A practical guide to hydrogels for cell culture

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There is growing appreciation of the role that the extracellular environment plays in regulating cell behavior. Mechanical, structural, and compositional cues, either alone or in concert, can drastically alter cell function. Biomaterials, and particularly hydrogels, have been developed and implemented to present defined subsets of these cues for investigating countless cellular processes as a means of understanding morphogenesis, aging, and disease. Although most scientists concede that standard cell culture materials (tissue culture plastic and glass) do a poor job of recapitulating native cellular milieus, there is currently a knowledge barrier for many researchers in regard to the application of hydrogels for cell culture. Here, we introduce hydrogels to those who may be unfamiliar with procedures to culture and study cells with these systems, with a particular focus on commercially available hydrogels.

Introduction: why hydrogels for cell culture?

Our collective understanding of many cell-based processes is derived from experiments performed on flat, unphysiologically stiff materials such as polystyrene and glass. Although the simplicity of these platforms is attractive, cells cultured in these environments tend to display aberrant behaviors: flattened shape, abnormal polarization, altered response to pharmaceutical reagents, and loss of differentiated phenotype (**Fig. 1**). Furthermore, these culture systems are typically two dimensional (2D)[1], whereas cells in the body are likely to receive signals not just at their ventral surface but in all three dimensions. Culture systems that better mimic the biological milieu are needed to bridge the gap between conventional cultures and complex native *in vivo* environments.

The field of biomaterials continues to advance the introduction of such complexity into cell culture systems, providing ways to control mechanical, compositional, and structural cues and thus more accurately represent features of native tissues[2]. A range of biomaterial systems have been developed toward this goal, including patterned glass substrates, elastomeric films, hydroxyapatite ceramics and fibrillar foams. However, hydrogels—water-swollen networks of polymers—have emerged as the most promising option for cell culture (**Fig. 2**) since they mimic salient elements of native extracellular matrices (ECMs), have mechanics similar to those of many soft tissues, and can support cell adhesion and protein sequestration[3].

Hydrogels have proven useful in a range of cell culture applications, revealing fundamental phenomena regulating cell behavior and providing tools for the expansion and directed differentiation of various cell types in ways not possible with conventional culture substrates. It would be impossible to cover all of these advances here, and that is not the intent of this Review. As one interesting example, however, seminal work from the Bissell lab has demonstrated that healthy mammary epithelial cells exhibit tumorigenic potential in conventional monolayer culture but assemble into multicellular spherical structures resembling healthy acini when encapsulated in a 3D basement membrane–derived hydrogel[4]. In separate experiments, embryonic stem cells (ESCs) typically spontaneously differentiate within a few days in conventional culture but show a more complex response in hydrogel culture: pluripotency markers can be maintained through control over hydrogel mechanics in the absence of leukemia inhibitory factor supplementation[5], or over hydrogel chemistry with the introduction of hyaluronic acid (HA)[6] and other glycosaminoglycans[7].

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BOX 1 KEY TERMS

Biomimetic. Imitates or replicates specific properties or features of natural tissues.

Chain-growth polymerization. Polymerization mode in which monomers are sequentially added onto the active site of a growing polymer chain.

Mesh size. The space between crosslinks in a polymer network, essentially the molecular porosity of the network.

Poisson's ratio. Negative ratio of the transverse to axial strain, where strain is the change in material length in response to an applied force divided by the original length.

RGD. Peptide often used in hydrogels to facilitate cell attachment containing the adhesive sequence arginine–glycine–aspartic acid found in fibronectin.

Rheology. Study of flow and deformation of materials in response to an applied force.

Step-growth polymerization. Polymerization mode in which multifunctional monomers and/or crosslinkers react gradually to form progressively higher-molecular-weight polymers.

In studies using hydrogels as models for drug screening, cells grown on stiff, collagen-rich substrates show greater resistance to chemotherapies than do cells on softer substrates[8]. In studies of mechanical influence on cell behavior, lung fibroblasts grown on stiff substrates undergo myofibroblast differentiation and retain a contractile phenotype even when later moved to soft substrates[9], implying that cells retain memory of past mechanical environments[10], which may bias subsequent experiments. Although these are just a few examples in which hydrogel culture platforms have been used, it is clear that cellular outcomes can be quite different in these systems than they are in standard culture.

The objective of this Review is to provide a guide for those who are interested in using hydrogels for cell culture but may not be sufficiently familiar with the technology. Although some hydrogels, in particular natural materials like collagen and Matrigel, are being explored by biologists, there are many other hydrogel options that can be used depending on the biological questions being asked. We will present an overview of various hydrogels that can be used for cell culture, ranging from simple 2D films (where cells sit atop a substrate) to more complex 3D systems (where cells are embedded within a hydrogel), as well as discussing advantages and disadvantages of such systems. We provide basic instruction on hydrogel formation and assessment and direct the user to references for commercially available systems that may be of particular interest. We caution that not all hydrogels are equal and will attempt to provide guidance here to their similarities and differences. Our hope is that this Review can serve as a primer to hydrogels for nonexperts and provide a roadmap for researchers for applying such culture systems to their cell studies.

Hydrogel fabrication and characterization

Fabricating hydrogels. Forming hydrogels for cellular experiments typically involves either encapsulation of viable cells within the material or fabrication of substrates using molds that are later seeded with cells. Hydrogel formation involves the transition of liquid precursor solutions into solid materials, which can be achieved using either physical (noncovalent) or chemical (covalent) crosslinking to assemble the hydrogel components. The majority of peptide- or protein-based systems are formed through self-assembly by physical crosslinking processes; in collagen hydrogels, for example, interactions between solubilized fibrils lead to fiber and network assembly over time. Peptide hydrogels are often engineered with amphiphilic or other complementary sequences that can self-assemble into supramolecular structures such as β-sheets during gelation[11]. Other natural materials may assemble through charge interactions: for instance, divalent cations induce gelation of anionic alginate polymers. Synthetic polymers have also been modified with various functional groups to enable physical crosslinking[12].

Chemical crosslinking of polymers can also be used for hydrogel formation. Chain-growth polymerizations (**Box 1**) may be initiated with one of various stimuli (such as redox initiation or photoinitiation) to induce the covalent reaction of reactive groups (such as acrylates, methacrylates or acrylamides) for rapid hydrogel formation[13]. When such preparations are to be used with cells, it is important to keep polymerization times short and use nontoxic initiators (for example, I2959 or lithium acylphosphinate salt for photopolymerization) so as to minimize cell death and maintain overall cellular function. Free radicals generated through photopolymerization have been reported to damage cells, especially sensitive primary cell types[14]; thus, it is important to investigate the compatibility of crosslinking procedures for any cell types of interest. Alternatively, step-growth polymerizations occur when two or more hydrogel precursors are combined that react directly upon mixing. Perhaps the most common reaction of this type for hydrogel formation is the Michael-type addition reaction between multifunctional monomers and crosslinkers. Again, it is important that the polymerization time and reagents be designed so that cell encapsulation occurs in a cytocompatible manner. In the examples included later in this Review, procedures optimized to preserve the viability of encapsulated cells are typically reported. In both examples of chemical crosslinking, gelation needs to occur fast enough to prevent the settling of cells during the encapsulation process.

Characterization of hydrogel properties. There are a variety of hydrogel properties that may be of interest to characterize, including mechanics, swelling, mesh size, and degradation. When purchasing commercial kits or following specific hydrogel recipes, these may already be known and will not need to be characterized by every user. However, it is important to understand how these features are characterized and how they may influence the utility of hydrogels for cell culture applications.

Hydrogel mechanical properties are important for the stability of the material in culture and may also influence cellular mechanotransduction (the conversion of mechanical information from the microenvironment into biochemical signaling), which in turn has consequences for cellular behaviors like spreading, migration, and stem cell differentiation[15]. Hydrogel mechanical properties are typically reported as either their shear modulus (G) or their elastic modulus (E), two values that are related to each other as a function of the material's Poisson's ratio (υ) as shown by the equation:

$$E = 2G(1 + \upsilon)$$

Most hydrogels are assumed to have a Poisson's ratio of around 0.45–0.5, meaning that $E \approx 3G$. Two dimensional hydrogel film

mechanics are typically assessed by atomic force microscopy (AFM), perhaps the most suitable technique for measuring substrate mechanical properties on a cellular scale because of the micron-sized cantilever probes used to indent the sample. Techniques such as compressive or tensile testing, which provide bulk mechanical properties by pushing or pulling a material, respectively, or other indentation methods may be used to characterize the mechanics of 3D hydrogels. The hydrogel elastic modulus is calculated from the applied stress and the resultant strain on the material within the linear elastic region of deformation. Indentation testing is well suited for many of the viscoelastic and poroviscoelastic natural materials used for tissue engineering, owing to the minimal sample preparation requirements and the ability to assay material properties at multiple length scales[16]. Time-dependent properties such as gelation time and shear modulus are measured using rheology, in which shear forces are applied in order to characterize the rate of hydrogel formation or the ability of a material to relax after gelation. For a more comprehensive review of hydrogel mechanical characterization techniques, the reader is referred elsewhere[17].

Another important hydrogel property is swelling, defined as the amount of water or buffer taken up into the hydrogel. This is a straightforward property to measure and is an indicator of the polymer network hydrophilicity as well as of the relative crosslinking density, with stiffer networks typically exhibiting lower swelling. The swelling properties can be useful as an indicator of batch-to-batch variations and consistency in hydrogel fabrication properties as well as to help understand whether the hydrogel mechanics are changing over time. The swelling ratio is measured by first allowing hydrogels to reach equilibrium swelling (typically by incubating at 37 °C for at least 24 h), blotting to remove excess solvent, and weighing to obtain the wet weight (M_w). The hydrogel is then dried to determine the dry weight (M_d). The mass-swelling ratio (Q_m) is typically defined as the ratio of wet weight to dry weight (M_w/M_d), whereas the volumetric swelling ratio (Q_v) is calculated from the mass swelling ratio and the densities of the hydrogel polymer (ρ_p) and solvent (ρ_s) using the following equation:

$$Q_v = 1 + \frac{\rho_p}{\rho_s}\left(\frac{M_w}{M_d} - 1\right)$$

The mesh size, or molecular porosity, of the hydrogel is typically on the nanometer scale and can influence nutrient flux throughout the matrix. It is correlated to hydrogel swelling behavior and mechanical properties, since lower swelling and higher modulus indicate a smaller mesh size. Although imaging techniques such as scanning electron microscopy (SEM) are commonly used to assess hydrogel microstructure, these techniques are inherently flawed for this application since the sample must be dried before analysis, which alters the native hydrogel structure. Alternatively, fluorescence recovery after photobleaching (FRAP)[18], DNA electrophoresis[19], or simply measurement of the diffusion of fluorescently tagged polymers entrapped within the hydrogel can be used to characterize mesh size and molecular transport. The mesh size of step-growth hydrogels can also be estimated using theoretical approaches[20]. More details on the characterization of hydrogel swelling ratio and mesh size can be found elsewhere[21].

Hydrogel degradation can lead to changes in mechanics and swelling over time, which in turn affect cell behaviors such as motility, spreading, and traction force generation[22]; whether or not hydrogel degradation is desirable depends on the goal of the study. Hydrogels typically degrade through either hydrolytic or enzymatic mechanisms, where hydrolysis occurs throughout the entire hydrogel and enzymatic degradation is local to the presented enzyme. Hydrolysis occurs with inclusion of hydrolytically unstable chemical bonds, and the rate of hydrolysis can be tuned by altering parameters such as the crosslinking density, which is controlled through the polymer concentration or extent of crosslinking during material fabrication. More advanced hydrogels have used external triggers such as ultraviolet (UV) light[23] to control degradation.

Natural hydrogels like collagen and fibrin degrade by cell-mediated proteases such as matrix metalloproteinases (MMPs). Synthetic hydrogels are increasingly being engineered with peptide

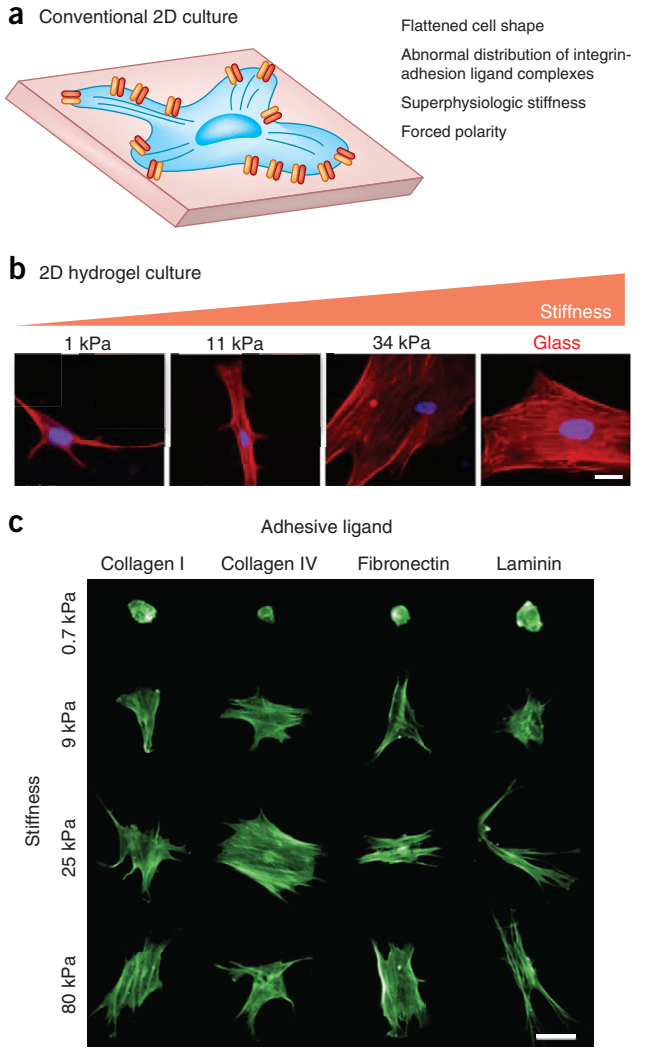

Figure 1 | Cell culture atop 2D hydrogels. (**a**) Conventional 2D culture on superphysiologically stiff plastic or glass substrates leads to cells displaying aberrant phenotypes. (**b**) Culturing cells on 2D hydrogel films has some of the same disadvantages as conventional methods but permits user-defined control of the substrate stiffness and adhesive ligand presentation. Human mesenchymal stem cells (MSCs) cultured on increasingly stiff 2D substrates display increasing spread area. Left to right: 1 kPa polyacrylamide (PA), 11 kPa PA, 34 kPa PA, and glass (~GPa). Scale bar, 10 μm. Images modified from ref. 65 with permission. (**c**) Substrate stiffness (*y* axis) and adhesive ligand type (*x* axis) combine to regulate MSC morphology. Human MSCs spread more with increasing stiffness, but cells on laminin-coated hydrogels are smaller than those on other ECM protein coatings. Images modified from ref. 64 with permission. Scale bar, 50 μm.

crosslinkers[24] that can be tailored to degrade in response to these same MMPs. Degradation kinetics are usually tracked by incubating hydrogels in buffer, collecting samples for analysis every few hours to several days, depending on anticipated degradation profiles, and monitoring degradation byproducts (for example, uronic acid for HA or soluble collagen). Degradation can also be quantified by labeling hydrogels with fluorophores and tracking the fluorescence of degradation byproducts or by monitoring changes in mechanical properties. It is important to note that even hydrogels that would be considered nondegradable on the time scale of most cell experiments, such as photocrosslinked poly(ethylene glycol), may eventually degrade; however, their stability at timescales useful for these studies makes them attractive for use.

Hydrogel sterilization. Hydrogels may be sterilized before cell culture using gamma or germicidal UV irradiation, ethylene oxide exposure, ethanol treatment of already formed hydrogels, or dense carbon dioxide gas sterilization[25]. For cell encapsulation, the precursor solutions must be sterilized before hydrogel formation. This can be accomplished either through filtering (if solutions are not too viscous) or through germicidal UV irradiation of the solution or dry polymer. While all of these approaches are effective, one must be careful to choose a technique that will not degrade, denature, or otherwise alter hydrogel physical properties. For example, extended UV treatment can denature collagen and promote peptide degradation within functionalized hydrogels[26], while gamma irradiation can degrade alginate[27]. Commercial hydrogel kit components are typically provided pre-sterilized or may include specific sterilization instructions.

Characterizing cell outcomes in hydrogel cultures

Isolating cells from hydrogels. Just as for cells cultured on conventional surfaces, cells cultured on or within hydrogels often need to be harvested as a means of propagation or to carry out molecular or cellular analyses. RNA and protein can be isolated from cells grown on 2D hydrogel substrates similarly to cells seeded on plastic or glass, although cell scraping may be more difficult. Sample preparation from hydrogel-embedded cells presents more technical challenges. Techniques used on tissue samples, including mechanical and/or enzymatic disruption, can be useful for liberating embedded cells but must be performed with great care to maintain the integrity of intracellular components while still generating sufficient yields. The specific properties of the hydrogel must be considered as well. For example, RNA isolation from polysaccharide matrices using guanidinium thiocyanate–based methods common in the Qiagen Mini Kits results in inferior RNA yields and quality compared to those obtained with Trizol and cationic surfactants like cetyltrimethylammonium bromide (CTAB)[28]. It may also be desirable to isolate intact cells for applications such as flow cytometry or when using hydrogels for cell expansion. Enzymatic degradation of naturally derived materials such as collagen (collagenase), fibrin (nattokinase), and HA (hyaluronidase) permits cell liberation, although care must be taken not to disrupt cell surface receptors through extended enzyme treatment. Once isolated, cells can be split and re-encapsulated in new hydrogels for further culture and expansion. More advanced materials and techniques (such as photodegradation) are also being applied to enable cell isolation from hydrogels where cell release is not currently possible[29].

Visualizing cells and biomolecules in hydrogels. Rather than extraction of cells for analysis, some hydrogel studies will require *in situ* cell imaging. Hydrogel films intended for microscopy are usually fabricated on glass coverslips to enable high-resolution imaging. Cells cultured on these 2D hydrogels can often be processed for immunohistochemistry in the same fashion as cells cultured conventionally, although care must be taken not to disturb the hydrogel attachment to coverslips during the staining and washing steps. Coverslip silanization to permit covalent attachment of the hydrogel directly to the coverslip surface is a common way to prevent this[30]. When preparing 3D hydrogels for imaging, standard immunostaining protocols can often be used, although incubation steps should be lengthened and/or include mechanical agitation to encourage adequate diffusion of the reagents into the hydrogel[31]. Many hydrogels are also optically transparent—including those specifically mentioned later here—and permit imaging using confocal microscopy.

While hydrogels are usually processed for histology in a manner similar to soft tissue samples, this may not be ideal: the dehydration and heating steps in paraffin embedding can result in hydrogel deformation and folding during sectioning[32], although careful processing can ameliorate these concerns[33]. Sugar-based solvents used in cryosectioning result in brittle embedding blocks that are difficult to cut due to elevated water content in hydrogels compared to that in many tissues. Recent evidence suggests that using alternatives to sucrose such as polyvinyl alcohol (PVA) and Optimum Cutting Temperature (OCT) compound during cryosectioning could improve histological assessment of hydrogels[34]. Other studies have used plastic resins such as glycol methacrylate as the embedding medium[35]. These materials tend to more faithfully preserve hydrogel structure but typically need to be stained whole mount before sectioning.

Selecting a hydrogel for cell culture

Hydrogels can be broadly classified as either natural or synthetic materials, with each classification carrying a distinct set of advantages and disadvantages. In this section we will discuss seven commonly used hydrogels: three naturally derived (collagen, fibrin, alginate), two synthetic (polyacrylamide, polyethylene glycol), and two hybrid materials that combine elements of synthetic and natural polymers (hyaluronic acid, polypeptides). We summarize commercial vendors and the advantages and disadvantages of using each material (**Table 1**) and include references for cell studies using these materials (**Supplementary Table 1**). Although other hydrogels have been used for cell culture (for example, chitosan[36], silk[37], PVA[38], dextran[39]), they require polymer synthesis before hydrogel formation; we focus here only on systems for which kits or hydrogel precursors can be directly purchased from vendors. Additionally, although this is beyond the scope of this Review, many of these hydrogels have applicability for *in vivo* studies, including cell delivery and soft tissue engineering[40].

Many factors should be considered when selecting a hydrogel; the most important for the typical biologist are adhesivity to cells, stability in culture, and biophysical properties such as hydrogel elastic modulus. Some materials, including collagen and fibrin, are typically used without further modification. Others, including PEG and HA, are often chemically modified to support specific crosslinking mechanisms; modified polymers can be purchased commercially or prepared in house. Additionally, some materials interact with cells through integrin–ligand interactions (for

Table 1 | Representative hydrogels that can be used for cell culture studies

Material	Example vendors	Notable material features
Natural materials		
Collagen	BD BioSciences, Advanced BioMatrix (PureCol, FibriCol), Vitrogen, Flexcell (Thermacol, Collagel)	Typically sourced from rat tail tendon or bovine skin and tendon Usually purchased in pepsin- or acid-solubilized form and stored at low pH and temperature Enzymatically degradable Exhibits structural and mechanical properties reminiscent of native tissues Presents native cell adhesion ligands
Fibrin	Baxter (Tisseel, Artiss), Johnson & Johnson (Evicel), Sigma	Typically sourced from human plasma Enzymatically degradable Provides good substrate for studying wound-healing phenomena *in vitro* Low mechanics limit utility
Alginate	NovaMatrix-3D, PRONOVA (FMC BioPolymer)	Derived from brown algae Must be modified with adhesive ligands for cell attachment Ionic crosslinking with divalent cations enables easy cell encapsulation and recovery Additional covalent crosslinking often needed for strength
Synthetic materials		
Polyacrylamide (PA)	Sigma	Wide range tuning of substrate mechanics Probably the most standardized material as far as protocols for making hydrogels and using for culture Suitable for 2D cell culture only
Polyethylene glycol (PEG)	QGel Inc. (QGel), Sigma, Cellendes (3-D Life Dextran-PEG or PVA-PEG), BioTime Inc. (PEGgel)	'Blank slate' synthetic material enables a wealth of user modifications Premodified versions and various molecular weights are readily available Can be engineered to present different adhesive ligands and to degrade via passive, proteolytic, or user-directed modes
Hybrid materials		
Hyaluronic acid (HA)	Lifecore (Corgel BioHydrogel), BioTime Inc. (HyStem), BRTI Life Sciences (Cell-Mate3D)	Usually produced via bacterial fermentation, but can also be sourced from animal products Wide variety and high degree of potential chemical modification enables considerable tunability Interacts with cell receptors but must be modified with adhesive ligands to permit cell attachment
Polypeptides	Corning (PuraMatrix), PepGel LLC (PGmatrix), Sigma (HydroMatrix)	Typically formed by self-assembly Useful in soft-tissue applications and in conjunction with other materials Protein engineering enables great design flexibility

example, collagen, fibrin, polypeptides) or other cell surface receptors (for example, HA), while others are considered more inert (for example, PEG, polyacrylamide). These and other material-specific design considerations will be discussed in the subsequent sections.

Beyond hydrogel selection, it is important to identify whether culturing cells in 2D (atop a hydrogel film) or in 3D (encapsulated within a hydrogel) is most appropriate. While this choice will primarily be influenced by the individual user's application of interest, there are additional considerations. In general, cells are less constrained in 2D than in 3D hydrogel environments (**Fig. 1**). 3D hydrogels may more accurately model the architecture of some tissues and present milieus that lead to more realistic cellular responses, especially in the context of pathophysiological environments[41] (**Fig. 2**). However, this again may vary depending on the specific application; cells such as epithelial cells or endothelial cells may naturally interact with more 2D-like substrates within their native environments. Nevertheless, in comparison to conventional culture surfaces, 2D hydrogels offer control over crucial environmental factors such as stiffness and presentation of adhesive ligands. While most hydrogels discussed here are suitable for either 2D or 3D cultures, materials like polyacrylamide are only usable in 2D due to toxicity of the precursor components.

Collagen. Collagen is the primary organic constituent of native tissues; about 90% of the 29 identified types of collagen in the human body are fibrillar. Type I collagen is the most common type and as such is the major structural component of many tissues[42]. This ubiquity makes collagen an attractive material for cell studies. Collagen hydrogels are mostly composed of type I collagen, although types II and III, and other constituents such as glycosaminoglycans, can also be incorporated. Gelatin, the amorphous form of collagen with the same amino acid sequence but lacking the triple helical character, is also a common hydrogel material. Collagen used in hydrogels is usually derived from solutions of acid- or pepsin-solubilized type I collagen, is often sourced from rat tail tendon, and is readily available from numerous vendors including BD Biosciences, Advanced BioMatrix, and Flexcell. These low-pH solutions are stored at low temperature to prevent spontaneous fibrillogenesis and gelation. Hydrogels are typically formed by raising the temperature and the pH to initiate collagen fibril self-assembly, which can occur in the presence of cells or culture medium[43]. Gelation occurs in about 30 min under physiological conditions and in shapes that can be flexibly molded. Temperature can critically affect hydrogel architecture, with lower gelation temperatures leading to the formation of larger fibrils[44]. These changes in hydrogel microstructure can

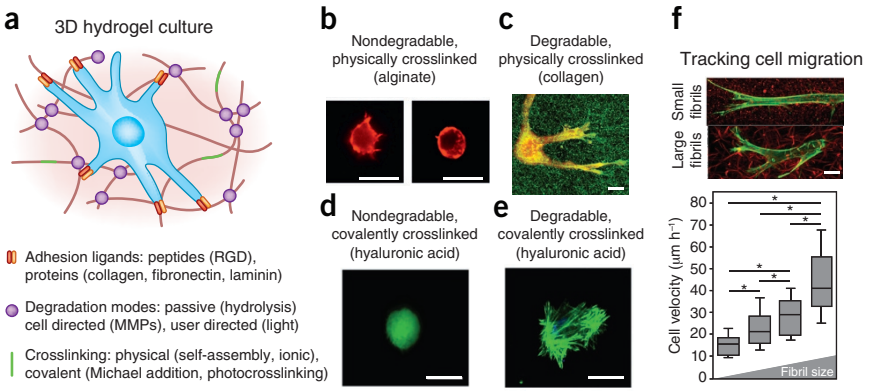

Figure 2 | 3D hydrogels for cell culture. (**a**) 3D hydrogels can be engineered to present a more realistic microenvironment to cells. Hydrogel design variables are indicated. (**b**) Mouse MSCs cultured in 3D alginate hydrogels display rounded morphology regardless of substrate stiffness. Left, 5 kPa; right, 110 kPa. Images modified from ref. 61 with permission. (**c**) Bovine dermal fibroblasts encapsulated in 3D collagen hydrogels spread at low stiffness (<1 kPa). Image modified from ref. 96 with permission. (**d**) Human MSCs cultured in a hyaluronic acid (HA) hydrogel are restricted from spreading regardless of substrate stiffness (shown here as ~4 kPa). Image modified from ref. 97 with permission. (**e**) Human MSCs cultured within a HA hydrogel with equivalent stiffness to that in **d** but crosslinked with MMP-degradable crosslinkers that permit cells to locally remodel their environment, generate tractions, and spread. Image modified from ref. 97 with permission. (**f**) Human foreskin fibroblast spreading and migration speed is influenced by collagen fibril size. Image modified from ref. 44 with permission. Scale bars, 10 μm.

critically influence cell behavior; for example, fibroblasts show less elongation and greater migration velocities in collagen matrices with larger fibrils[44] (**Fig. 2f**).

The primary advantage of collagen is its biomimetic properties: collagen hydrogels are cytocompatible, are amenable to cell adhesion without modification, and present a native viscoelastic environment to resident cells (**Fig. 2c**). Collagen hydrogels have a rich history of use as model cellular microenvironments for studies on topics ranging from mesenchymal stem cell (MSC) differentiation[45] to carcinoma cell reprogramming[46]. Collagen suffers from some important drawbacks, shared by other natural materials, that include low stiffness, limited long-term stability, and batch-to-batch variability. Collagen hydrogel mechanics are dictated by the collagen concentration, but this is coupled to changes in the adhesive ligand density, which limits independent control of these features. It is also difficult to produce collagen hydrogels with higher stiffnesses (>1 kPa) without extensive chemical crosslinking, which fundamentally alters the degradability of collagen fibrils. As a result, culturing cells in collagen hydrogels for long times results in significant contraction of the matrix, although this phenomenon can also be leveraged for studies ranging from modular tissue assembly[47] to measurement of cell contractile forces[48]. Despite some drawbacks, collagen is an excellent choice for *in vitro* studies of cell behavior such as migration[41,44] (**Fig. 2f**) in a tissue-like environment.

Other collagen-containing hydrogels have been used in cell studies, most notably Matrigel. Matrigel is a basement membrane–derived preparation extracted from Engelbreth–Holm–Swarm (EHS) mouse sarcoma tumors that is composed primarily of laminin, type IV collagen, and entactin, with various other constituents including proteoglycans and growth factors[49]. Corning sells Matrigel as a frozen protein solution that is diluted to a working concentration in PBS (~3–4 mg ml^{-1}) and self-assembles into a hydrogel at physiological temperatures. Matrigel offers many of the advantages of collagen and other natural hydrogels and has been used to study cell migration, angiogenesis, and tumor development[49]. Significant drawbacks include Matrigel's tumorigenic origin, diverse composition, and batch-to-batch variability in terms of mechanical and biochemical properties, which in turn brings a significant level of uncertainty to cellular experiments[50]. For these reasons, the more well-defined and tunable hydrogels discussed here may be more suitable for cell culture.

Fibrin. Fibrin is a natural polymer formed during wound coagulation[51]. Selective cleavage of the dimeric glycoprotein fibrinogen by the serine protease thrombin results in the formation of fibrin molecules that interact through a series of disulfide bonds[52]. Additional fibrin crosslinking is provided by factor XIIIa, a form of factor XIII also activated by thrombin. The wide use of fibrin sealants in the medical community provides several options for acquiring fibrin material for cell culture applications (typically sourced from human plasma), including Tisseel and Artiss (Baxter), as well as Evicel (Johnson & Johnson). The individual hydrogel components fibrinogen and thrombin can also be purchased separately from Sigma and other vendors to provide the user with greater design flexibility. For example, increased thrombin content relative to fibrinogen results in fibrin hydrogels with thinner fibrils and smaller pores[53]. Fibrin's role as a natural matrix critically involved in hemostasis and wound healing make it useful as an *in vitro* tool to study these and related phenomena, including angiogenesis[54] and platelet mechanosensing[55]. Fibrin has also been used as a temporary scaffold to guide cell-mediated collagenous tissue assembly[56,57]. However, fibrin's extreme susceptibility to protease-mediated degradation limits its use for long-term cell cultures.

Alginate. Alginate is a polysaccharide derived from brown algae that has been applied in industries as varied as food, textiles, printing, and pharmaceuticals[58]. Alginate consists of β-D-mannuronic acid M units and α-L-guluronic acid G units[59] assembled as block copolymers with regions composed either homogeneously of M or G units or with alternating G and M units[58,60]. Unlike collagen and fibrin, alginate must be modified with an adhesive ligand such as RGD to enable cell attachment.

Alginate is notable for its ability to form hydrogels via ionic crosslinking, making it easily amenable to cell encapsulation, as well as to cell recovery for downstream applications. Alginate's ionic crosslinks are formed using divalent cations such as calcium, magnesium, or barium to promote the formation of ionic bridges between alginate G units[58]. Although the formation of alginate hydrogels is possible through covalent crosslinking[60], most commercial approaches, such as the NovaMatrix-3D cell culture system, use ionic crosslinking. The NovaMatrix kit consists of an air-dried alginate foam disk preformed inside well plates of varying sizes and lyophilized alginate that can be mixed with cells and culture media. The alginate–cell solution is then added to the foam disk, where residual cations in the

disk promote ionic crosslinking and gelation. Ion chelation with an isotonic solution later allows facile hydrogel dissolution and cell harvesting. Alginate solutions can also be purchased from vendors such as PRONOVA. The ability of alginate to be easily dissolved for cell recovery makes it attractive for studying cell-material interactions in 3D. However, cells normally remain rounded, as they cannot degrade the matrix[61] (**Fig. 2b**).

Polyacrylamide. Polyacrylamide (PA) is a synthetic polymer with a rich history of use in molecular biology and, more recently, cell culture applications[62]. PA hydrogels are produced by reacting acrylamide monomer and bisacrylamide crosslinker, usually in the presence of ammonium persulfate (APS) and tetramethylethylenediamine (TEMED). These components are readily available from Sigma and other commercial vendors. APS serves as a source of free radicals, while TEMED is a catalyst to initiate redox radical polymerization of the PA. PA hydrogels are typically fabricated as thin films bound to coverslips functionalized with aminosilanes, which can be prepared in house or purchased from vendors (for example, Schott). Protein conjugation to the PA hydrogel surface to enable cell attachment is usually achieved using a bifunctional crosslinker such as sulfo-SANPAH, although other approaches including hydrazine modification of polyacrylamide amide groups for coupling to aldehyde or ketone groups on oxidized proteins have been used[63]. Detailed protocols on functionalizing coverslips, tuning monomer and crosslinker amounts to produce specific hydrogel mechanics, and conjugating adhesive ligands to the surface of the hydrogels have been published elsewhere[30].

The appeal of PA hydrogels for cell culture is the existence of well-established protocols for the fabrication of hydrogels with tunable stiffness and coupling of proteins[30,62]. The ability to independently modulate hydrogel stiffness and adhesive ligand presentation can lead to more complete understanding of complex cell responses to these inputs and is difficult to accomplish with natural materials. For example, many cell types such as human MSCs spread and proliferate more with increasing stiffness, but MSCs on laminin-coated hydrogels spread less than on other ECM protein coatings, illustrating how the combination of hydrogel stiffness and adhesive ligand presentation can be engineered to modify cell response[64] (**Fig. 1c**). PA hydrogels do not inherently interact with cell surface receptors or integrins, permitting user-defined control of these interactions. One major disadvantage is that PA cannot be used to encapsulate cells in 3D, because of the toxicity of the hydrogel precursors. In our view, PA is ideally suited for mechanobiology studies in which hydrogel stiffness needs to be finely controlled. This has been exemplified by elegant studies that illustrate the influence of substrate stiffness on cell motility[62], spreading[62,65], and differentiation[65,66]. The simple fabrication procedure also makes PA hydrogels an excellent choice for investigators without much experience in hydrogel substrates.

Polyethylene glycol. The synthetic polymer polyethylene glycol (PEG) is advantageous because of its hydrophilicity and relative inertness; PEG is often referred to as a 'blank slate' material. It shows relatively low protein adsorption and is thus amenable to user-defined crosslinking chemistry and presentation of ligands to cells. Like other materials discussed here, PEG can be modified with a wealth of different functional groups, and hydrogels can be formed using a variety of chain-growth, step-growth, or mixed-mode polymerization techniques[13], giving the user more design flexibility than most of the other polymers discussed here. The utility of PEG has been demonstrated in a diverse set of cell culture applications[23,24,67,68] including studies of stem cell differentiation, mechanobiology, and angiogenesis. In particular, PEG is an excellent choice for photoencapsulation experiments[69,70].

PEG can be purchased from common vendors like Sigma in a variety of molecular weights and with several chemical modifications, including (meth)acrylates that are needed for crosslinking. In addition to its use as a base hydrogel, chemically modified PEG is often used to crosslink other polymeric materials. For example, Cellendes sells hydrogel kits based on PEG-thiol crosslinkers and maleimide-functionalized dextran or PVA; maleimides react rapidly with thiols under physiological conditions to form hydrogels. The PEG crosslinkers are available in either nondegradable or MMP-degradable forms. Other, more specialized PEG hydrogel kits exist, including the QGel assay kit for drug screening. This kit includes PEG (functionalized with or without the adhesive ligand RGD) and peptide crosslinkers containing sequences that degrade via MMPs. Hydrogels are formed by simply mixing the components together[24]. As PEG is very cytocompatible and the reaction conditions are mild, cells for encapsulation can also be introduced at this step. PEG hydrogels have also been formed with cells through photoencapsulation techniques, including with osteoblasts[69] and chondrocytes[70]. BioTime Inc. sells a photopolymerizable kit called PEGgel that includes PEG-diacrylate and a photoinitiator. The PEG-diacrylate, photoinitiator, and cells are mixed together and gelled via UV light exposure for a prescribed time.

Hyaluronic acid. Hyaluronic acid (HA) is a nonsulfated glycosaminoglycan composed of a repeating disaccharide unit of glucuronate and *N*-acetylglucosamine[71]. HA is distributed throughout many tissues, including skin, cartilage, and brain, and is known to play an important role in development, wound healing, and disease[72]. Although HA can be isolated from animal tissue such as rooster combs, animal-free production of HA can be achieved via microbial fermentation in *Escherichia coli*. Notable HA vendors include Lifecore and BioTime. HA can be purchased from Lifecore as a sodium hyaluronate, permitting user modification of the structure before forming a hydrogel. Lifecore also markets an HA hydrogel kit called Corgel BioHydrogel, which includes a tyramine-substituted HA that is enzymatically crosslinked using peroxidase to form a quick-setting hydrogel[73]. BioTime sells a line of HyStem HA hydrogel kits composed of thiolated HA and PEG-diacrylate crosslinker. In addition to these components, HyStem kits can also be purchased with collagen (to permit cell adhesion) and/or heparin (for sequestration of growth factors and other therapeutic proteins). BRTI Life Sciences offers a simple system (Cell-Mate3D) composed of HA and chitosan in which the hydrogel assembles through electrostatic interactions between negatively charged carboxyl groups on HA and positively charged amino groups on chitosan. Cells can be mixed and encapsulated directly into the hydrogel. Notably, the BRTI Life Sciences website includes protocols for RNA isolation, protein extraction, and microscopy in conjunction with this kit.

HA has several important advantages as a hydrogel platform, including its biological relevance and chemical tunability. The ability to modify HA to present functional groups enabling a range of

crosslinking chemistries is perhaps its biggest advantage and has been reviewed elsewhere[74]. This permits formation of a range of hydrogel systems that can be processed into 2D films, 3D free-swelling hydrogels, nanofibers, and injectable materials. The versatility of crosslinking chemistries available with HA has also enabled the fabrication of hydrogels with a wide range of mechanics suitable for cell studies as with hydrogels such as PA and PEG. In particular, HA hydrogels whose mechanics can be changed in a user-directed manner have proven useful for cellular mechanotransduction investigations[22,75]. Tuning HA hydrogel degradability with the incorporation of MMP-cleavable crosslinks showed that a degradable environment was required for traction force generation, as well as stem cell spreading and osteogenic differentiation in a covalently crosslinked hydrogel environment[22] (**Fig. 2d**,**e**). Although unmodified HA does not support integrin-mediated cell adhesion, HA interacts with numerous cell surface markers, including CD44 and RHAMM (CD168)[76]. This can be a double-edged sword as HA's cellular interactions and important role in numerous physiological and pathological processes may complicate experimental results.

Polypeptides. Advances in the design and synthesis of custom peptide sequences and non-natural amino acids provide an intriguing tool kit for the design of synthetic hydrogels with user-defined properties[77]. There are many methods to fabricate peptide-based hydrogels, but perhaps the most common involves peptide self-assembly into supramolecular nanostructures. Several polypeptide hydrogel formulations are commercially available, including PuraMatrix (Corning), PGmatrix (PepGel LLC)[78], and HydroMatrix (Sigma). PuraMatrix is composed of an amphiphilic 16 residue peptide containing a repeating arginine-alanine-aspartate-alanine sequence (RADARADARADARADA). This peptide self assembles into β-sheets in the presence of monovalent cations and forms a stable network with fibril diameter and pore size on the order of tens of nanometers, analogous to native ECM. Other common peptide-derived hydrogels have applied a similar assembly principle including EAK16 (AEAEAKAKAEAEAKAK)[79] and KLD12 (KLDLKLDLKLDL)[80].

Polypeptide hydrogels are intriguing for cell culture applications in that they are synthetic materials with tunable properties that can be engineered to exhibit many of the advantages of naturally derived polymers, including interactions with cells, assembly into hierarchical structures reminiscent of native proteins[81], and degradability, while also being able to decouple the effects of these parameters (unlike with natural matrices). A critical disadvantage of peptide-based materials at this point is their expense: cost is prohibitive for large-scale culture systems. As with naturally derived protein materials, it is difficult to form hydrogels that are stable for long periods of time.

Future perspective

Although the systems described in this Review are already much more complex than standard tissue culture plastic or glass, the properties of hydrogels are constantly evolving in an effort to match the complexity of native tissues. The approaches discussed here, including most commercially available systems, are primarily macroscale constructs with static properties, but there has been tremendous progress in the design of dynamic hydrogels that are responsive and/or instructive to resident cells[82]. Thermoresponsive hydrogels such as poly-*N*-isopropylacrylamide (pNIPAm) have been used to efficiently harvest cell populations[83]. Additionally, the convergence of microscale technologies for cell culture[84] with tunable hydrogel designs has enabled diverse studies including the investigation of cell migration in microfluidic hydrogels[85] and high-throughput screening platforms for probing cell-material interactions[86]. Of particular interest for the mechanobiology community are a range of mechanically dynamic hydrogels that can be stiffened[22,75], softened[23], or reversibly stiffened and softened[87] to probe cellular responses. These mechanically dynamic substrates enable investigations of the influence of mechanical dosing on cell behavior in a manner similar to what has been done with soluble factors for decades.

Beyond dynamic properties, fibrous[88,89] and viscoelastic[90,91] hydrogels that more accurately recreate the complex structural and mechanical milieus found in tissues are emerging as useful cell culture substrates. This also includes spatial patterning, where numerous biochemical and biophysical signals can be introduced in a heterogeneous manner to control populations of single cell types or co-cultures[92,93]. Techniques are also advancing for engineering heterogeneity and including multiple cell types within 3D constructs. These include newly developed methods where hydrogels act as bio-inks to print cells either in a layer-by-layer manner from a 2D substrate[94] or directly in 3D space within another hydrogel[95]. As these types of platforms progress, they will likely become available to a wider audience. But in the meantime, it is important to maintain an open, collaborative dialog between cell biologists and materials scientists and engineers so that the next generation of hydrogel systems will be equipped to tackle important challenges of increasing biological complexity.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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