Johnstone, L. F. Caldera, H.-P. Shih, D. A. Tirrell, H. T. Ku, *Front. Bioeng. Biotechnol.* *11*, **2023**.
[64] F. Sun, W.-B. Zhang, A. Mahdavi, F. H. Arnold, D. A. Tirrell, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 11269.
[65] J. Wu, P. Li, C. Dong, H. Jiang, Bin Xue, X. Gao, M. Qin, W. Wang, Bin Chen, Y. Cao, *Nat. Commun.* **2018**, *9*, 620.
[66] K. Wu, H. Bai, Y.-T. Chang, R. Redler, K. E. McNally, W. Sheffler, T. J. Brunette, D. R. Hicks, T. E. Morgan, T. J. Stevens, A. Broerman, I. Goreshnik, M. DeWitt, C. M. Chow, Y. Shen, L. Stewart, E. Derivery, D. A. Silva, G. Bhabha, D. C. Ekiert, D. Baker, *Nature* **2023**, *616*, 581.
[67] M. Baumgart, M. Röpke, M. E. Mühlbauer, S. Asami, S. L. Mader, K. Fredriksson, M. Groll, A. P. Gamiz-Hernandez, V. R. I. Kaila, *Nat. Commun.* **2021**, *12*, 1895.
[68] J. Hu, J. Li, J. Jiang, L. Wang, J. Roth, K. N. McGuinness, J. Baum, W. Dai, Y. Sun, V. Nanda, F. Xu, *Nat. Commun.* **2022**, *13*, 6761.
[69] D. Dranseike, Y. Ota, T. G. W. Edwardson, E. A. Guzzi, M. Hori, Z. R. Nakic, D. V. Deshmukh, M. D. Levasseur, K. Mattli, C. M. Tringides, J. Zhou, D. Hilvert, C. Peters, M. W. Tibbitt, *Acta Biomater.* **2024**, *117*, 107.
[70] R. Mout, R. C. Bretherton, J. Decarreau, S. Lee, N. Gregorio, N. I. Edman, M. Ahlrichs, Y. Hsia, D. D. Sahtoe, G. Ueda, A. Sharma, R. Schulman, C. A. DeForest, D. Baker, *Proc. Natl. Acad. Sci. USA* **2024**, *121*, 2309457121.
[71] J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Zdek, A. Potapenko, A. Bridgland, C. Meyer, S. A. A. Kohl, A. J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, et al., *Nature* **2021**, *596*, 583.
[72] J. L. Watson, D. Juergens, N. R. Bennett, B. L. Trippe, J. Yim, H. E. Eisenach, W. Ahern, A. J. Borst, R. J. Ragotte, L. F. Milles, B. I. M. Wicky, N. Hanikel, S. J. Pellock, A. Courbet, W. Sheffler, J. Wang, P. Venkatesh, I. Sappington, S. V. Torres, A. Lauko, V. De Bortoli, E. Mathieu, S. Ovchinnikov, R. Barzilay, T. S. Jaakkola, F. DiMaio, M. Baek, D. Baker, *Nature* **2023**, *620*, 1089.
[73] A. D. Martin, A. B. Robinson, A. F. Mason, J. P. Wojciechowski, P. Thordarson, *Chem. Commun.* **2014**, *50*, 15541.
[74] A. D. Martin, J. P. Wojciechowski, A. B. Robinson, C. Heu, C. J. Garvey, J. Ratcliffe, L. J. Waddington, J. Gardiner, P. Thordarson, *Sci. Rep.* **2017**, *7*, 43947.
[75] G. Yang, T. Huang, Y. Wang, H. Wang, Y. Li, K. Yu, L. Dong, *J. Biomater. Sci. Polym. Ed.* **2018**, *29*, 1812.
[76] J. Kisiday, M. Jin, B. Kurz, H. Hung, C. Semino, S. Zhang, A. J. Grodzinsky, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 9996.
[77] Q. Li, G. Qi, X. Liu, J. Bai, J. Zhao, G. Tang, Y. S. Zhang, R. Chen-Tsai, M. Zhang, D. Wang, Y. Zhang, A. Atala, J.-Q. He, X. S. Sun, *Adv. Funct. Mater.* **2021**, *31*, 2104046.
[78] A. Faroni, V. L. Workman, A. Saiani, A. J. Reid, *Adv. Healthcare Mater.* **2019**, *8*, 1900410.
[79] S. Koutsopoulos, S. Zhang, *Acta Biomater.* **2013**, *9*, 5162.
[80] X. Mu, L. Shi, S. Pan, L. He, Y. Niu, X. Wang, *ACS Omega* **2020**, *5*, 16568.
[81] S. Zhang, C. Lockshin, R. Cook, A. Rich, *Biopolymers* **1994**, *34*, 663.
[82] C. J. Barrow, A. Yasuda, P. T. Kenny, M. G. Zagorski, *J. Mol. Biol.* **1992**, *225*, 1075.
[83] E. F. Banwell, E. S. Abelardo, D. J. Adams, M. A. Birchall, A. Corrigan, A. M. Donald, M. Kirkland, L. C. Serpell, M. F. Butler, D. N. Woolfson, *Nat. Mater.* **2009**, *8*, 596.
[84] A. Saiani, A. Mohammed, H. Frielinghaus, R. Collins, N. Hodson, C. M. Kielty, M. J. Sherratt, A. F. Miller, *Soft Matter* **2008**, *5*, 193.
[85] J. P. Schneider, D. J. Pochan, B. Ozbas, K. Rajagopal, L. Pakstis, J. Kretsinger, *J. Am. Chem. Soc.* **2002**, *124*, 15030.
[86] P. W. J. M. Frederix, G. G. Scott, Y. M. Abul-Haija, D. Kalafatovic, C. G. Pappas, N. Javid, N. T. Hunt, R. V. Ulijn, T. Tuttle, *Nat. Chem.* **2015**, *7*, 30.
[87] A. K. Nguyen, T. G. Molley, E. Kardia, S. Ganda, S. Chakraborty, S. L. Wong, J. Ruan, B. E. Yee, J. Mata, A. Vijayan, N. Kumar, R. D. Tilley, S. A. Waters, K. A. Kilian, *Nat. Commun.* **2023**, *14*, 6604.
[88] T. Xu, J. Wang, S. Zhao, D. Chen, H. Zhang, Y. Fang, N. Kong, Z. Zhou, W. Li, H. Wang, *Nat. Commun.* **2023**, *14*, 3880.

[89] J. Wu, B. R. Liyarita, H. Zhu, M. Liu, X. Hu, F. Shao, *ACS Appl. Mater. Interfaces* **2021**, *13*, 49705.
[90] D. Athanasiadou, N. Meshry, N. G. Monteiro, A. C. Ervolino-Silva, R. L. Chan, C. A. McCulloch, R. Okamoto, K. M. M. Carneiro, *Proc. Natl. Acad. Sci. USA* **2023**, *120*, 2220565120.
[91] Y.-H. Peng, S.-K. Hsiao, K. Gupta, A. Ruland, G. K. Auernhammer, M. F. Maitz, S. Boye, J. Lattner, C. Gerri, A. Honigmann, C. Werner, E. Krieg, *Nat. Nanotechnol.* **2023**, *18*, 1463.
[92] S. R. Deshpande, R. Hammink, R. K. Das, F. H. T. Nelissen, K. G. Blank, A. E. Rowan, H. A. Heus, *Adv. Funct. Mater.* **2016**, *26*, 9075.
[93] R. Cruz-Acuña, A. Mulero-Russe, A. Y. Clark, R. Zent, A. J. García, *J. Cell Sci.* **2019**, *132*, jcs226639.
[94] O. Chaudhuri, S. T. Koshy, C. Branco da Cunha, J.-W. Shin, C. S. Verbeke, K. H. Allison, D. J. Mooney, *Nat. Mater.* **2014**, *13*, 970.
[95] R. S. Stowers, A. Shcherbina, J. Israeli, J. J. Gruber, J. Chang, S. Nam, A. Rabiee, M. N. Teruel, M. P. Snyder, A. Kundaje, O. Chaudhuri, *Nat. Biomed. Eng.* **2019**, *3*, 1009.
[96] N. Huebsch, E. Lippens, K. Lee, M. Mehta, S.-T. Koshy, M.-C. Darnell, R. M. Desai, C.-M. Madl, M. Xu, X. Zhao, O. Chaudhuri, C. Verbeke, W.-S. Kim, K. Alim, A. Mammoto, D.-E. Ingber, G.-N. Duda, D.-J. Mooney, *Nat. Mater.* **2015**, *14*, 1269.
[97] E. Y. Du, M. Jung, J. Skhinas, M. A. K. Tolentino, J. Noy, N. Jamshidi, J. L. Houng, K. C. Tjandra, M. Engel, R. Utama, R. D. Tilley, M. Kavallaris, J. J. Gooding, *ACS Appl. Biol. Mater.* **2023**, *6*, 4603.
[98] F. Charbonier, D. Indana, O. Chaudhuri, *Curr. Protoc.* **2021**, *1*, e124.
[99] S. Nam, V. K. Gupta, H.-P. Lee, J. Y. Lee, K. M. Wisdom, S. Varma, E. M. Flaum, C. Davis, R. B. West, O. Chaudhuri, *Sci. Adv.* **2019**, *5*, eaaw6171.
[100] S. Nam, R. Stowers, J. Lou, Y. Xia, O. Chaudhuri, *Biomaterials* **2019**, *200*, 15.
[101] I. A. Marozas, K. S. Anseth, J. J. Cooper-White, *Biomaterials* **2019**, *223*, 119430.
[102] B. M. Richardson, D. G. Wilcox, M. A. Randolph, K. S. Anseth, *Acta Biomater.* **2019**, *83*, 71.
[103] D. D. McKinnon, D. W. Domaille, J. N. Cha, K. S. Anseth, *Adv. Mater.* **2014**, *26*, 865.
[104] J. Lou, S. Friedowitz, K. Will, J. Qin, Y. Xia, *Adv. Mater.* **2021**, *33*, 2104460.
[105] B. Yang, K. Wei, C. Loebel, K. Zhang, Q. Feng, R. Li, S. H. D. Wong, X. Xu, C. Lau, X. Chen, P. Zhao, C. Yin, J. A. Burdick, Y. Wang, L. Bian, *Nat. Commun.* **2021**, *12*, 3514.
[106] H. Chen, J. Zhang, W. Yu, Y. Cao, Z. Cao, Y. Tan, *Angew. Chem., Int. Ed.* **2021**, *60*, 22332.
[107] R. K. Das, V. Gocheva, R. Hammink, O. F. Zouani, A. E. Rowan, *Nat. Mater.* **2016**, *15*, 318.
[108] B. Yang, K. Wei, C. Loebel, K. Zhang, Q. Feng, R. Li, S. H. D. Wong, X. Xu, C. Lau, X. Chen, P. Zhao, C. Yin, J. A. Burdick, Y. Wang, L. Bian, *Nat. Commun.* **2021**, *12*, 3514.
[109] K. M. Wisdom, K. Adebowale, J. Chang, J. Y. Lee, S. Nam, R. Desai, N. S. Rossen, M. Rafat, R. B. West, L. Hodgson, O. Chaudhuri, *Nat. Commun.* **2018**, *9*, 4144.
[110] H.-P. Lee, F. Alisafaei, K. Adebawale, J. Chang, V. B. Shenoy, O. Chaudhuri, *Sci. Adv.* **2021**, *7*, eabd4058.
[111] S. Nam, J. Lee, D. G. Brownfield, O. Chaudhuri, *Biophys. J.* **2016**, *111*, 2296.
[112] C. Liu, N. Morimoto, L. Jiang, S. Kawahara, T. Noritomi, H. Yokoyama, K. Mayumi, K. Ito, *Science* **2021**, *372*, 1078.
[113] T. A. Ulrich, A. Jain, K. Tanner, J. L. MacKay, S. Kumar, *Biomaterials* **2010**, *31*, 1875.
[114] K. H. Vining, A. Stafford, D. J. Mooney, *Biomaterials* **2019**, *188*, 187.
[115] J. Kim, J. Feng, C. A. R. Jones, X. Mao, L. M. Sander, H. Levine, B. Sun, *Nat. Commun.* **2017**, *8*, 842.
[116] A. D. Doyle, F. W. Wang, K. Matsumoto, K. M. Yamada, *J. Cell Biol.* **2009**, *184*, 481.
[117] M. D. Davidson, K. H. Song, M.-H. Lee, J. Llewellyn, Y. Du, B. M. Baker, R. G. Wells, J. A. Burdick, *ACS Biomater. Sci. Eng.* **2019**, *5*, 3899.
[118] M. D. Davidson, M. E. Prendergast, E. Ban, K. L. Xu, G. Mickel, P. Mensah, A. Dhand, P. A. Janmey, V. B. Shenoy, J. A. Burdick, *Sci. Adv.* **2021**, *7*, eabi8157.
[119] E. Prince, Z. Chen, N. Khuu, E. Kumacheva, *Biomacromolecules* **2021**, *22*, 2352.
[120] J. D. Hartgerink, E. Beniash, S. I. Stupp, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5133.
[121] A. C. Farsheed, A. J. Thomas, B. H. Pogostin, J. D. Hartgerink, *Adv. Mater.* **2023**, *35*, 2210378.
[122] J. R. Lovett, M. J. Derry, P. Yang, F. L. Hatton, N. J. Warren, P.-W. Fowler, S. P. Armes, *Chem. Sci.* **2018**, *9*, 7138.
[123] A. Pardo, S. M. Bakht, M. Gomez-Florit, R. Rial, R. F. Monteiro, S. P. B. Teixeira, P. Taboada, R. L. Reis, R. M. A. Domingues, M. E. Gomes, *Adv. Funct. Mater.* **2022**, *32*, 2208940.
[124] D. S. W. Benoit, A. R. Durney, K. S. Anseth, *Biomaterials* **2007**, *28*, 66.
[125] A. K. Jha, K. M. Tharp, J. Ye, J. L. Santiago-Ortiz, W. M. Jackson, A. Stahl, D. V. Schaffer, Y. Yeghiazarians, K. E. Healy, *Biomaterials* **2015**, *47*, 1.
[126] L. Rijns, M. J. Hagelaars, J. J. B. van der Tol, S. Loerakker, C. V. C. Bouten, P. Y. W. Dankers, *Adv. Mater. n/a*, 2300873.
[127] B. Trappmann, B. M. Baker, W. J. Polacheck, C. K. Choi, J. A. Burdick, C. S. Chen, *Nat. Commun.* **2017**, *8*, 371.
[128] C. M. Madl, B. L. LeSavage, R. E. Dewi, C. B. Dinh, R. S. Stowers, M. Khariton, K. J. Lampe, D. Nguyen, O. Chaudhuri, A. Enejder, S. C. Heilshorn, *Nat. Mater.* **2017**, *16*, 1233.
[129] R. Oria, T. Wiegand, J. Escribano, A. Elosegui-Artola, J. J. Uriarte, C. Moreno-Pulido, I. Platzman, P. Delcanale, L. Albertazzi, D. Navajas, X. Trepat, J. M. García-Aznar, E. A. Cavalcanti-Adam, P. Roca-Cusachs, *Nature* **2017**, *552*, 219.
[130] J. Huang, S. V. Gräter, F. Corbellini, S. Rinck, E. Bock, R. Kemkemer, H. Kessler, J. Ding, J. P. Spatz, *Nano Lett.* **2009**, *9*, 1111.
[131] M. Zhang, Q. Sun, Y. Liu, Z. Chu, L. Yu, Y. Hou, H. Kang, Q. Wei, W. Zhao, J. P. Spatz, C. Zhao, E. A. Cavalcanti-Adam, *Biomaterials* **2021**, *268*, 120543.
[132] Q. Liu, S. Zheng, K. Ye, J. He, Y. Shen, S. Cui, J. Huang, Y. Gu, J. Ding, *Biomaterials* **2020**, *263*, 120327.
[133] R. H. Utama, V. T. G. Tan, K. C. Tjandra, A. Sexton, D. H. T. Nguyen, A. P. O'Mahony, E. Y. Du, P. Tian, J. C. C. Ribeiro, M. Kavallaris, J. J. Gooding, *Macromol. Biosci.* **2021**, *21*, 2100125.
[134] E. A. Aisenbrey, W. L. Murphy, *Nat. Rev. Mater.* **2020**, *5*, 539.
[135] R. Cruz-Acuña, M. Quirós, A. E. Farkas, P. H. Dedhia, S. Huang, D. Siuda, V. García-Hernández, A. J. Miller, J. R. Spence, A. Nusrat, A. J. García, *Nat. Cell Biol.* **2017**, *19*, 1326.
[136] O. Chaudhuri, J. Cooper-White, P. A. Janmey, D. J. Mooney, V. B. Shenoy, *Nature* **2020**, *584*, 535.
[137] H. T. Zhang, T. Hiiragi, *Annu. Rev. Cell Dev. Biol.* **2018**, *34*, 405.
[138] D. R. Hunt, K. C. Klett, S. Mascharak, H. Wang, D. Gong, J. Lou, X. Li, P. C. Cai, R. A. Suhar, J. Y. Co, B. L. LeSavage, A. A. Foster, Y. Guan, M. R. Amieva, G. Peltz, Y. Xia, C. J. Kuo, S. C. Heilshorn, *Adv. Sci.* **2021**, *8*, 2004705.
[139] M. M. Capeling, M. Czerwinski, S. Huang, Y.-H. Tsai, A. Wu, M. S. Nagy, B. Juliar, N. Sundaram, Y. Song, W. M. Han, S. Takayama, E. Alsberg, A. J. Garcia, M. Helmrath, A. J. Putnam, J. R. Spence, *Stem Cell Rep.* **2019**, *12*, 381.
[140] R. Curvello, D. Alves, H. E. Abud, G. Garnier, *Mater. Sci. Eng., C: Mater. Biol. Appl.* **2021**, *124*, 112051.
[141] M. Diba, S. Spaans, S. I. S. Hendrikse, M. M. C. Bastings, M. J. G. Schotman, J. F. van Sprang, D. J. Wu, F. J. M. Hoeben, H. M. Janssen, P. Y. W. Dankers, *Adv. Mater.* **2021**, *33*, 2008111.
[142] N. J. Treacy, et al., *Bioact. Mater.* **2023**, *21*, 142.

[143] A. Elosegui-Artola, A. Gupta, A. J. Najibi, B. R. Seo, R. Garry, C. M. Tringides, I. de Lázaro, M. Darnell, W. Gu, Q. Zhou, D. A. Weitz, L. Mahadevan, D. J. Mooney, *Nat. Mater.* **2023**, *22*, 117.
[144] B. A. Nerger, S. Sinha, N. N. Lee, M. Cheriyan, P. Bertsch, C. P. Johnson, L. Mahadevan, J. V. Bonventre, D. J. Mooney, *Adv. Mater.* *36*, 2308325.
[145] C. M. Nelson, *AIChE J.* **2012**, *58*, 3608.
[146] P. Kollmannsberger, C. M. Bidan, J. W. C. Dunlop, P. Fratzl, *Soft Matter* **2011**, *7*, 9549.
[147] C.-P. Heisenberg, Y. Bellaïche, *Cell* **2013**, *153*, 948.
[148] N. Gjorevski, M. Nikolaev, T. E. Brown, O. Mitrofanova, N. Brandenberg, F. W. DelRio, F. M. Yavitt, P. Liberali, K. S. Anseth, M. P. Lutolf, *Science* **2022**, *375*, eaaw9021.
[149] N. Pentinmikko, R. Lozano, S. Scharaw, S. Andersson, J. I. Englund, D. Castillo-Azofeifa, A. Gallagher, M. Broberg, K.-Y. Song, A. Sola Carvajal, A. T. Speidel, M. Sundstrom, N. Allbritton, M. M. Stevens, O. D. Klein, A. Teixeira, P. Katajisto, *Sci. Adv.* **2022**, *8*, eabm1847.
[150] P. Srivastava, S. Romanazzo, C. Kopecky, S. Nemec, J. Ireland, T. G. Molley, K. Lin, P. B. Jayathilaka, E. Pandzic, A. Yeola, V. Chandrakanthan, J. Pimanda, K. Kilian, *Adv. Sci.* **2023**, *10*, 2203614.
[151] R. H. Utama, L. Ataputta, A. P. O'Mahony, C. M. Fife, J. Baek, T. Allard, K. J. O'Mahony, J. C. C. Riberi, K. Gaus, M. Kavallaris, J. J. Gooding, *Science* **2020**, *23*, 101621.
[152] F. M. Yavitt, B. E. Kirkpatrick, M. R. Blatchley, K. F. Speckl, E. Mohagheghian, R. Moldovan, N. Wang, P. J. Dempsey, K. S. Anseth, *Sci. Adv.* **2023**, *9*, eadd5668.
[153] K. I. Ritzau-Reid, S. J. P. Callens, R. Xie, M. Cihova, D. Reumann, C. L. Grigsby, L. Prados-Martin, R. Wang, A. C. Moore, J. P. K. Armstrong, J. A. Knoblich, M. M. Stevens, *Adv. Mater.* **2023**, *35*, 2300305.
[154] L. Kandilogiannakis, E. Filidou, I. Drygiannakis, G. Tarapatzi, S. Didaskalou, M. Koffa, K. Arvanitidis, G. Bamias, V. Valatas, V. Paspaliaris, G. Kolios, *Stem Cells Int.* **2021**, *2021*.
[155] G.-W. He, L. Lin, J. DeMartino, X. Zheng, N. Staliarova, T. Dayton, H. Begthel, W. J. van de Wetering, E. Bodewes, J. van Zon, S. Tans, C. Lopez-Iglesias, P. J. Peters, W. Wu, D. Kotlarz, C. Klein, T. Margaritis, F. Holstege, H. Clevers, *Cell Stem Cell* **2022**, *29*, 1333.
[156] M. Magni, B. Bossi, P. Conforti, M. Galimberti, F. Dezi, T. Lischetti, X. He, R. A. Barker, C. Zuccato, I. Espuny-Camacho, E. Cattaneo, *Int. J. Mol. Sci.* **2022**, *23*, 13159.
[157] L. Moroni, T. Boland, J. A. Burdick, C. De Maria, B. Derby, G. Forgacs, J. Groll, Q. Li, J. Malda, V. A. Mironov, C. Mota, M. Nakamura, W. Shu, S. Takeuchi, T. B. F. Woodfield, T. Xu, J. J. Yoo, G. Vozzi, *Trends Biotechnol.* **2018**, *36*, 384.
[158] M. Jung, S. Ghamrawi, E. Y. Du, J. J. Gooding, M. Kavallaris, *Adv. Healthcare Mater.* **2022**, *11*, 2200690.
[159] H. Gudapati, M. Dey, I. Ozbolat, *Biomaterials* **2016**, *102*, 20.
[160] F. L. C. Morgan, L. Moroni, M. B. Baker, *Adv. Healthcare Mater.* **2020**, *9*, 1901798.
[161] R. J. Mondschein, A. Kanitkar, C. B. Williams, S. S. Verbridge, T. E. Long, *Biomaterials* **2017**, *140*, 170.
[162] R. L. Truby, J. A. Lewis, *Nature* **2016**, *540*, 371.
[163] J. A. Brassard, M. Nikolaev, T. Hübscher, M. Hofer, M. P. Lutolf, *Nat. Mater.* **2021**, *20*, 22.
[164] K. T. Lawlor, J. M. Vanslambrouck, J. W. Higgins, A. Chambon, K. Bishard, D. Arndt, P. X. Er, S. B. Wilson, S. E. Howden, K. S. Tan, F. Li, L. J. Hale, B. Shepherd, S. Pentoney, S. C. Presnell, A. E. Chen, M. H. Little, *Nat. Mater.* **2021**, *20*, 260.
[165] D. L. L. Ho, S. Lee, J. Du, J. D. Weiss, T. Tam, S. Sinha, D. Klinger, S. Devine, A. Hamfeldt, H. T. Leng, J. E. Herrmann, M. He, L. G. Fradkin, T. K. Tan, D. Standish, P. Tomasello, D. Traul, N. Dianat, R. Ladi, Q. Vicard, K. Katikireddy, M. A. Skylar-Scott, *Adv. Healthcare Mater.* **2022**, *11*, 2201138.
[166] M. A. Skylar-Scott, J. Y. Huang, A. Lu, A. H. M. Ng, T. Duenki, S. Liu, L. L. Nam, S. Damaraju, G. M. Church, J. A. Lewis, *Nat. Biomed. Eng.* **2022**, *6*, 449.
[167] V. H. M. Mouser, F. P. W. Melchels, J. Visser, W. J. A. Dhert, D. Gawlitta, J. Malda, *Biofabrication* **2016**, *8*, 035003.
[168] K. S. Lim, B. S. Schon, N. V. Mekhileri, G. C. J. Brown, C. M. Chia, S. Prabakar, G. J. Hooper, T. B. F. Woodfield, *ACS Biomater. Sci. Eng.* **2016**, *2*, 1752.
[169] G. Cidonio, C. R. Alcala-Orozco, K. S. Lim, M. Glinka, I. Mutreja, Y.-H. Kim, J. I. Dawson, T. B. F. Woodfield, R. O. C. Oreffo, *Biofabrication* **2019**, *11*, 035027.
[170] D. Petta, A. R. Armiento, D. Grijpma, M. Alini, D. Eglin, M. D'Este, *Biofabrication* **2018**, *10*, 044104.
[171] B. G. Soliman, G. C. J. Lindberg, T. Jungst, G. J. Hooper, J. Groll, T. B. F. Woodfield, K. S. Lim, *Adv. Healthcare Mater.* **2020**, *9*, 1901544.
[172] K. E. Inostroza-Brito, E. Collin, O. Siton-Mendelson, K. H. Smith, A. Monge-Marcet, D. S. Ferreira, R. P. Rodríguez, M. Alonso, J. C. Rodríguez-Cabello, R. L. Reis, F. Sagués, L. Botto, R. Bitton, H. S. Azevedo, A. Mata, *Nat. Chem.* **2015**, *7*, 897.
[173] V. Yesilyurt, M. J. Webber, E. A. Appel, C. Godwin, R. Langer, D. G. Anderson, *Adv. Mater.* **2016**, *28*, 86.
[174] S. M. Hull, J. Lou, C. D. Lindsay, R. S. Navarro, B. Cai, L. G. Brunel, A. D. Westerfield, Y. Xia, S. C. Heilshorn, *Sci. Adv.* **2023**, *9*, eade7880.
[175] H. Liu, P. Chansoria, P. Delrot, E. Angelidakis, R. Rizzo, D. Rütsche, L. A. Applegate, D. Loterie, M. Zenobi-Wong, *Adv. Mater.* **2022**, *34*, 2204301.
[176] H. Joukhdar, Z. Och, H. Tran, C. Heu, G. M. Vasquez, N. Sultana, M. Stevens, S. Dokos, K. S. Lim, M. S. Lord, J. Rnjak-Kovacina, *Adv. Mater. Technol.* **2023**, *8*, 2201642.
[177] S. Romanazzo, T. G. Molley, S. Nemec, K. Lin, R. Sheikh, J. J. Gooding, B. Wan, Q. Li, K. A. Kilian, I. Roohani, *Adv. Funct. Mater.* **2021**, *31*, 2008216.
[178] C. B. Highley, K. H. Song, A. C. Daly, J. A. Burdick, *Adv. Sci.* **2018**, *6*, 1801076.
[179] G. K. Jalandhra, T. G. Molley, T. Hung, I. Roohani, K. A. Kilian, *Acta Biomater.* **2023**, *156*, 75.
[180] N. D. Caprio, M. D. Davidson, A. C. Daly, J. A. Burdick, *Adv. Mater.* **2024**, *36*, 2312226.
[181] L. Ouyang, J. P. K. Armstrong, Q. Chen, Y. Lin, M. M. Stevens, *Adv. Funct. Mater.* **2020**, *30*, 1908349.
[182] B. G. Soliman, A. Longoni, M. Wang, W. Li, P. N. Bernal, A. Cianciosi, G. C. J. Lindberg, J. Malda, J. Groll, T. Jungst, R. Levato, J. Rnjak-Kovacina, T. B. F. Woodfield, Y. S. Zhang, K. S. Lim, *Adv. Funct. Mater.* **2023**, *33*, 2210521.
[183] P. Suwannakot, S. Nemec, N. G. Peres, E. Y. Du, K. A. Kilian, K. Gaus, M. Kavallaris, J. J. Gooding, *ACS Biomater. Sci. Eng.* **2023**, *9*, 1362.
[184] K. Jin, H. Li, M. Liang, Y. Li, L. Wang, Y. Fan, *Regener. Biomater.* **2023**, *10*, rbad050.
[185] B. A. Aguado, W. Mulyasasmita, J. Su, K. J. Lampe, S. C. Heilshorn, *Tissue Eng., Part A* **2012**, *18*, 806.
[186] M. Mateu-Sanz, C. V. Fuenteslópez, J. Uribe-Gomez, H. Jostein Haugen, A. Pandit, M. Ginebra, O. Hakimi, M. Krallinger, A. Samara, *Trends Biotechnol.* **2023**, *42*.
[187] M. C. Branco, D. J. Pochan, N. J. Wagner, J. P. Schneider, *Biomaterials* **2009**, *30*, 1339.
[188] M. Nikolaev, O. Mitrofanova, N. Broguiere, S. Geraldo, D. Dutta, Y. Tabata, B. Elci, N. Brandenberg, I. Kolotuev, N. Gjorevski, H. Clevers, M. P. Lutolf, *Nature* **2020**, *585*, 574.

**Bram G. Soliman** is a Postdoctoral Researcher at the University of New South Wales, Australia. He completed his Ph.D. at the University of Otago under the supervision of Associate Professor Khoon Limon designing biofabrication strategies for the simultaneous control over macro- and microenvironment physical features in engineered tissues, for which he was recognized with the Division of Health Sciences Award for Outstanding Ph.D. Thesis. His current research currently focusses on developing reductionist in vitro models to investigate the roles of cell–cell and cell–matrix interactions in breast cancer drug resistance and metastasis.

**Ashley K. Nguyen** is a postdoctoral researcher in the Department of Bioengineering at the University of San Diego (UCSD). She completed her Ph.D. under Professor Kristopher A. Kilian at the University of New South Wales in Sydney, Australia. She is currently working in the laboratory of Professor Brian Aguado, with research interests including dynamic biomaterials, pluripotent stem cells, and X-chromosome inactivation and gene escape.

**Justin Gooding** is a Scientia Professor in the School of Chemistry at the University of New South Wales and a National Health and Medical Research Council Leadership Fellow. He is a recipient of the David Craig Medal from the Academy of Science, a three time Eureka Prize winner and a two time winner of the International Biosensors and Bioelectronics award. He is the inaugural editor-in-chief of the journal *ACS Sensors*. He leads a research team of over 40 researchers interested in surface modification and nanotechnology for biosensors for medical applications, electrocatalysis and 3D cell printing.

**Kristopher A. Kilian** is Professor of Chemistry and Materials Science and Engineering at the University of New South Wales and Co-Director of the Australian Centre for NanoMedicine. He is a recipient of the Cornforth Medal from the Royal Australian Chemical Institute, the NIH Ruth L. Kirchstein National Research Service Award, the National Science Foundation's CAREER award, a Young Innovator of Cellular and Molecular Bioengineering, and the Australian Research Council Future Fellowship. His research interests include stem cell engineering, microphysiological systems and the design and development of dynamic hydrogels for biotechnology and biomedical applications.