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Review article

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Light-based fabrication and 4D customization of hydrogel biomaterials

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Abstract

Light has become an essential tool to make and manipulate living systems in the increasingly intertwined fields of cell biology and materials science. With the ever-expanding interdisciplinary nature of current scientific research and the ongoing hunt for orthogonal, high-precision stimuli for biomaterial synthesis and modification, light has emerged as the gold standard with its low cytotoxicity and high bioorthogonality, enabling the modulation of properties in both 3D space and time (that is, 4D). Not only can light govern when and where changes occur, dosage modulation permits control over the extent of material customization, providing a route to engineered constructs approaching the 4D complexity of native tissue. Recent technological innovations span advances in stereolithography, digital light processing, volumetric bioprinting, multiphoton lithography and grayscale fabrication. Material chemistries have matched pace with the technologies: novel photochemistries permit the building of dynamic materials with complex mechanical and biochemical functionalities, such as on-demand protein activation, rapid gel formation/degradation and immobilization/release of signalling factors. Herein, we discuss the union of rapid light-based manufacturing and photoresponsive chemistries and highlight future opportunities using photochemistry in the design and user-defined customization of hydrogel biomaterials. We anticipate that these areas will continue to evolve in tandem and be influenced by new insights from traditionally disparate disciplines (such as protein engineering and inorganic chemistry), facilitating further discoveries in cellular development and disease progression, as well as orchestrating advanced tissue construction.

Sections

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Key points

- Light is a uniquely powerful stimulus for creating and modifying hydrogel biomaterials with full spatiotemporal control in the presence of cells.
- Diverse photochemistries have been developed that proceed rapidly, bioorthogonally and reversibly, increasingly with a focus on wavelength selectivity and protein/inorganic-based species.
- Novel light-based manufacturing tools such as volumetric printing and multiphoton lithography have drastically increased the speed and precision of material customization.
- Light-responsive materials whose physicochemical properties can be (reversibly) modulated have enabled the study of dynamic processes such as fibrosis and mechanomemory.
- Photopatterned formation and/or modification of materials opens new doors for engineering structurally complex and heterogeneous functional tissues.

Introduction

Tissue development, organization and homeostasis, as well as pathological progression, are governed by complex interactions between cells and their surrounding environment. Comprised of a mesh of proteins, polysaccharides, proteoglycans and other signalling molecules, the extracellular matrix (ECM) physically and biochemically supports and guides the function of resident cells. The ECM's 3D spatial heterogeneity is evident amongst and within tissues, leading to differential cell populations, tissue organization and distinct organ function. Moreover, such physicochemical properties vary over time, preceding and accompanying both normal and pathological processes. Although conventional 2D culture has proven useful in elucidating the biochemical signalling pathways activated by user-defined ECM components, such methods suffer from an intrinsic inability to recapitulate the 4D complexity of native tissue (that is, its variations in both 3D space and time). In vivo animal models alternatively enable the study of the dynamic and more natural interactions of cells and the ECM; however, they are costly and difficult to control, and they have varying applicability to human biology. Hydrogels — water-swollen polymeric networks derived from synthetic and/or natural precursors — combine many of the benefits while sidestepping the limitations of these two conventional platforms.

Hydrogels balance reductionism and intricacy: they are based on comparatively simple polymeric backbones but can be engineered to respond to a myriad of stimuli, providing a route to mimic biology's native heterogeneity. Owing to its unique ability to customize and reprogramme materials over both time and space, light has been catapulted into the spotlight for advanced biomaterial fabrication[1]. As the fields of tissue engineering and disease modelling have approached a new level of complexity, the material platforms, as well as the chemistries necessary for this level of control, have followed closely in suit. Here, we review advances that leverage light to create and/or post-synthetically tune biomaterials, placing emphasis on chemistries and fabrication methodologies introduced over the past 5 years that have opened new doors for probing and directing biology in 4D[1].

Light-based chemistries

Hydrogel photoresponsiveness typically arises from functional groups that have been intentionally incorporated within the system as opposed to stemming from some intrinsic property of the polymers themselves. In such systems, absorbed photons can induce material changes through the catalysis of primary photochemical events. Although most photochemistries proceed through a one-photon-one-reaction-type mechanism, others can be used to drive self-propagating reactions that enable rapid and large-scale material alteration with comparatively few photons. Here, we focus our discussion on primary photochemical reactions that drive bond formation, scission and isomerization[2] (Table 1).

Making bonds

Acrylates, methacrylates and chain-growth polymerizations. Given its ease, rapidity and cytocompatibility, as well as the facile access to synthetic monomer precursors, photoinitiated radical polymerization has established itself as the most popular type of photoreaction in the biomaterials community. These bond-forming chemistries originate from industry, in which ultraviolet (UV) curing of acrylates was popularized in the late 1960s for use in adhesives and coatings. In radical chain-growth polymerization, a light-exposed photoinitiator (a molecule or complex that absorbs photons upon exposure to light and forms reactive species that can initiate bond formation/scission reactions) cleaves or abstracts a hydrogen atom to generate active radicals (Box 1); these active centres then propagate across alkene-containing monomers, creating carbon-centric radicals that can go on to react with other alkenes in a self-propagating mechanism. Radical recombination results in reaction termination[3]. Light-responsive polymerizations for creating hydrogels emerged in the mid-1990s, with multiple seminal works modifying linear polyethylene glycol (PEG) with acrylate end groups via a reaction with acryloyl chloride. PEG was copolymerized with α-hydroxy acid and capped with acrylate end groups, enabling controlled degradation via hydrolysis and in situ photopolymerization in rabbit models of injured arteries[4,5]. These efforts demonstrated the ease of use and utility of light for rapidly making hydrogels, establishing the foundation for the now extensive light-based hydrogel community.

Methacrylate/methacryloyl functionalization of natural biomaterials such as gelatin (to obtain 'GelMA'), hyaluronic acid, collagen and dextran is readily obtained via a simple reaction between methacrylic anhydride and free amines; such modifications enable the control of network formation, creating stiffer and more uniform stand-alone materials than those otherwise obtained using natural biomolecule-based gels[6–12]. Of note, acrylate and similarly photopolymerizable acrylamide groups can be made to react with cysteines in a base-catalysed Michael-type addition or through photoinitiated mixed-mode radical polymerization, enabling the incorporation of native biomolecules[13–15]. Mixed-mode polymerization combined with enzymatic degradation has found application in photoexpansion microscopy[16]. However, the major drawback of using chain-growth polymerization remains the heterogeneity in network formation, which leads to decreased mechanical integrity and increased rates of erosion[17].

Photoclick reactions

Thiol-based additions. Stepwise network formation — processes achieved through polyaddition/polycondensation reactions of complementary reactive groups — yields greater material homogeneity at the molecular level. The thiol–ene photoreaction has proven the most popular of such chemistries, as it affords increased

Table 1 | Common light-sensitive chemistries utilized in hydrogel synthesis and tuning

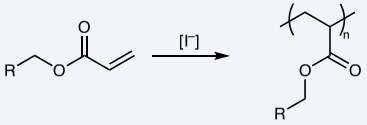
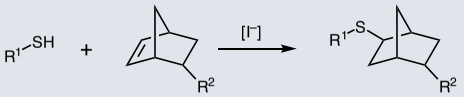
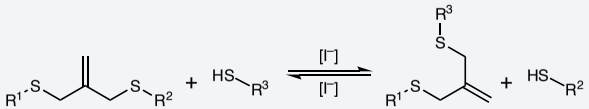
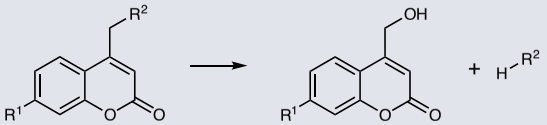
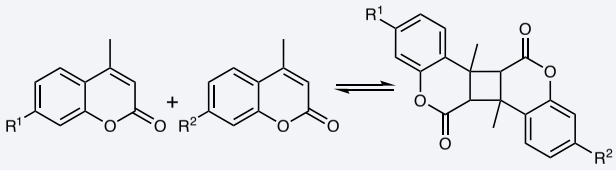
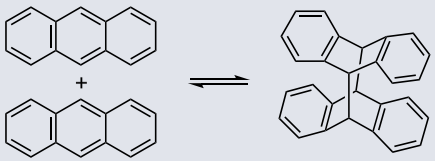
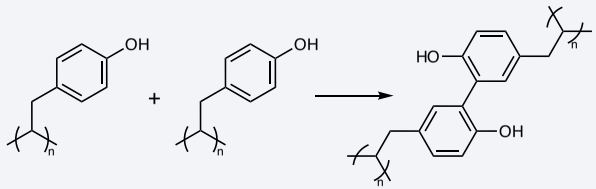
Reaction	Representative mechanism	Synthetic feasibility	Cytocompatibility	Material stiffening/formation	Material softening/degradation	Biochemical functionalization	Biochemical release
Acrylate polymerization		Good	Yes	✓	–	✓	–
Thiol–ene		Good	Yes	✓	–	✓	–
AFCT		Good	Yes	✓	✓	✓	✓
Coumarin degradation		Moderate	Yes	–	✓	–	✓
Coumarin dimerization		Moderate	Yes	✓	✓	–	–
Anthracene dimerization		Good	Yes	✓	–	–	–
Tyrosine dimerization		Good	Yes	✓	–	–	–

Table 1 (continued) | Common light-sensitive chemistries utilized in hydrogel synthesis and tuning

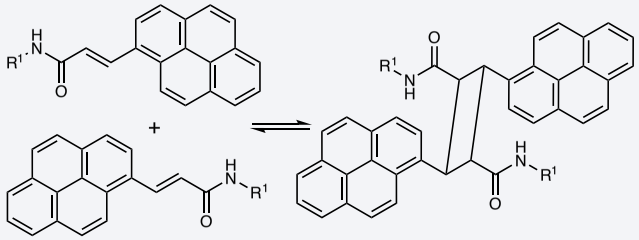
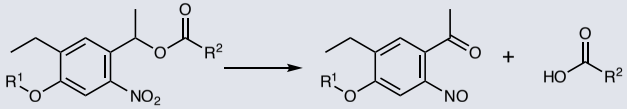
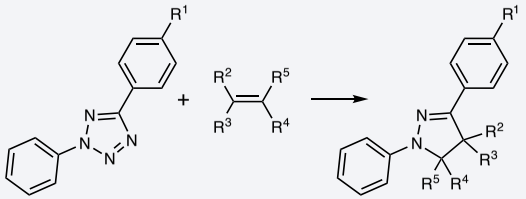
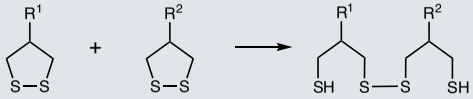
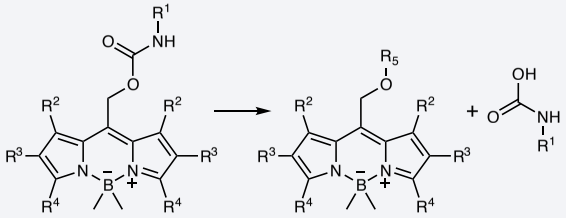
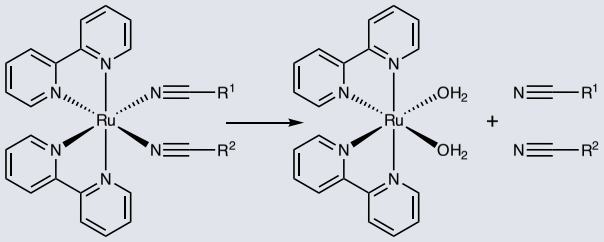
Reaction	Representative mechanism	Synthetic feasibility	Cytocompatibility	Material stiffening/formation	Material softening/degradation	Biochemical functionalization	Biochemical release
SP		Good	Yes[a]	✓	✓	–	–
oNB		Moderate	Yes	✓	✓	✓	✓
NITEC		Poor	No[b]	✓	–	✓	–
Dithiolane		Good	Yes	✓	✓	✓	–
BODIPY		Poor	Yes	✓	–	–	–
Ru		Moderate	Yes[a]	–	✓	–	✓

Table 1 (continued) | Common light-sensitive chemistries utilized in hydrogel synthesis and tuning

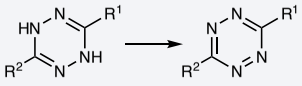
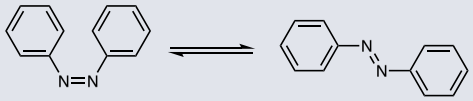
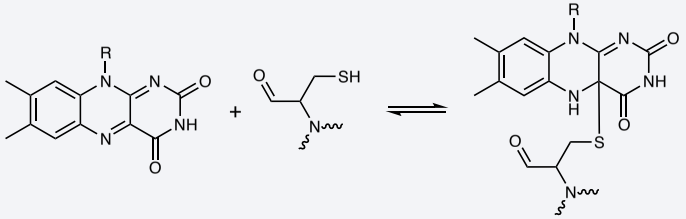
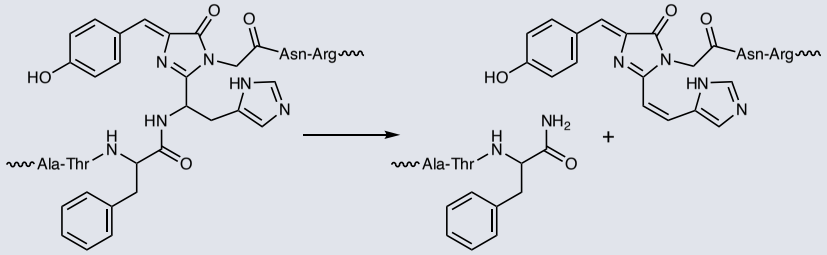
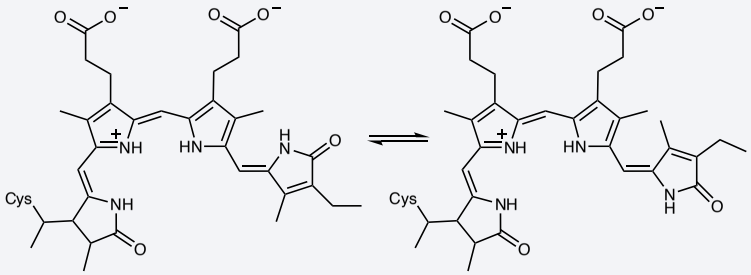
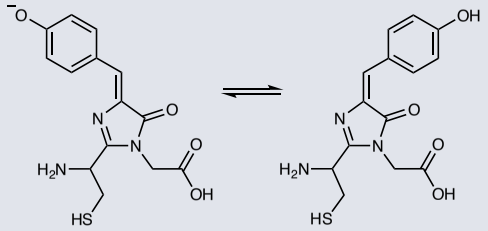
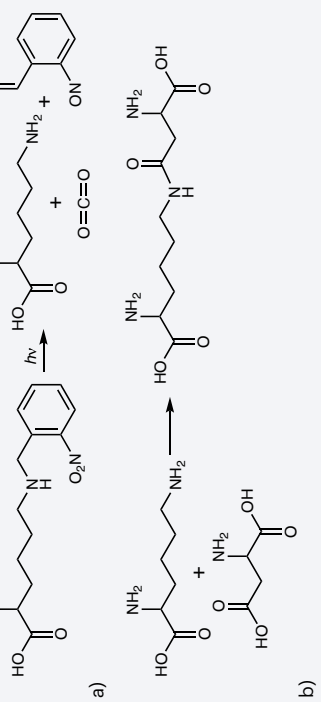
Reaction	Representative mechanism	Synthetic feasibility	Cytocom-patibility	Material stiffening/ formation	Material softening/ degradation	Biochemical function-alization	Biochemi-cal release
DHTz		Moderate	Yes	✓	–	✓	–
Azobenzene isomerization		Poor	Yes	✓	✓	✓	✓
LOVTRAP		NA	Yes	✓	✓	✓	✓
PhoCl		NA	Yes	–	–	–	✓
Cph1		NA	Yes	✓	✓	–	–
Dronpa145N		NA	Yes	✓	✓	–	–

Table 1 (continued) | Common light-sensitive chemistries utilized in hydrogel synthesis and tuning

Reaction	Representative mechanism	Synthetic feasibility	Cytocompatibility	Material stiffening/formation	Material softening/degradation	Biochemical functionalization	Biochemical release
LASL		NA	Yes	–	–	✓	–

AFCT, addition–fragmentation chain transfer; BODIPY, boron-dipyrromethene; DHTz, dihydrotetrazine; Dronpa145N, a monomeric fluorescent photoswitchable protein isolated from coral; LASL, light-activated SpyLigation; LOVTRAP, LOV2 trap and release of protein; NA, not applicable; NITEC, nitrile imine-mediated tetrazole and ene 1,3-dipolar cycloaddition; oNB, *ortho*-nitrobenzyl; PhoCl, photocleavable protein; Ru, ruthenium; SP, spiropyran. [a]Potential cytocompatibility concerns. [b]Reaction has been conducted with cells present; however, it has not been shown for biomaterials.

reaction cytocompatibility via shorter-lived free radicals accompanying polymerization[18]. Although thiols react with linear alkenes in the presence of a radical initiator, this addition typically does not occur in a 1:1 ratio outside of very special cases (for example, allyl ethers). This mixed-mode reaction proceeds simultaneously through both step-growth (that is, thiol–ene – a type of polymerization mechanism involving complementary reactive groups in which bifunctional or multifunctional monomers undergo successive reaction with another monomer to first form a dimer, then a trimer and then oligomers) and chain-growth (that is, alkene homopolymerization – wherein monomer molecules add one at a time to the active site of a growing polymer chain) mechanisms. To circumvent this, norbornene, a highly strained, bicyclic alkene that preferentially undergoes a 1:1 stepwise addition with a thiol radical, was introduced[19]. PEG thiol–ene hydrogels were first formed and used for therapeutic delivery[20] and for encapsulating pancreatic β-cells[18]. Robust methods exist to functionalize gelatin, collagen, hyaluronic acid and, more recently, carboxymethylcellulose, while retaining native cellular affinity and bioactivity[21–25]. The ease of modification of various natural materials with -ene groups and the robustness of this reaction have enabled many biological labs with limited synthetic accessibility to enter the biomaterial and biofabrication space.

As thiol–ene chemistry continues to gain popularity for making materials, several groups have considered how hydrogel stability relates to the polymer functionalization routes and ene type. Traditional ester-linked norbornene–thiol networks degrade on the order of days to weeks, in a context in which the hydrolysis rate is dependent on the charge of the crosslinking peptides[26]. Towards the design of more hydrolytically stable networks, alloxycarbonyl groups have been used for thiol–ene reactions. The alloxycarbonyl group is a common protecting group for the primary amine on lysine; simply incorporating this protected amino acid into a peptide sequence enables crosslinking with PEG-tetrathiol or patterning of thiol-containing biomolecules[27,28]. 1,2-Dithiolanes were also proposed as dynamic thiol–ene covalent crosslinkers that undergo light-induced ring-opening polymerization and form linear, reversible disulfide crosslinks without a photoinitiator. However, in the presence of a photointiator and an alkene (such as norbornene), dithiolanes can form permanent thioether bonds, enabling control over the ratio of dynamic and static crosslinks[29,30]. Despite being new, we surmise that dithiolane chemistry will gain more traction in the field, owing to its easily obtainable starting materials and the possibility for post-gelation modification via reversible bond formation, which is not accessible with more traditional thiol–ene chemistries. Other reviews have analysed the application of thiol–ene chemistries in photoclick reactions and drug delivery[31,32].

Cycloadditions. [2 + 2] and [4 + 4] dimerizations and [1,3]-dipolar cycloadditions have emerged as prominent players in the photopolymerization space. Included among the first cycloadditions for hydrogel formation is the coumarin dimerization for crosslinking polyoxazoline hydrogels[33]. Coumarin undergoes a [2 + 2] cycloaddition via a cyclobutane linkage under 365 nm light, but cycloreverts upon short-wave UV irradiation (≤254 nm), yielding soluble monomers. This reaction cyclicity can be leveraged to develop recyclable hydrogel materials and nanoparticles; however, the use of short-wave UV irradiation precludes the reversibility of this system in a cellular context[34–37]. Two-photon cycloreversion (532 nm) may be a possible workaround, but its cytocompatibility remains untested[38]. Anthracene, another polycyclic aromatic hydrocarbon, also undergoes a [4 + 4] cycloaddition under 365 nm light

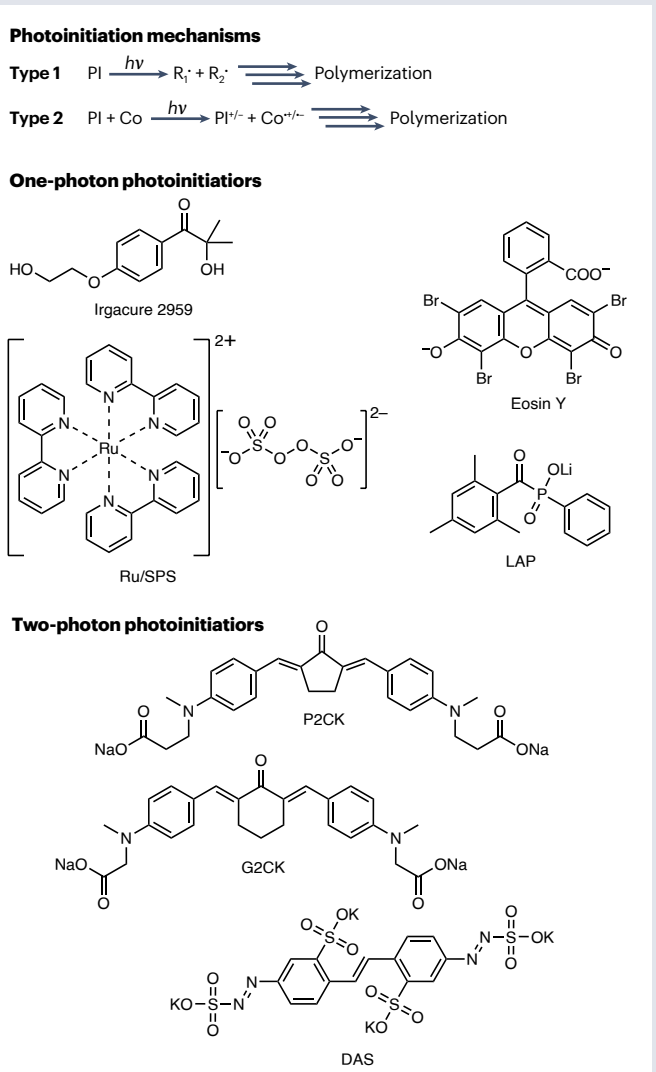
Box 1 | Photoinitiators

One key factor in many photopolymerizations is the photoinitiating system (PIS), which can involve various combinations of photoinitiators—molecules that create reactive species when exposed to light—and/or photosensitizers—light-absorbing molecules that can alter a photochemical reaction. Photointiators are divided into two broad classes: type I photoinitiators directly cleave into two radicals that can initiate polymerization, whereas type II species generate radicals in the presence of co-initiators in a multistep sequence[31,227] (see the figure). Type II photoinitiation processes can proceed in response to lower energy visible light, but they tend to be slower and less efficient due to the presence of competitive reactions during photoexcitation involving the monomer, co-initiator and oxygen. By contrast, type I processes proceed efficiently but typically require higher-energy photons (ultraviolet (UV) light) to cleave a bond.

Single-photon PIS: Two type I photoinitiators dominate the biomaterials field: 1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propanone (that is, I2959)[228] and the phosphine-derived lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP)[227]. Despite being popular owing to its cytocompatibility, I2959 is poorly soluble in water under ambient conditions; although theoretically soluble near 2 wt% (~89 mM), high concentrations often require extensive sonication (lasting even days or weeks) to be achieved. Additionally, although I2959 is responsive to UV light, its molar extinction coefficient—the measure of how strongly a compound absorbs light at a given wavelength—at 365 nm is low (4 M^{-1} cm^{-1}) and trails off entirely by 370 nm, necessitating greater light dosage for complete polymerization. LAP displays higher solubility, polymerization rates and absorbance above 400 nm than I2959, enabling efficient and cytocompatible violet light-based polymerization[227]. LAP is the current standard for most in vitro applications.

Type II visible light-responsive alternatives include eosin Y, a common dye used in histology[4,5,229], typically alongside a coinitiator and accelerant species (for example, 1-vinyl-2-pyrrolidinone) with a peak absorption of 517 nm. However, because eosin Y can abstract hydrogens from thiols, this initiator can be used for direct visible light-based polymerization of thiol–norbornene systems without other species[230]. Similarly, the ruthenium(II) tris-bipyridyl dication and sodium persulfate (Ru–SPS) complex that has gained traction in the biofabrication space due to the lower cost of visible light sources for commercial digital light processing printers, can respond to 450 nm light[231]. The Ru–SPS system has seen use for crosslinking protein-based systems such as decellularized lung extracellular matrix[232] and cartilage-like proteins[233], as well as methacrylated bovine serum albumin (BSA) and gelatin[234,235]. Although this system enables photoinitiation of crosslinking reactions squarely in the visible spectrum, undesired component radical formation, particularly when used with thiol–ene coupling, can occur in the dark[236].

Two-photon PIS: In these reactions, a PIS must absorb two photons from the near-infrared region to generate free radicals directly or indirectly for subsequent polymerization. Critically, for efficient reactions, these molecules must have a high two-photon absorption cross section—the probability of the simultaneous absorption of two photons by an atom—as well as a high initiating efficiency. As intramolecular charge transfer is the driving force for these reactions, these species are characterized by strong donor–acceptor groups and highly conjugated, coplanar π-systems, with a low fluorescent quantum yield, meaning that most of the absorbed photons are not converted to emitted light, yielding instead a high population of active states that can participate in two-photon photopolymerization[237]. To make these photoinitiators water-soluble, a series of type II cyclic benzylidene ketone-based molecules (for example, G2CK and P2CK) (see the figure) were developed to enable the two-photon photopolymerization of GelMA (methacrylate/methacryloyl-functionalized gelatin); however, cell viability is very low in biofabricated structures, purportedly due to peroxyl radicals formed from the photoinitiator scission[238]. More recently, aryl diazosulfones (such as 4,4′-(1,2-ethenediyl)



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bis[2-(3-sulfophenyl)diazenesulfonate), which cleave rapidly and unimolecularly, thus reducing the formation of undesired singlet oxygen species, have been reported with enhanced cytocompatiblity[176]. The current lack of commercial availability of such water-soluble photoinitiators has largely limited their usage in the biomaterials space.

and reverts to the monomeric state thermally or under ≤300 nm light. This reaction has been used for forming and photostiffening PEG hydrogels[39–41]. By placing an electron-rich group at the 9-position of the anthracene ring, dimerization can be further red-shifted to occur under exposure to wavelengths of light in the 400–500 nm range[41]. Similarly, dibenzocyclooctyne (DBCO)-functionalized polymers also undergo a radical-mediated cycloaddition[42–44].

Nitrile imine-mediated tetrazole and ene 1,3-dipolar cycloaddition, first reported in 1967 (ref. 45), has been rediscovered and repurposed for photoactivatable bioconjugation; however, its potential remains relatively untapped. In this [3 + 2] cycloaddition reaction, a tetrazole-containing compound photolyses upon exposure to 302–365 nm light, releases nitrogen gas and forms a reactive a nitrile imine moiety that undergoes rapid and highly specific conjugation with an electron-deficient alkene or alkyne to form a stable pyrazoline-based linkage[46,47]. The rate of the reaction can be increased, primarily by tuning the highest occupied molecular orbital (HOMO) energy of the tetrazole by adding electron-donating methoxy substituents at the *para* position of the aryl rings; moreover, the wavelength of light triggering cycloaddition can be red-shifted to the near-infrared (NIR) region by substituting the aryl rings for naphthalene functionalities or using upconverting nanoparticles[48–50]. These cycloadditions have been used for labelling proteins and nucleotides in live bacterial and mammalian cells, as well as in mouse models with both single and multiphoton techniques[49,51,52]. This reaction has been used to assemble macromolecular structures such as block copolymers, films and PEG hydrogels[50,53,54]. The lack of catalyst, full bioorthogonality (a class of reactions that proceed rapidly and selectively in biological environments, without reaction with native biochemical residues), fast gelation times (<5 min) and spatial control over polymerization make this a potentially attractive system for future applications, yet synthesis of tetrazole reagents is not as accessible as other more popular chemistries[53].

The desire for multiplexing stimuli and in vivo crosslinking for implants has necessitated the development of red-shifted step-growth reactions. For these applications, expanded π-conjugated systems with higher absorption bands that lower the energy required for a π–π* transition have been developed. Styryl pyrene systems have been used for stepwise PEG hydrogel formation, as they undergo a [2 + 2] cycloaddition at 410 nm, which can be reversed at 330–340 nm, enabling orthogonal coupling and decoupling of polymer chains[55,56]. However, water solubility and cytocompatibility concerns remain. An acrylamidylpyrene system was developed to address these limitations, dimerizing at wavelengths in the 420–490 nm range. Interestingly, although not apparent from the UV–visible (UV–Vis) spectra, the acrylamidylpyrene cycloaddition reactivity is sufficiently red-shifted from that of styryl pyrene, enabling the stepwise stiffening of the hydrogel system by including both styryl pyrene and acrylamidylpyrene on PEG star polymers[57]. Further efforts have continued to push the boundaries of red-shifted cycloadditions, such as that of styrylquinoxaline, which can be activated by green light (up to 550 nm) to form 1 cm-thick gels[58].

Tyrosine dimerization. To easily crosslink protein-based hydrogels, tyrosine dimerization has commonly been implemented to form covalent bi-phenolic bonds in natural materials such as silk and mussel-adhesive proteins[59,60]. Although this reaction is naturally catalysed by a large group of metalloenzymes (such as horseradish peroxidase), it can also be photo-triggered in the presence of a type II photoinitiator[61]. These chemistries are not limited to protein-based hydrogels: by including tyrosine moieties on polymers such as polyvinyl alcohol, growth factors can be non-specifically tethered to gels and released based on the hydrolytic degradation of the overall material[62]. For example, dynamically stiffening PEG–peptide hydrogels can be developed: the initial network was created with a stepwise thiol–ene reaction, and upon incubation with the photoinitiator flavin mononucleotide, secondary crosslinking between tyrosines included in the peptide crosslinkers occurred at 440 nm (ref. 63). This strategy offers a simple and robust method for crosslinking hydrogels with visible light. However, natural materials such as collagen contain few tyrosine residues, causing only small changes in their mechanical properties; this chemistry for biomaterial assembly may become more popular with the advent of rationally designed protein crosslinkers.

Making via breaking (photouncaging and photooxidizing chemistries). Here, we introduce the concept of 'making via breaking': the idea that chemistries can be rendered inactive by a protecting group that can be 'broken off', enabling the unhindered 'making' or formation of a new bond. Many such approaches involve photocages — chemical moieties that are cleaved off in response to light and thus expose a reactive group for subsequent click reactions. Although these chemistries have been primarily used for biochemical patterning, some have also been used for material photopolymerization. Initial efforts were focused on photocaging thiols and amines.

Ortho-nitrobenzyl (*o*NB) is arguably the most popular photoprotecting group in synthetic chemistry. The photorelease mechanism proceeds once an incident photon breaks the π-bond in the nitro-group, causing the excitation of the substrate to a diradical state. The reaction then proceeds via an *aci*-nitro intermediate state, as the nitrogen radical abstracts a proton from the benzylic carbon; the ground state tautomer intermediates can then undergo a cyclization reaction to form a five-membered ring that includes the nitrogen. The photoprotecting group is then cleaved off, yielding 2-nitrosobenzaldehyde, a molecule of carbon dioxide and the newly deprotected functional moiety[31]. The related 2-(2-nitrophenyl) propyloxycarbonyl photoprotecting group has been used for photocaging alkoxyamines, which, upon uncaging, can form stable oxime linkages with benzaldehydes, for both patterning biomolecules[64,65] and crosslinking of step-growth-type hydrogels[66]. 2-(2-Nitrophenyl) propyloxycarbonyl has additionally seen use in caging amines that react with azolactones in photomediated ring-opening reactions, generating pH-responsive hydrogel actuators[67]. *o*NB-caged lysines have also been applied to cage the spontaneous and covalent ligation of SpyCatcher

protein and its peptide partner SpyTag; this 'light-activated SpyLigation' has been leveraged for patterned immobilization and functional assembly of proteins in hydrogels[68].

Coumarin-based photocages have been explored as an alternative to *o*NB groups owing to their fast cleavage rates, red-shifted absorption, two-photon sensitivity and biocompatibility[31]. For example, the phototrigger group (7-methoxycoumarinyl)methyl was used for caging small molecules such as cAMP and glutamate, which could be uncaged in physiologically relevant buffer and in brain slices[69,70]. The reaction range can be further red-shifted to encompass wavelengths as high as 600 nm by extending the π-system and including a heterocycle moiety with a quaternary nitrogen[71]. To improve multiphoton-based uncaging, the 6-bromo-7-hydroxycoumarin sulfhydryl protecting group was used for patterning more complex biomolecules[72,73]; this same photocaging group can also mask amines[74].

Although nitrobenzyl and coumarin-based systems remain popular and the most widely applied strategies for photocaging reactive groups, several other photocaging systems have been proposed. For example, a water-soluble cyclopentadienone–norbornadiene photocage undergoes a retro Diels–Alder cleavage upon exposure to 365 nm light, losing carbon monoxide and a substituted benzene by-product while forming a reactive cyclopentadiene that can undergo Diels–Alder cycloaddition with a maleimide[75,76]. This platform was used to modify 3D-printed PEG-diacrylate hydrogels post-polymerization at 405 nm with maleimide-bearing biomolecules[75]. Alternatively, photo-strain-promoted azide-alkyne cycloaddition was introduced to provide spatial control for a popular form of click chemistry; by appending a cyclopropenone cage sensitive to 350 nm light, photoexcitation yields a dibenzocyclooctyne that reacts with an azide to form a stable triazole bond[77]. This chemistry was used for the multiphoton patterning of AlexaFluor molecules in mouse intestinal crypts and polyacrylamide hydrogels[78,79]. However, this platform has not yet seen wide adoption for biomaterials, despite being an example of bioorthogonal click chemistry.

Photocaging the inverse-demand Diels–Alder addition between a tetrazine and alkene or alkyne dienophiles has also been demonstrated[80]. Dihydrotetrazine (DHTz) undergoes oxidation under red light in the presence of a photosensitizer (a compound belonging to a class of molecules that absorb light to expedite a photochemical reaction) to form a tetrazine, which then reacts rapidly with moieties such as *trans*-cyclooctynes or norbornenes. This reaction was used to spatially immobilize RGD — a cell-adhesive peptide derived from fibronectin — modified with *trans*-cyclooctynes on DHTz-containing fibres[80]. Additionally, multi-arm PEG monomers can be functionalized with either DHTz or norbornene to polymerize hydrogels through 1 cm dermal tissue models using 625 nm light[81]. However, the tetrazine moiety is prone to reduction in media. As such, a furan precursor that generates an aldehyde upon exposure to red light (625 nm) along with a photosensitizer that would react with a hydroxylamine was developed; this system proved to be more stable and enable crosslinking behind a biomimetic phantom[82].

Another promising set of candidates, boron-dipyrromethene (BODIPY)-based photocages, have been widely used to develop more red-shifted chemistries to achieve maximum tissue penetration with low phototoxicity. For example, density functional theory computational studies revealed *meso*-substituted BODIPY scaffolds as ideal photocages, with high molar extinction coefficients (~50,000–70,000 M^{-1} cm^{-1}), good thermal stability (survives boiling for 1 h with no degradation) and tuneable absorptions throughout the visible spectrum[83]. Photorelease studies demonstrated the ability to unmask carboxylic acids and amines with green light (>500 nm)[83]. This family of BODIPY-derived photocages was further expanded by adding conjugate moieties to the α-positions of the dipyrromethene groups; this further red-shifted maximum absorption values to between 580 and 690 nm and afforded quantum yields similar to those of *o*NB groups[84]. However, these compounds suffer from low aqueous solubility; therefore, a heterocycle moiety with electron-withdrawing sulfonate groups was proposed[85]. For example, a four-arm PEG with a BODIPY thioether was synthesized that would undergo thiol-yne addition with four-arm PEG-propiolate and unmasking of the thioether upon green light illumination (530 nm). This system demonstrated excellent mechanics, stability and cell viability[86]. Following this work, synthesis and characterization of BODIPY photocages was also reported for amines, hydrazines and aminooxys for use in mild and rapid hydrogel formation. The gelation of a poly(ethylene glycol methacrylate) scaffold was controlled stepwise with BODIPY-protected amines that reacted with phthalaldehyde moieties on a modified poly(ethylene glycol methacrylate) copolymer[87]. Furan/DHTz oxidation and BODIPY cages are the most red-shifted chemistries currently used for the controlled assembly of macromolecular structures; however, many more visible light and NIR photocages, such as those based on vitamin B12 or cyanine, have been proposed for applications such as in vitro and in vivo biosensors and as drug-photocages, which may prove useful for identifying new biomaterial chemistries[88]. For now, difficult syntheses of these novel cages proves to be a barrier for wider adoption.

Breaking bonds — photo-clip reactions

As we have considered making bonds with the help of light (often through 'click'-like processes), we must also consider the converse: breaking bonds with light, which we refer to as 'photo-clip' reactions, a further extension of the term coined by the Johnson group[89]. Due to its inherent bioorthogonality to cellular processes, light has often been used as an input for breaking down hydrogels for cellular release or for delivery of small molecules and biologics. Although this side of the biomaterials field has seen less overall development than the photopolymerization space, a few key chemistries based on *o*NB, coumarin and ruthenium stand out and are highlighted below.

oNB. Although *o*NB-based cages have been used extensively to form or subsequently decorate hydrogels, these species can also be used as photodegradable linkers when inserted between species. *o*NB linkers have been included in both peptide and PEG crosslinkers for the controlled degradation of inner channels to mimic vasculature and direct neural outgrowth[90,91], modulate hydrogel mechanics[92,93] and release cells[94,95]. A series of photodegradable *o*NB tethers with various functionalities at the benzylic position and varying number of aryl ether groups were further developed to enable the complex, multiplexed release profiles of model therapeutics[96], or to release small molecules and proteins[97,98]. While these previous examples explored binary release or degradation, grayscale degradation to modulate surface mechanics was first reported in an *o*NB-crosslinked gel film[99]. Two-photon lithography further enables grayscale control over 4D protein photorelease from hydrogels[100]. Although *o*NB generally exhibits low responsiveness to multiphoton activation, using photosensitizers (such as P2CK) (Box 1) can greatly accelerate photocleavage[101].

Coumarins. Coumarins uniquely exhibit both photo-click and photo-clip reaction mechanisms. This propensity must be carefully considered when breaking down macromolecular assemblies.

Therefore, few reports exist of coumarin photocages in the hydrogel backbone. Click-formed PEG and polyacrylamide gels have both been crosslinked with coumarin-based crosslinkers that degrade on demand with 365 and 405 nm light, as well as two-photon irradiation at wavelengths up to 860 nm (refs. 102,103). Coumarin linkers have also been used for the release of a model antibody[104] and to modify a polyazophene-based polymer that upon exposure generated a macromolecular photoacid that rapidly hydrolytically degraded the polyphosphazene backbone[105]. As such, coumarins are excellently suited as a photolabile motif for biomolecule release in which the dimerization reaction is not counter-productive and less so for structural degradation.

Ruthenium. In the search for photolabile groups susceptible to low-energy light, some recent biomaterials systems have embraced inorganic chemistry. Ru polypyridyl complexes exhibit the unique ability to exchange a pyridine ligand upon exposure to visible light in a radical-free manner. Moreover, their optical properties are tied to the ligand identity around the Ru core, enabling the development of complexes that absorb over the entire visible spectrum and into the NIR[106]. Ru-based crosslinkers have been created bearing azide[107], aldehyde[108], amino-methyl pyridine[109] and poly(4-vinylpyridine) moieties[110], allowing for click-based or free-radical polymerization hydrogel assembly and have been used to release TEM1 β-lactamase, as well as fibroblasts, with light wavelengths ranging from 450 nm to 617 nm (refs. 107,108). The photodegradation of gels mixed with various Ru-based crosslinkers can be multiplexed through complex tissue up to 2 cm-thick[107]; these gels have also been observed to rapidly photosoften via multiphoton-based photodegradation[100]. Nonetheless, the potential cytotoxicity of the by-products of degraded Ru-based crosslinkers, as manifested by Ru's active use in photodynamic therapies as an antitumour agent, will be important to fully resolve for this chemistry to flourish.

Photoresponsive proteins. An alternative approach for photo-clip chemistry involves light-response proteins. The major benefit of recombinant platforms is the ability to genetically encode properties of interest without having to pursue difficult syntheses; however, this does require a distinct set of skills and equipment for the purification of proteins. Many of these systems can be clicked into conventional synthetic or natural precursors, or recombinantly expressed as large fusion constructs with flanking spacing domains (such as elastin-like polypeptides) and then assembled via orthogonal reactions such as SpyTag/SpyCatcher[111] or SnoopTag/SnoopCatcher[112]. One photo-clip protein system is CarH, a transcriptional regulator controlling bacterial carotenoid synthesis that tetramerizes in the presence of adenosylcobalamine in the dark but will dissociate upon exposure to green (522 nm) light[113,114]. Alternatively, photocleavable protein (PhoCl) is a green fluorescent protein that undergoes irreversible backbone scission near its C terminus upon exposure to 400 nm light and has been used to selectively release C-terminally-fused proteins and peptides from hydrogels[115,116].

Reversible bond exchange or isomerization

As researchers attempt to further recapitulate the 4D complexity of biological processes, reversible chemistries for sequentially tethering/releasing biomolecules or cyclically modulating mechanics are garnering interest. Although some click/clip-type chemistries can be performed iteratively (for example, visible thiol–ene followed by *o*NB degradation)[117] – enabling a single cycle of forming one bond and then breaking another chemically distinct bond[98,118] – there is growing interest in systems offering full reversibility.

E/Z isomerizations of azobenzenes and other systems. Azobenzenes represent the most prominent synthetic route for endowing hydrogels with switchable properties. Azobenzenes convert from the thermodynamically stable E conformation to the less stable Z isomer when exposed to UV light (~340 nm); they then revert back to the E conformation upon irradiation with blue light (~450 nm)[119]. This reaction has since been red-shifted by *ortho*-halogenating the benzene moieties[120,121]. The isomerization changes the local packing of the intermolecular microenvironment and alters cation–π interactions, enabling modulation of the non-covalently-assembled complexes. Using this photoswitch in tandem with host–guest chemistry enables sol–gel modulation, as azobenzene can act as a guest of β-cyclodextrin in the *trans* configuration but not in the *cis* configuration[122–124]. Alternatively, the E/Z isomerization of an azobenzene group can be taken advantage of to control boronic acids/ester interconversion, enabling the generation of reversibly stiffened hydrogel networks. This reaction can be red-shifted (626 nm) by replacing the fluorine atoms with methyl ether groups[125,126].

Most work with photoswitches (chemical groups with two states that can be cycled between via light exposure) targets the classical switching between states A and B using wavelengths that maximize the macroscopic response – specifically the full phase transition. However, in a supramolecularly assembled hydrogel system, the E/Z isomerization of arylazo-bis(*o*-methylated)pyrazole can be wavelength-gated; that is, although illumination with 536 nm light would fully convert the compound to the E conformation and illumination with 375 nm light would fully convert it to the Z conformation, intermediate wavelengths would yield a mixture of E and Z isomers[127]. Gels could undergo more than 30 photoswitching cycles with very little mechanical fatigue and access a wide range of stiffnesses. This study represents the first instance of wavelength-dependent mechanical properties in the hydrogel field, although we expect that similar approaches could be applied to other systems.

Addition–fragmentation chain transfer. Drawing inspiration from common chain-transfer agents used in the polymer field, this chemistry leverages allyl sulfide crosslinking groups that can rearrange via an addition–fragmentation chain transfer process. A single photon cleaves the type I photoinitiator, which then generates thiyl radicals from free thiols in the system; these thiyl radicals can then attack the allyl sulfide moiety, causing the formation of an unstable intermediate, which rapidly undergoes β-scission and eliminates an alkylthiol[128]. Alternating cycles of thiol–ene addition can lead to reversible patterning of peptides[129], proteins[130] and viscoelasticity[131]. By flooding the system with excess free thiols such as methoxy-PEG-thiol or glutathione, the network can also be rapidly photodegraded[128,132–134]. However, unproductive side reactions cause this chemistry to lose over 50% of its potential reactivity with each cycle, limiting the reversibility.

Photoresponsive proteins. Stimuli-responsive protein pairs are an excellent option for endowing biomaterials systems with reversibility. One of the more common protein-based reversible systems is based on the LOV2 trap and release of protein (LOVTRAP)[135]: in this system, the small, engineered Zdark protein has a high affinity for the dark state of LOV2, a photosensor domain from the oat plant *Avena sativa* phototropin 1, and a low affinity for the light state (450–490 nm). Originally, this

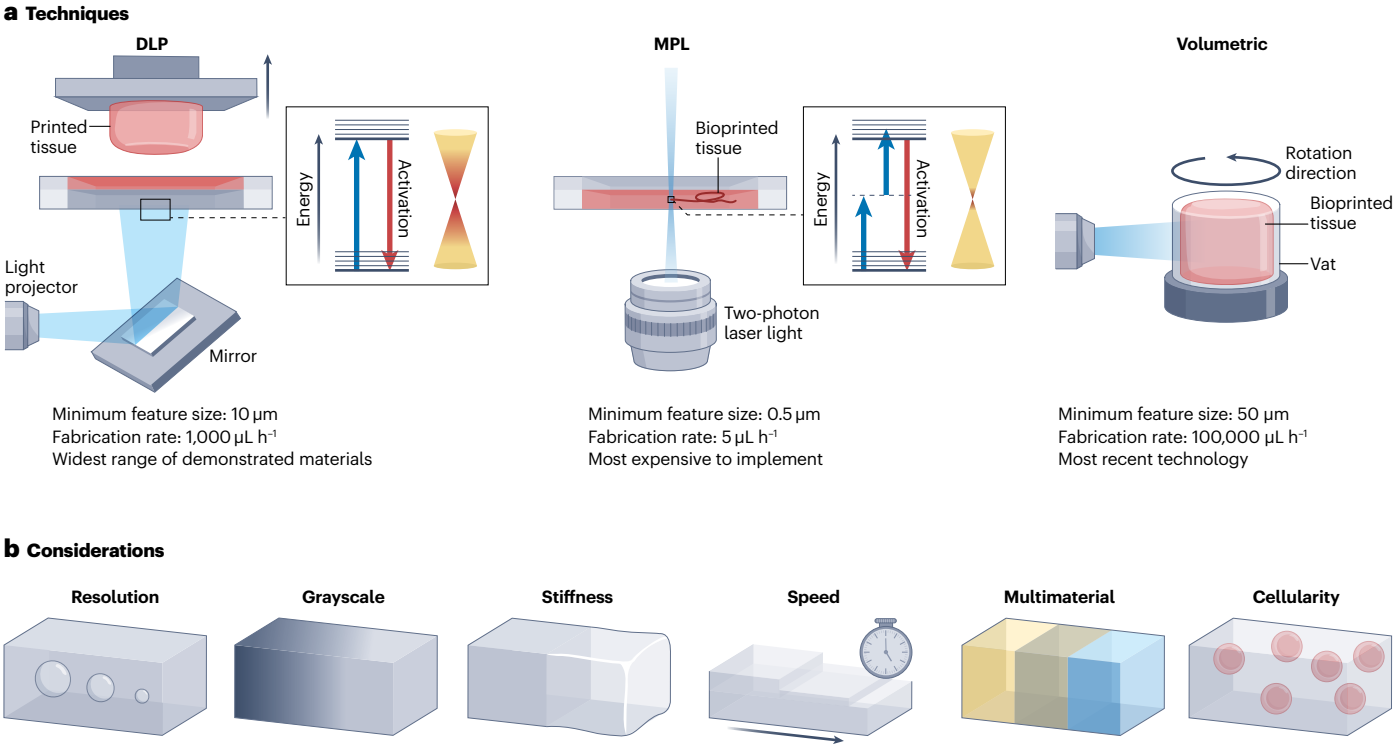

Fig. 1 | Light-based fabrication methods. a,**b**, Light-based fabrication methods can be broadly divided into three different patterning methods (part **a**), with several key considerations highlighted (part **b**). DLP, digital light processing; MPL, multiphoton lithography.

technique and its derivatives were developed for the precise and reversible control of protein activation kinetics, but it has since been applied to the photomediated assembly of recombinant hydrogels[136], the reversible decoration of hydrogels with grayscale control[137,138] and the cyclic modulation of mechanics[139,140]. Other photosensitive pairs exist as well: the photosensory module of the cyanobacterial receptor Cph1 dimerizes with 660 nm light and returns to its monomeric form with 740 nm light; it has also been coupled to PEG vinyl sulfone hydrogels[141]. The Cph1-R472A mutant further widens the dynamic range of gel mechanics as compared with the original Cph1-Y263F version[142]. One major drawback to the original Cph1 system was that the gelation conditions required extended times at cold temperatures in an oxygen-free environment, although this may be addressed with further iterations of this system. Finally, Dronpa145N — a monomeric fluorescent protein isolated from coral that tetramerizes under 400 nm light and disassembles under 500 nm light — has been similarly included in recombinant hydrogel systems to control gelation and degradation[143,144].

Light-based fabrication and modification

To fully harness the growing library of powerful photochemistries to make and modify materials, complementary technological advances have been made in how light is selectively delivered to samples. Recent efforts have sought to print large 3D structures rapidly at high resolution and geometric complexity. As several recent reviews have discussed bioprinting as a whole[145–147], here we focus on only the most exciting and recently developed methods, including digital light processing (DLP), volumetric printing, two-photon lithography and grayscale fabrication (Fig. 1).

SLA and DLP

Single-photon laser stereolithography (SLA) and DLP both rely on single-photon-activatable photochemistries to build structures from user-inputted 3D models. In both methods, thin layers of the polymer precursor are sequentially exposed to light and polymerized in a layer-by-layer fashion stacked into a 3D structure. SLA utilizes laser rastering to polymerize 2D shapes within each layer, whereas DLP illuminates the complete 2D region of interest simultaneously, achieving minimum features of ~10 µm (refs. 148,149). DLP traditionally utilizes arrays of digitally controlled micrometre-scale mirrors that deflect an incoming light to generate a projected image, enabling substantially faster patterning[150]. DLP is now being conducted using commercial light projector systems (typically 405 nm) in conjunction with visible light-based photoinitiating system, further decreasing costs relative to SLA[151]. Given its relatively low cost, material flexibility and micrometre-scale resolution across large areas (>1 cm^2), DLP is generally favoured for biomaterial printing. However, the technique is unable to achieve cellular resolution and can require long print times due to its layer-by-layer nature.

Because DLP proceeds through sequential layer polymerization, photons unabsorbed in the intended build layer can cause undesired curing and low resolutions in the z dimension; forced light attenuation through inclusion of photoabsorbers within the resin mixture has proven a simple and effective way to increase resolution[152]. In a similar vein, refractive index matching using contrast agents (such as Iodixanol) can minimize heterogeneous light scattering caused by cellularized bioinks, improving resolution[153]. Early DLP used acrylate chemistries, but it has now switched to thiol–ene chemistry to generate cellularized and vascularized constructs[154].

The heterogeneity of tissues is important to physiological function, and, as such, it has been an area of research interest in bioprinting. Early methods manually exchanged bioresin between layers[148,150,155], although more recent methods have attempted to automate this process via microfluidics[156,157] or by leveraging meniscus effects to pin bioink between the formed construct and the build platform[158]. DLP can also be readily combined with other types of bioprinting, such as with direct ink writing, to enable multi-material printing of non-photoactive materials[159]. Finally, although they have not been applied in the context of living cells, multiwavelength methods combining DLP with light sheet-based planar activation of polymer precursors offer exciting routes towards rapidly achieving very high-resolution prints (~25 µm)[160,161].

Light-based bioprinting has fewer commercially available options than extrusion bioprinting; however, SLA/DLP-based machines are available including the TissueRay (Tissue Labs), the Smart Print UV (Microlight 3D), the Bionova X (Cellink) and the Lumen X (Cellink).

Volumetric printing

A shortcoming of many light delivery methods is their layer-based nature; this poses practical challenges, both in printing complex geometries (for example, overhangs) and with regard to the potential for long fabrication times that scale linearly with print thickness (such as layer numbers). As an alternative, volumetric printing uses tomography to drive the photoreaction in 3D. By illuminating the prepolymer solution from several angles, either using multiple light sources or through sample rotation, the cumulative buildup of light dosage triggers photopolymerization only in desired 3D locations[162,163]. Using this method, GelMA scaffolds can be fabricated in less than 1 min for millilitre-scale structures with ~40 µm minimum feature size[164]. Refractive index matching can further improve resolution, enabling printing of organoid-containing constructs[165]. Volumetric printing has also been combined with extrusion printing for the generation of multicellular constructs[166]. Additionally, gelatin-norbornene–thiol crosslinker bioinks have been demonstrated that enable the spatial functionalization of structured volumetric prints and secondary volumetric functionalization of gelatin/PEG prints with thiolated dyes and vascular endothelial growth factor (VEGF)[167]. For example, a gelatin-norbornene/PEG–SH bioink was developed for generating cellularized constructs and multi-material vascularized structures[168,169]. The patterning speeds of volumetric printing are substantially higher than those of other techniques, but the applicability of volumetric printing is limited by the small radial dimensions (~1 cm) and limited resolution of printed internal voids.

Although previously a niche technique, emerging interest in volumetric printing has led to the commercial availability of Tomolite (Readily3D) — using a 405 nm light source and a rotating stage — which has been used in several publications to date[167–169].

Multiphoton-based fabrication

Although SLA/DLP and volumetric printing both enable the creation of tissue-scale structures, they fail to reach sub-cellular resolution or recapitulate small vascular structures. As a powerful alternative, multiphoton-mediated processes use two or more near-simultaneously absorbed incident photons to achieve a two-stage photoactivation. Through this mechanism, in conjunction with controlled raster scanning of the laser focal point, activation can be achieved in discrete volumes with sub-micrometre resolution in 3D. Although the fabrication time scales directly with construct size and is generally slower than alternative techniques, multiphoton-based processes have been used extensively for both additive and subtractive manufacturing.

From an additive perspective, controlled two-photon polymerization is most commonly achieved via radical-mediated polymerization initiated using a multiphoton-sensitive photoinitiating system. For biomaterials, this was first demonstrated in the photodecoration of hydrogels using bioactive peptides to drive cell invasion[170] and to create acellularized PEG-diacrylate scaffolds[171]. Although early attempts were largely incompatible with live cells, likely due to the high photonic fluxes required to initiate poorly absorbing photoinitiating system and/or the potential generation of toxic radicals during the polymerization process[172,173], development of more photoreactive chemistries and next-generation photoinitiators have lessened these concerns[174–176]. An important innovation was the development of 4,4′-(1,2-ethenediyl) bis[2-(3-sulfophenyl)diazenesulfonate, which enabled the two-photon polymerization of cell-containing prepolymers[176]. Implementation with a gelatin-norbornene/dithiothreitol bioink generated enzymatically degradable constructs with a patterning speed of 1 m s^{-1} (refs. 174,175). Furthermore, two-photon polymerization has been used to generate gel-within-gel matrices, useful for guiding cell outgrowth and organoid morphology[177], as well as to optically print biomaterials within living animals[178,179].

Multiphoton lithography has also been used for subtractive manufacturing — in which well-defined 3D voids are sculpted within bulk materials through degradation-mediated processes — often achieving geometries that are difficult to reach with additive techniques (such as overhangs and small-diameter channels)[180]. When implemented using high-powered lasers to drive focal point-localized cavitation, chemical bonds can be non-specifically broken through direct photoablation. Although ablation-based methods are compatible with most biomaterials (for example collagen and fibrin)[181–183], this approach necessitates large laser dwell times, which limits fabrication scales/times, and it cannot be performed in a cytocompatible manner to degrade materials surrounding cells. Incorporation of photodegradable bonds within the hydrogel's polymeric backbone can speed up degradation by many orders of magnitude, enabling more rapid and cytocompatible subtraction-based fabrication. The now conventional *o*NB-based linkers have dominated this space[28,92], with improved photodegradation kinetics achieved using photosensitizers[101] or inorganic-based photodegradable linkers[107]. Multiphoton-based degradation has been applied to create complex vasculature[90], guide organoid formation and tissue geometry[184], and customize the biochemical landscape of hydrogels[185]. Because photoablation does not require the integration of specific photolabile groups, it has proven easier to implement and more widely utilized, despite photodegradation-based methods offering greater material customization. Trading speed for precision, multiphoton lithography uniquely accesses sub-cellular patterning resolutions but suffers from extended fabrication times.

Multiphoton patterning systems are commercially available; however, they generally cost more than other commercial bioprinters due to the need for high-powered lasers. The Quantum X bio (NanoScribe/Cellink) promises resolutions up to 100 nm with a suite of available bioresins using a fixed-wavelength light source. There are also commercially available multiphoton microscopes, including the Bergamo II (Thorlabs) and the Stellaris 8 Dive (Leica); however, they require substantial modifications to be adapted for multiphoton lithography.

Grayscale lithography

Most of the presented methods generate constructs with homogeneous physicochemical properties, yet biological tissues present

variable mechanical and biochemical properties that photolithographic processes can potentially recreate. DLP and multi-material printing have been used to generate mechanical and biochemical gradients through the exchange of different bioinks; however, such methods afford only stepwise material variations and with resolutions limited by the layer height. Similar methods have been demonstrated with volumetric printing, but again, the property changes are limited by the composition of the different bioinks[168]. Taking advantage of the light dose-dependence of many photochemistries, in conjunction with variably powered multiphoton lithography, our group recently introduced 'grayscale image z-stack-guided multiphoton optical-lithography' (GIZMO)[100]. GIZMO uniquely affords grayscale biochemical/biophysical customization of biomaterials in full 3D space and at subcellular resolutions and across mm^3 scales. By continuously varying laser light intensity throughout raster scanning based on inputted grayscale image stacks, GIZMO achieves arbitrarily graded and complex material modulation without increased patterning times. We believe that image-guided photopatterning methods such as GIZMO will push the limits of light-based fabrication and 4D customization of precisely engineered hydrogel biomaterials. The GIZMO technique has been made open-source and is directly compatible with many commercial two-photon microscopes.

Post-synthetic hydrogel tuning

The ECM, in addition to acting as a physical scaffolding for cells, imparts many biological, biochemical and topographical cues that regulate cell behaviour[186]. Although 2D culture models have been instrumental in better understanding cellular function and dysregulation, they are unable to accurately capture the 3D nature of the human body. As questions increase in complexity, so should the models, which must be able to capture the dynamic nature of such processes. Relatively simple chemistries in conjunction with photolithographic patterning have proven extremely powerful in answering complex questions. Here, we describe recent innovations in using light-sensitive chemistries for 4D tuning of hydrogel properties (for example, stiffening, softening and reversible mechanics) (Fig. 2), with a focus on the types of biological questions that have been probed and what, for now, remains a goal.

Optical tuning of hydrogel mechanics

As cells experience tensile, compressive and shear forces, these mechanical signals are transduced into biochemical signals via integrin-mediated signalling pathways in a process known as mechanotransduction. The main cellular components involved in sensing and regulating ECM mechanics include integrins, cytoskeletal and signalling proteins associated with focal adhesions, the actomyosin cytoskeleton, and the signalling components that regulate assembly of these structures. Examples of these signalling components include focal adhesion kinase (FAK), Rho GTPases and their downstream effectors, Rho-associated protein kinase (ROCK), and myosin light chain kinases, as well as other transcriptional effectors such as yes-associated protein (YAP) and the transcriptional coactivator with PDZ-binding motif (TAZ)[187]. As these signalling pathways are critical in many physiological processes ranging from cellular growth, proliferation and motility, photoresponsive hydrogel models have become indispensable for better understanding the dynamic role of ECM mechanics.

Informing disease models. Many of the aforementioned pathways are also dysregulated in diseases (for example in cancer, fibrosis, diabetes and cardiovascular disease), resulting in matrix stiffening caused by increased ECM deposition, crosslinking and fibre alignment[188]. However, use of supraphysiologically stiff, conventional tissue culture polystyrene may obscure the role of ECM rigidity; therefore, 3D hydrogel systems are popularly utilized to mimic more in vivo-like conditions.

Hydrogels based on anthracene and DBCO photodimerization, as well as thiol–ene secondary photopolymerization, have been used to increase Young's moduli on demand to study myoblast, valvular interstitial cell (VIC) and muscle stem cell activation[40,42,43,189,190]. Increasing the Young's modulus from 10 to 50 kPa increased fibroblast area and enhanced nuclear localization of nuclear factor of activated T cells (NFAT) as compared with the static controls. Although it had been speculated that calcineurin–NFAT signalling was mechanosensitive and involved in fibroblast activation, this platform enabled the tracking of this translocation event on physiological timescales, which was previously impossible in other models[40]. Similarly, the role of BAG3 as a mechanotransducer was identified in myoblasts and a chaperone of the YAP–TAZ complex, further informing how YAP–TAZ is controlled[44]. Other topics, including the mechanotransduction basis of human mesenchymal stem cell (MSC) lineage commitment, and pancreatic cancer cell spheroid formation and metabolic activity, have been explored with photostiffening platforms[63,191].

Photosoftening hydrogels can also be used to survey cellular mechanomemory — the ability of cells to remember past mechanical cues long after their removal. Allyl sulfide and *o*NB-based gel systems that enable various dosages of a stiff environment have been used to investigate YAP–TAZ activation and specific epigenetic modulators of chromatin condensation in human MSCs and myofibroblasts[133,192,193]. These translocation events and epigenetic modifications could be reversed when cells were briefly exposed to a stiff environment but not when culture was carried out for longer periods of time[133].

Although photostiffening and softening techniques have independently shed light on mechanotransduction phenomena, models that can both stiffen and soften are valuable for studying disease development and resolution, as well as cyclical loading processes. For example, photoswitchable azobenzene-modified polyacrylamide gels have been used to study human MSC spreading[194] and to examine the gene expression levels of the cell surface marker E-cadherin in MCF-7 breast cancer cells, the downregulation of which has been correlated with metastasis[195]. Photoresponsive protein-based hydrogels have also been leveraged to study 3D myofibroblast activation, with cycled gels exhibiting increased transcriptional levels of periostin and αSMA[139], as well as human MSC transcriptome changes in response to cycling of mechanics[141]. Yet, this area remains ripe for investigation; despite cyclical changes in bulk elastic moduli being uncommon in the body, non-invasive methods and materials that capture cyclical mechanical loading experienced by cells in soft tissues (such as in cartilage, heart, lung, gastrointestinal tract and blood vessels) will be powerful in studying the mechanotransduction events occurring in these environments.

The contributions of ECM mechanics to promoting fibrosis, atherogenesis and cancer malignancy are becoming widely appreciated; however, many questions remain unanswered. Current systems explore the consequences of mechanical changes and kinetics of mechanotransduction pathways. Still, data indicate that matrix stiffening precedes many of these pathological changes[188]; questions remain concerning the biochemical basis of this initial stiffening. Co-culture models in tandem with dynamic hydrogel platforms are necessary to examine the paracrine crosstalk leading up to these events.

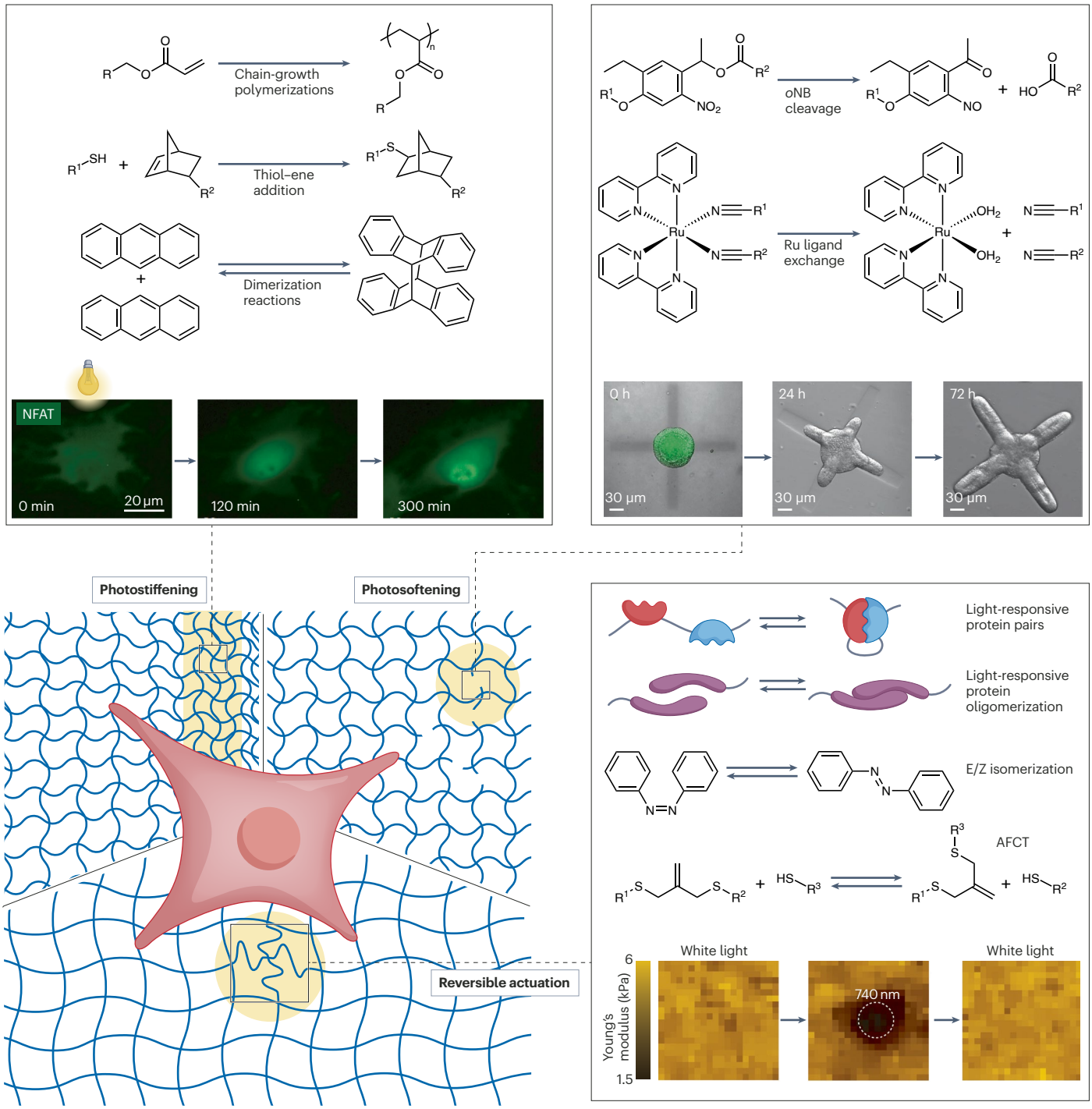

Fig. 2 | Mechanical properties of hydrogels for influencing cell fate and morphology can be controlled by light. Hydrogels can photostiffen around cells in response to light by initially including unreacted functional groups, such as acrylates, alkenes or dimerizing moieties such as anthracene, and they can be used to study mechanotransduction pathways, such as nuclear factor of activated T cells (NFAT) localization. Additionally, hydrogels crosslinked with photolabile moieties, such as ruthenium or *ortho*-nitrobenzyl (*o*NB), can be used to selectively degrade voids to direct organoid growth. Finally, materials based on reversible bond exchange chemistries, such as the addition–fragmentation chain transfer (AFCT) of allyl sulfides and phytochrome-based protein pairs have been successfully demonstrated to transiently change stiffness in response to light exposure. Upper left panel reprinted with permission from ref. 40, Wiley. Upper right panel reprinted with permission from ref. 184, AAAS. Bottom right panel reprinted with permission from ref. 141, Wiley.

Directing organoid growth. Another avenue in which photosensitive chemistries have seen a rise in interest has been in the engineered organoid space. Approaches for patterning geometric confinement as well as the pliability of the surrounding matrix have benefited greatly from thiol–ene, nitrobenzyl and allyl sulfide-based chemistries[177,196]. Including *o*NB moieties into a PEG hydrogel enabled channel photodegradation around the embedded intestinal organoid, which permitted epithelial symmetry breaking and control over bud formation[184]. The degree of crypt formation as a function of matrix degradation was quantified in an allyl-sulfide system and demonstrated that around 15% degradation of the initial network yielded the ideal mechanical conditions for crypt formation[132]. Modulating the surrounding viscoelasticity further influences cell shape, notably without changing the hydrogel stiffness[197,198]. For example, photomodulated viscoelasticity around intestinal organoids yielded epithelial curvature followed by a tension-induced membrane potential gradient and YAP nuclear translocation[134]. Dynamic materials will continue to play a major role in the further development of these advanced platforms.

Optical tuning of hydrogel biochemical cues

The immobilization or release of biochemical cues (such as bioactive small molecules, peptides and proteins) has been a large focus in the fields of tissue engineering and regenerative medicine, and, accordingly, many photoresponsive platforms have been developed with this goal in mind (Fig. 3). Although light has been used extensively for triggered therapeutic release from biomaterials[106], here we focus on photochemical strategies used to control biomolecule presentation within 3D hydrogel models.

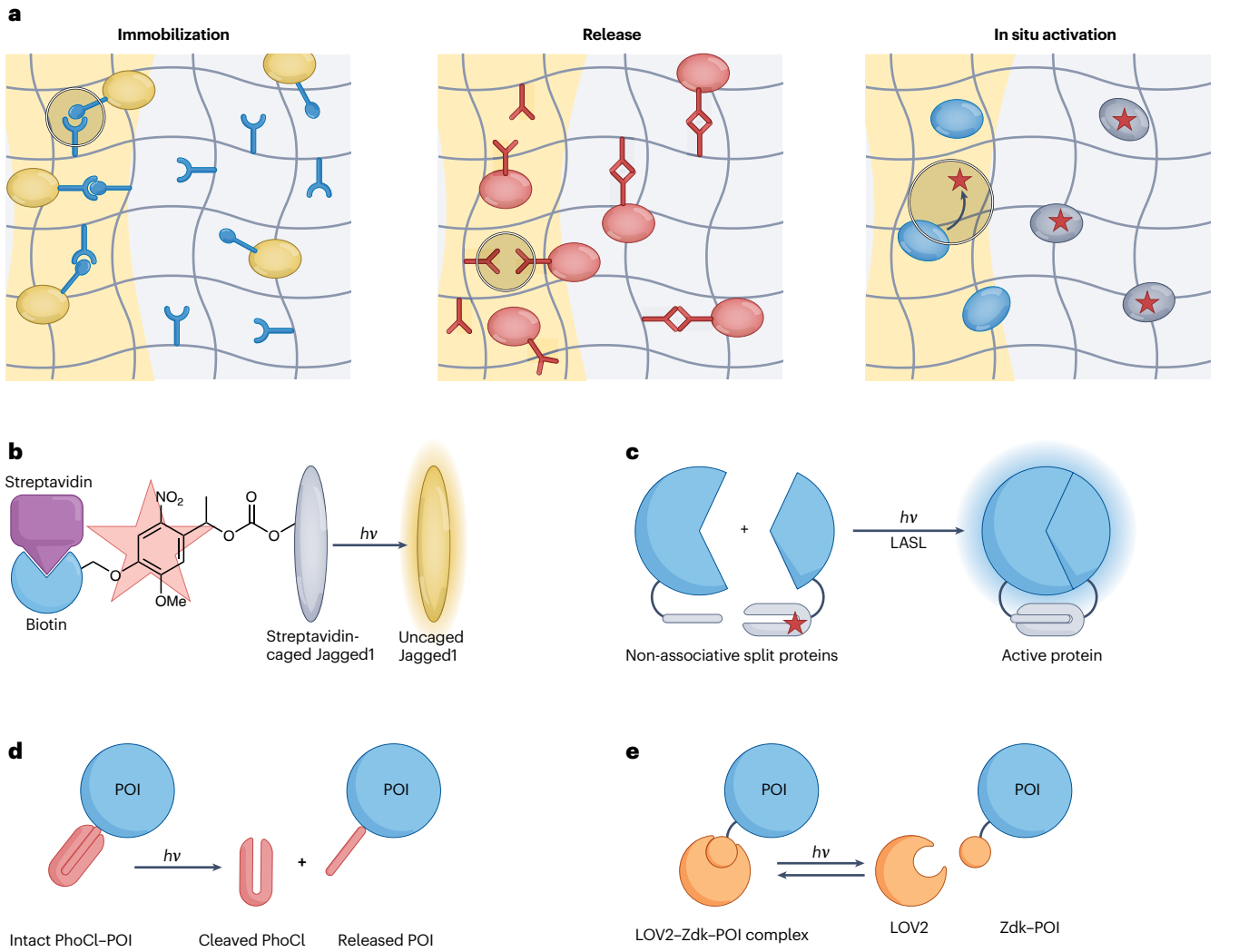

Fig. 3 | Light-modulated biochemical activity and composition of hydrogels. a, Proteins, and similarly peptides, can be immobilized (via acrylate and thiol–ene addition), released (through breakage of either chemical or genetically encoded crosslinkers) or in situ-activated. **b**, Jagged1 (and other proteins) can be photocaged through a bulky, *ortho*-nitrobenzyl (*o*NB) tethered moiety, which can be removed by irradiation with 365 nm light, yielding the active protein. **c**, Light-activated SpyLigation (LASL) enables the irreversible assembly of split proteins via photocaged SpyCatcher and SpyTag. **d**, Photocleavable protein (PhoCl) enables the irreversible release of a genetically encoded photoreleasable protein of interest (POI). **e**, POIs can be reversibly tethered to a hydrogel backbone through the LOV2 trap and release of protein (LOVTRAP) system. Zdk, small, engineered Zdark protein. Adapted from ref. 68, Springer Nature Limited.

Spatiotemporal control over peptide presentation for directed cell adhesion and migration. Patterning peptide motifs such as RGD — the canonical cell-adhesion peptide motif derived from fibronectin — or IKVAV — derived from laminin — has proven to be one of the most accessible ways to control cell function and organization. Initial studies focused on uncaging reactive groups on the hydrogel itself, liberating anchoring points to selectively immobilize biomolecules[199–201]. Alternatively, direct photopolymerization of modified peptides inside a gel provides a straightforward way to spatially modify the biochemical properties; here, only functionalizing the peptide with reactive handles (which in many cases is as simple as including a cysteine residue during peptide synthesis) is required for reaction with hydrogel-forming chemistries such as acrylates or norbornenes. Additionally, RGD modified with an *o*NB or the genetic photocleavable tether PhoCl enables subtractive patterning[116]. However, because these methods rely on the diffusion of peptides in/out of the gel during patterning, modification times are heavily dictated by biomaterial geometry.

Sidestepping limitations intrinsic to diffusion-based strategies, more recent efforts have focused on the direct photoactivation of peptides. In general, a bulky photocage is included on a critical amino acid, thus blocking the peptide's function; upon light exposure, the native peptide is generated, near-instantaneously 'activating' the peptide. In this way, cyclic RGD has been transdermally uncaged, promoting vascularization of the hydrogel in a mouse model[202]. Similarly, two-photon uncaging of cyclic RGD in the presence of soluble VEGF led to directed human umbilical vein endothelial cell migration and sprouting in the exposed areas; non-illuminated areas exhibited round phenotypes, despite the presence of VEGF, highlighting the synergy between adhesion and angiogenic pathways[203]. Alternatively, this strategy can be applied for the in vitro photouncaging of a VEGF peptidomimetic (QK) with similar results[204]. Given the broad availability of solid-phase peptide synthesis and the orthogonality of typical photoprotecting groups (for example, *o*NB and 4,5-dimethoxy-2-nitrobenzyl) to amino acid coupling reactions, this strategy is well-suited for the precise manipulation of cellular environments with more advanced image-guided photopatterning techniques (such as GIZMO).

Reversible patterning of peptides such as RGD has been proposed via allyl sulfide, supramolecular and azobenzene, and LOV2-Jα chemistries; this approach has enabled the in-depth study of focal adhesion assembly/disassembly and the proposal of trypsin-free passaging platforms[129,205–207]. Intriguingly, in one study, anionic polymers were functionalized with azobenzene moieties and RGD motifs; NIR-upconverted UV light caused swelling of the microgels and increased RGD bioavailability, facilitating the adhesion and pro-regenerative polarization of macrophages, whereas irradiation with visible light caused deswelling of the microgels and burying of the RGD motifs, resulting in a pro-inflammatory phenotype[208]. Although prior studies involving reversible peptide presentation have largely been implemented in 2D culture, functional studies in this regard may yield important findings regarding focal adhesion formation in 3D.

4D-controlled protein presentation to guide cell function and morphogenesis. Although the same chemistries used to pattern peptides in gels can be implemented for protein immobilization, the installation of photoreactive handles necessitates careful thought. Protein functionalization has been conventionally achieved via stochastic chemistries such as those based on *N*-hydroxysuccinimide or maleimides, but such labelling strategies often dramatically impair protein activity and yield a heterogeneously modified population[209]. As such, site-specific modification strategies are critical for preserving protein functionality and native structure throughout biomaterial decoration. Terminal functionalization can be accomplished by many protein semi-synthesis techniques; in this regard, the bacterial transpeptidase sortase, which recognizes and cleaves the sorting sequence LPXTG and installs custom polyglycine tags, has emerged as a popular tool[98,210]. Additionally, N-terminally biotinylated proteins, as well as streptavidin bioconjugates, can be readily prepared or purchased, enabling more generalizable immobilization strategies.

The simplest strategies to biochemically decorate gels with proteins again involve additive patterning: proteins can be modified with a click handle such as DBCO or their binding partners can be modified with photosensitive moieties such as acrylates. For example, FGF2 and TGFβ1 were modified with azide and tetrazine moieties, respectively, that could react with spatially photopatterned binding partners to control VIC activation in a single hydrogel network. VICs in the FGF2 regions were quiescent, whereas those in the TGFβ1 regions became activated myofibroblasts[211]. Patterning BMP2 in a similar manner in conjunction with TGFβ1 produced patterned VIC nodule formation, offering insights into synergistic pathways in aortic valve calcification[211]. In another method, methacrylated heparin was spatially immobilized in an acrylate-modified alginate gel, enabling patterning of heparin-binding proteins such as VEGF and BMP2 in a generalizable manner for controlled human MSC differentiation[212].

Reactive sites can also be uncaged on the hydrogel substrate. For example, using 6-bromo-7-hydroxycoumarin-caged agarose enabled spatial immobilization of maleimide-streptavidin and maleimide-barnase in distinct volumes; subsequent reactions with biotinylated ciliary neurotrophic factor and barstar-sonic hedgehog enabled directing of neural precursor cell migration and organization[73]. Similarly, highly epidermal growth factor receptor (EGFR)-expressing MDA-MB-468 breast cancer cells invaded deeper into a hyaluronic acid gel photochemically modified with EGF gradients as compared with an unmodified gel[213]. Using two-photon lithography in conjunction with a 7-dicarboxymethylaminocoumarin (DCMAC)-caged polyglycine substrate enabled the photopatterned sortase-mediated immobilization of an LPXTG-modified avidin complex; the avidin could be further decorated with biotinylated proteins, a strategy that was used to pattern nerve growth factor for controlled axon guidance[210]. Additionally, photocaged cyclopentadienes have been used for immobilizing EGF onto natural matrices for studying extracellular-signal-regulated kinase (ERK) activity[76]. Photomediated oxime ligation has also been utilized for uncaging reactive groups on both synthetic and natural hydrogels[64,65,98,214]. For example, this chemistry was first used within natural protein-based gels (such as fibrin and collagen I) by patterning Delta-1 protein to spatially control Notch signalling (a critical pathway in embryonic development, regulation of tissue homeostasis and adult stem cell maintenance) in embedded U2OS osteosarcoma cells[64]. Pairing this chemistry with *o*NB-modified tethers enables a single cycle of protein immobilization and release[65,98]. This chemistry can also be used in conjunction with multiphoton lithography to control EGF–EGFR ligand–receptor endocytosis with sub-cellular resolution[98]. However, each of these methods rely on the diffusion of active protein throughout the gel during the patterning process; like with patterned immobilization of peptides, embedded cells are flood-exposed to soluble and potentially bioactive cues during diffusion that may lead to spatially uncontrolled cell activation.

Rather than simply immobilizing/releasing biomolecules within biomaterials, several exciting strategies have been introduced to directly

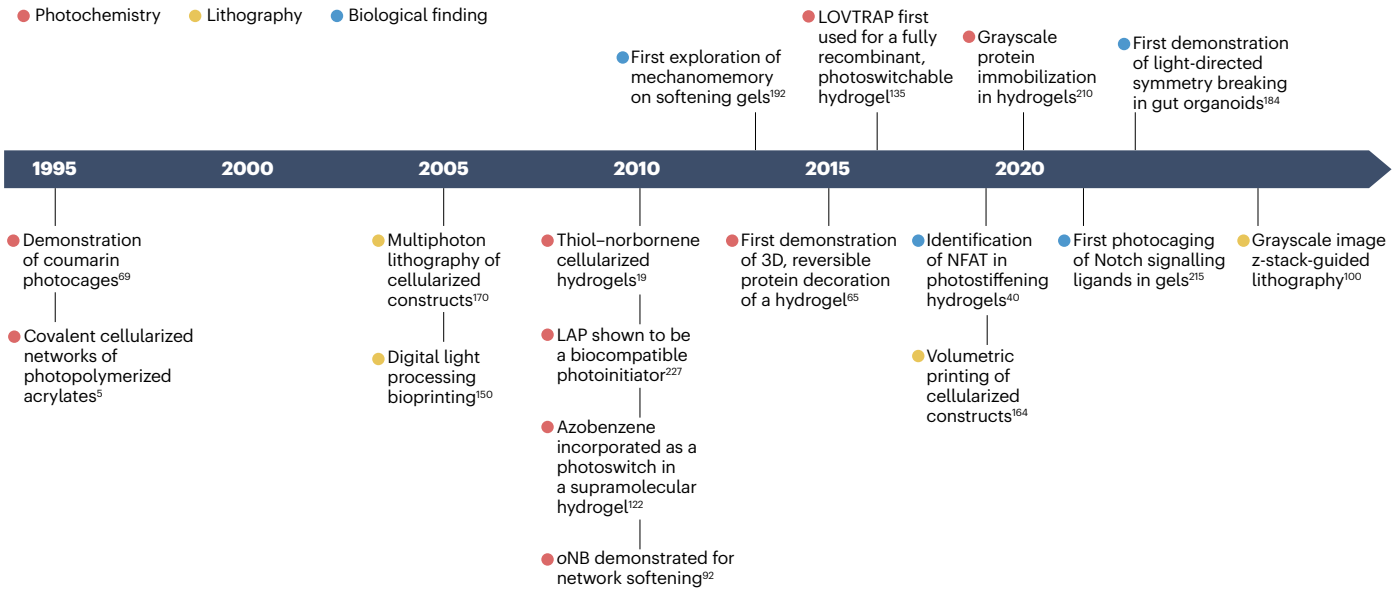

Fig. 4 | Timeline of key milestones in the field. Timeline highlighting important advances in photochemistries, hydrogel demonstrations and patterning techniques over the past three decades[5,19,40,65,69,92,100,122,135,150,164,170,184,192,210,215,227]. LAP, lithium phenyl-2,4,6-trimethylbenzoylphosphinate; LOVTRAP, LOV2 trap and release of protein; NFAT, nuclear factor of activated T-cells; *o*NB, *ortho*-nitrobenzyl.

photoactivate functional proteins in gels. For example, photocaged SpyCatcher and SpyTag can be genetically fused onto non-associative split protein pairs; upon light exposure, light-activated SpyLigation permitted irreversible reconstitution and functional assembly of split proteins in biomaterials, in solution and in cellulo[68]. In addition to patterning activation of fluorescent and luminescent proteins via mask-based and multiphoton-based lithography, we demonstrated patterned genome editing of primary fibroblasts via irreversible photoassembly of a split Cre recombinase. Although this strategy is generalizable, it still relies on the diffusion of a non-reactive half. Conversely, uncaging proteins in situ approaches near-instantaneous protein activation. For example, caging Jagged1 (a Notch signalling ligand) with a bulky, biotin–streptavidin–*o*NB conjugate sterically inhibited cell-receptor interactions. In this way, cholangiocyte differentiation of hepatoblasts could be controlled in hyaluronic acid gels[215]. A similar cage was used for caging tethered EGF[216]. Although this strategy can be readily applied for the traceless photoactivation of many proteins, its lack of genetic encodability precludes its usage in many cellular contexts. 'Living materials' have also been reported that co-encapsulate living mammalian cells with engineered bacteria capable of optogenetically producing and secreting bioactive proteins on demand[217]. In this manner, gel-seeded fibroblasts were able to uptake bacteria-secreted fluorescent proteins upon light exposure.

Outlook

As the engineering goals become more advanced, the tools must also match pace — indeed, since the early 2010s, there has been an explosion of novel chemistries and manufacturing techniques that have afforded unprecedented control over the cellular microenvironment (Fig. 4). Further development and deeper analysis of photochemistries, as well as techniques to pattern their activation, are required to enable faster, higher resolution and more complex tissue construction.

Building on the recent successes of BODIPY, DHTz and ruthenium-based systems[86,87,107], chemical reactions that respond directly to visible and/or NIR light remain of prime interest. These systems promise a rapid response to low-energy and tissue-penetrating wavelengths of light, which is a feature that makes them exciting both from the perspective of intravital printing and rapid material customization. Moreover, the ability to photocontrol processes across a wide range of optical spectra opens doors for multiplexed and independent control over distinct physicochemical properties within a given material. In addition to new reaction development, photosensitizers and/or upconverting nanoparticles are likely to find use in effectively red-shifting tried-and-true reaction schemes[197,218,219].

Notably, a considerable mismatch has been reported between UV–Vis spectra and photochemical reactivity, in which activities were substantially red-shifted relative to that expected based on the relevant absorption spectra[220,221]. Given this pressing discrepancy, using tuneable laser systems to systematically construct photochemical action plots to evaluate new and tried-and-true photochemical activities should become the new standard in the field. This method was used to multiplex the degradation of three distinct crosslinkers within a narrow wavelength range (λ = 325–420 nm) by balancing kinetics and reactivity[222]. Although most of this work has looked at single-photon reactions, a deep scrutiny of two-photon-related action plots is also critical towards progression in multiphoton lithography.

Using orthogonal wavelengths to increase precision and multiplex modification is a largely underexplored design space. Multiplexed hydrogel degradation and stiffening has been demonstrated; however, few reports show coupled biochemical and mechanical modulation[107,222]. One way to integrate technological and chemical advances is xolography, which leverages the geometrically orthogonal delivery of two distinct wavelengths alongside specialized photoinitators to improve resolution compared with traditional DLP[160,161]. Although not yet applied to biologically relevant materials, xolography potentially offers better resolution than DLP, with faster speeds than multi-photon lithography.

Technical developments in lithographic light delivery (such as GIZMO) now enable the finely tuned grayscale control of topographical, mechanical and biochemical properties in 3D and across diverse scales. Similarly exciting, volumetric printing enables structured materials to be created and/or modified almost instantaneously. Engineered waveguides now permit light stimulation at previously inaccessible tissue depths. Again, in the ongoing quest to speed up and expand the scale of these processes, more sensitive chemical moieties, photoinitiators and photosensitizers that permit rapid polymerization/degradation with high precision will be required.

Although biological systems are four-dimensionally complex in both their biochemical and biophysical nature, exceptionally few strategies to date have investigated their synergic/antagonistic effects. From our perspective, the future of this field lies in designing platforms that examine the interplay of biochemical and mechanical heterogeneity in a high-throughput manner[223–225]. Furthermore, we believe that next-generation models need to take advantage of these photochemistries to impart such physicochemical heterogeneity both in space and time. For instance, solid tumours are known to exhibit dramatic stiffening as the disease progresses[188]. Co-culture models with cancer cells at the core and other cells associated with the microenvironment, such as fibroblasts surrounding the 'tumour', could undergo dramatic changes in mechanics over the culture period to provide insights into paracrine crosstalk in these dynamic environments. Similarly, other fibrotic diseases would benefit from spatiotemporally controlled 3D models[226].

Although further development of light-based patterning techniques and chemistries will continue to advance our capacity for complex patterning, existing technologies, such as grayscale and multi-material printing, can already recreate a substantial portion of biological complexity at the micrometre scale to modulate cell function. Therefore, applying existing technologies and chemistries through collaborations with biologists and clinicians is an important next step for the translation of this field. We urge that increasing the impact of existing technologies and novel future directions will only emerge if all stakeholders are included via collaborations and industrial partnerships. Overall, the toolkit of light-based chemistries and patterning technologies is robust and primed for increasing adoption and application.

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References

1. Ruskowitz, E. R. & DeForest, C. A. Photoresponsive biomaterials for targeted drug delivery and 4D cell culture. *Nat. Rev. Mater.* **3**, 17087 (2018).
2. Li, L., Scheiger, J. M. & Levkin, P. A. Design and applications of photoresponsive hydrogels. *Adv. Mater.* **31**, 1807333 (2019).
3. Chatani, S., J. Kloxin, C. & Bowman, N. C. The power of light in polymer science: photochemical processes to manipulate polymer formation, structure, and properties. *Polym. Chem.* **5**, 2187–2201 (2014).
4. Hill-West, J. L., Chowdhury, S. M., Slepian, M. J. & Hubbell, J. A. Inhibition of thrombosis and intimal thickening by in situ photopolymerization of thin hydrogel barriers. *Proc. Natl Acad. Sci. USA* **91**, 5967–5971 (1994).
5. West, J. L. & Hubbell, J. A. Photopolymerized hydrogel materials for drug delivery applications. *React. Polym.* **25**, 139–147 (1995).
6. Van Den Bulcke, A. I. et al. Structural and rheological properties of methacrylamide modified gelatin hydrogels. *Biomacromolecules* **1**, 31–38 (2000).
7. Nichol, J. W. et al. Cell-laden microengineered gelatin methacrylate hydrogels. *Biomaterials* **31**, 5536–5544 (2010).
8. Benton, J. A., DeForest, C. A., Vivekanandan, V. & Anseth, K. S. Photocrosslinking of gelatin macromers to synthesize porous hydrogels that promote valvular interstitial cell function. *Tissue Eng. Part A* **15**, 3221–3230 (2009).
9. Burdick, J. A., Chung, C., Jia, X., Randolph, M. A. & Langer, R. Controlled degradation and mechanical behavior of photopolymerized hyaluronic acid networks. *Biomacromolecules* **6**, 386–391 (2005).
10. Park, Y. D., Tirelli, N. & Hubbell, J. A. Photopolymerized hyaluronic acid-based hydrogels and interpenetrating networks. *Biomaterials* **24**, 893–900 (2003).
11. Brinkman, W. T., Nagapudi, K., Thomas, B. S. & Chaikof, E. L. Photo-cross-linking of type I collagen gels in the presence of smooth muscle cells: mechanical properties, cell viability, and function. *Biomacromolecules* **4**, 890–895 (2003).
12. Kim, S. H. & Chu, C. C. Synthesis and characterization of dextran-methacrylate hydrogels and structural study by SEM. *J. Biomed. Mater. Res.* **49**, 517–527 (2000).
13. Elbert, D. L. & Hubbell, J. A. Conjugate addition reactions combined with free-radical cross-linking for the design of materials for tissue engineering. *Biomacromolecules* **2**, 430–441 (2001).
14. Cramer, N. B. & Bowman, C. N. Kinetics of thiol–ene and thiol–acrylate photopolymerizations with real-time fourier transform infrared. *J. Polym. Sci. A Polym. Chem.* **39**, 3311–3319 (2001).
15. Salinas, C. N. & Anseth, K. S. Mixed mode thiol–acrylate photopolymerizations for the synthesis of PEG–peptide hydrogels. *Macromolecules* **41**, 6019–6026 (2008).
16. Günay, K. A. et al. Photo-expansion microscopy enables super-resolution imaging of cells embedded in 3D hydrogels. *Nat. Mater.* **22**, 777–785 (2023).
17. Tibbitt, M. W., Kloxin, A. M., Sawicki, L. A. & Anseth, K. S. Mechanical properties and degradation of chain and step-polymerized photodegradable hydrogels. *Macromolecules* **46**, 2785–2792 (2013).
18. Lin, C.-C., Raza, A. & Shih, H. PEG hydrogels formed by thiol-ene photo-click chemistry and their effect on the formation and recovery of insulin-secreting cell spheroids. *Biomaterials* **32**, 9685–9695 (2011).
19. Fairbanks, B. D. et al. A versatile synthetic extracellular matrix mimic via thiol-norbornene photopolymerization. *Adv. Mater.* **21**, 5005–5010 (2009).
20. Aimetti, A. A., Machen, A. J. & Anseth, K. S. Poly(ethylene glycol) hydrogels formed by thiol-ene photopolymerization for enzyme-responsive protein delivery. *Biomaterials* **30**, 6048–6054 (2009).
21. Mũnoz, Z., Shih, H. & Lin, C.-C. Gelatin hydrogels formed by orthogonal thiol–norbornene photochemistry for cell encapsulation. *Biomater. Sci.* **2**, 1063–1072 (2014).
22. Soliman, B. G. et al. Development and characterization of gelatin-norbornene bioink to understand the interplay between physical architecture and micro-capillary formation in biofabricated vascularized constructs. *Adv. Healthc. Mater.* **11**, 2101873 (2022).
23. Gramlich, W. M., Kim, I. L. & Burdick, J. A. Synthesis and orthogonal photopatterning of hyaluronic acid hydrogels with thiol-norbornene chemistry. *Biomaterials* **34**, 9803–9811 (2013).
24. Yang, K. et al. Photo-crosslinked mono-component type II collagen hydrogel as a matrix to induce chondrogenic differentiation of bone marrow mesenchymal stem cells. *J. Mater. Chem. B* **5**, 8707–8718 (2017).
25. McOscar, T. V. C. & Gramlich, W. M. Hydrogels from norbornene-functionalized carboxymethyl cellulose using a UV-initiated thiol-ene click reaction. *Cellulose* **25**, 6531–6545 (2018).
26. Shih, H. & Lin, C.-C. Cross-linking and degradation of step-growth hydrogels formed by thiol–ene photoclick chemistry. *Biomacromolecules* **13**, 2003–2012 (2012).
27. Sawicki, L. A. & Kloxin, A. M. Design of thiol–ene photoclick hydrogels using facile techniques for cell culture applications. *Biomater. Sci.* **2**, 1612–1626 (2014).
28. DeForest, C. A. & Anseth, K. S. Cytocompatible click-based hydrogels with dynamically tunable properties through orthogonal photoconjugation and photocleavage reactions. *Nat. Chem.* **3**, 925–931 (2011).
29. Nelson, B. R. et al. Photoinduced dithiolane crosslinking for multiresponsive dynamic hydrogels. *Adv. Mater.* https://doi.org/10.1002/adma.202211209 (2023).
This article reports the first application of 1,2-dithiolanes as dynamic covalent photocrosslinkers in hydrogel biomaterials, demonstrating the versatility of this chemistry for dynamic stiffening, softening and biomolecule immobilization.
30. Nelson, B. R. et al. Facile physicochemical reprogramming of PEG-dithiolane microgels. *Adv. Healthc. Mater.* https://doi.org/10.1002/adhm.202302925 (2023).
31. Fairbanks, B. D. et al. Photoclick chemistry: a bright idea. *Chem. Rev.* **121**, 6915–6990 (2021).
32. Kharkar, P. M., Rehmann, M. S., Skeens, K. M., Maverakis, E. & Kloxin, A. M. Thiol–ene click hydrogels for therapeutic delivery. *ACS Biomater. Sci. Eng.* **2**, 165–179 (2016).
33. Chujo, Y., Sada, K. & Saegusa, T. Polyoxazoline having a coumarin moiety as a pendant group. Synthesis and photogelation. *Macromolecules* **23**, 2693–2697 (1990).
34. Tamate, R. et al. Photo-dimerization induced dynamic viscoelastic changes in ABA triblock copolymer-based hydrogels for 3D cell culture. *Chem. Mater.* **28**, 6401–6408 (2016).
35. Kabb, C. P., O'Bryan, C. S., Deng, C. C., Angelini, T. E. & Sumerlin, B. S. Photoreversible covalent hydrogels for soft-matter additive manufacturing. *ACS Appl. Mater. Interfaces* **10**, 16793–16801 (2018).
36. Chen, Y. et al. Light-enabled reversible self-assembly and tunable optical properties of stable hairy nanoparticles. *Proc. Natl Acad. Sci. USA* **115**, E1391–E1400 (2018).
37. Zhu, C. N. et al. Reconstructable gradient structures and reprogrammable 3D deformations of hydrogels with coumarin units as the photolabile crosslinks. *Adv. Mater.* **33**, 2008057 (2021).
38. Inacker, S., Schipplick, L., Kahler, P. & Hampp, N. Upgrading the toolbox: two-photon absorption induced cleavage of coumarin dimers for light-based 4D printing. *Macromol. Rapid Commun.* **44**, 2300217 (2023).
39. Zheng, Y. et al. PEG-based hydrogel synthesis via the photodimerization of anthracene groups. *Macromolecules* **35**, 5228–5234 (2002).
40. Günay, K. A. et al. PEG–anthracene hydrogels as an on-demand stiffening matrix to study mechanobiology. *Angew. Chem. Int. Ed.* **58**, 9912–9916 (2019).

41. Truong, V. X., Li, F. & Forsythe, J. S. Versatile bioorthogonal hydrogel platform by catalyst-free visible light initiated photodimerization of anthracene. *ACS Macro Lett.* **6**, 657–662 (2017).
42. Silver, J. S. et al. Injury-mediated stiffening persistently activates muscle stem cells through YAP and TAZ mechanotransduction. *Sci. Adv.* **7**, eabe4501 (2021).
43. Brown, T. E. et al. Secondary photocrosslinking of click hydrogels to probe myoblast mechanotransduction in three dimensions. *J. Am. Chem. Soc.* **140**, 11585–11588 (2018).
44. Günay, K. A. et al. Myoblast mechanotransduction and myotube morphology is dependent on BAG3 regulation of YAP and TAZ. *Biomaterials* **277**, 121097 (2021).
45. Clovis, J. S., Eckell, A., Huisgen, R. & Sustmann, R. 1.3-Dipolare Cycloadditionen, XXV. Der Nachweis des freien Diphenylnitrilimins als Zwischenstufe bei Cycloadditionen. *Chem. Ber.* **100**, 60–70 (1967).
46. Wang, Y., Hu, W. J., Song, W., Lim, R. K. V. & Lin, Q. Discovery of long-wavelength photoactivatable diaryltetrazoles for bioorthogonal 1,3-dipolar cycloaddition reactions. *Org. Lett.* **10**, 3725–3728 (2008).
47. Dietrich, M. et al. Photoclickable surfaces for profluorescent covalent polymer coatings. *Adv. Funct. Mater.* **22**, 304–312 (2012).
48. Wang, Y., Song, W., Hu, W. J. & Lin, Q. Fast alkene functionalization in vivo by photoclick chemistry: HOMO lifting of nitrile imine dipoles. *Angew. Chem. Int. Ed.* **48**, 5330–5333 (2009).
49. Yu, Z., Ohulchanskyy, T. Y., An, P., Prasad, P. N. & Lin, Q. Fluorogenic, two-photon-triggered photoclick chemistry in live mammalian cells. *J. Am. Chem. Soc.* **135**, 16766–16769 (2013).
50. Lederhose, P. et al. Near-infrared photoinduced coupling reactions assisted by upconversion nanoparticles. *Angew. Chem. Int. Ed.* **55**, 12195–12199 (2016).
51. Liu, H. et al. A nucleoside derivative 5-vinyluridine (VrU) for imaging RNA in cells and animals. *Bioconjugate Chem.* **30**, 2958–2966 (2019).
52. Wu, Y., Guo, G., Zheng, J., Xing, D. & Zhang, T. Fluorogenic "photoclick" labeling and imaging of DNA with coumarin-fused tetrazole in vivo. *ACS Sens.* **4**, 44–51 (2019).
53. Fan, Y., Deng, C., Cheng, R., Meng, F. & Zhong, Z. In situ forming hydrogels via catalyst-free and bioorthogonal "tetrazole–alkene" photo-click chemistry. *Biomacromolecules* **14**, 2814–2821 (2013).
54. Li, S. et al. Redox-sensitive and intrinsically fluorescent photoclick hyaluronic acid canogels for traceable and targeted delivery of cytochrome c to breast tumor in mice. *ACS Appl. Mater. Interfaces* **8**, 21155–21162 (2016).
55. Truong, V. X., Li, F., Ercole, F. & Forsythe, J. S. Wavelength-selective coupling and decoupling of polymer chains via reversible [2 + 2] photocycloaddition of styrylpyrene for construction of cytocompatible photodynamic hydrogels. *ACS Macro Lett.* **7**, 464–469 (2018).
56. Marschner, D. E. et al. Visible light [2 + 2] cycloadditions for reversible polymer ligation. *Macromolecules* **51**, 3802–3807 (2018).
57. Kalayci, K., Frisch, H., Barner-Kowollik, C. & Truong, V. X. Wavelength-dependent stiffening of hydrogel matrices via redshifted [2+2] photocycloadditions. *Adv. Funct. Mater.* **30**, 1908171 (2020).
58. Kalayci, K., Frisch, H., Truong, V. X. & Barner-Kowollik, C. Green light triggered [2+2] cycloaddition of halochromic styrylquinoxaline — controlling photoreactivity by pH. *Nat. Commun.* **11**, 4193 (2020).
59. Cui, X. et al. Rapid photocrosslinking of silk hydrogels with high cell density and enhanced shape fidelity. *Adv. Healthc. Mater.* **9**, 1901667 (2020).
60. Jeon, E. Y. et al. Rapidly light-activated surgical protein glue inspired by mussel adhesion and insect structural crosslinking. *Biomaterials* **67**, 11–19 (2015).
61. Partlow, B. P., Applegate, M. B., Omenetto, F. G. & Kaplan, D. L. Dityrosine cross-linking in designing biomaterials. *ACS Biomater. Sci. Eng.* **2**, 2108–2121 (2016).
62. Atienza-Roca, P. et al. Visible light mediated PVA-tyramine hydrogels for covalent incorporation and tailorable release of functional growth factors. *Biomater. Sci.* **8**, 5005–5019 (2020).
63. Liu, H., Nguyen, H. D. & Lin, C. Dynamic PEG–peptide hydrogels via visible light and FMN-induced tyrosine dimerization. *Adv. Healthc. Mater.* **7**, 1800954 (2018).
64. Batalov, I., Stevens, K. R. & DeForest, C. A. Photopatterned biomolecule immobilization to guide three-dimensional cell fate in natural protein-based hydrogels. *Proc. Natl Acad. Sci. USA* **118**, e2014194118 (2021).
65. DeForest, C. A. & Tirrell, D. A. A photoreversible protein-patterning approach for guiding stem cell fate in three-dimensional gels. *Nat. Mater.* **14**, 523–531 (2015).
66. Farahani, P. E., Adelmund, S. M., Shadish, J. A. & DeForest, C. A. Photomediated oxime ligation as a bioorthogonal tool for spatiotemporally-controlled hydrogel formation and modification. *J. Mater. Chem. B* **5**, 4435–4442 (2017).
67. Fitzgerald, E. R., Mineo, A. M., Pryor, M. L. & Buck, M. E. Photomediated post-fabrication modification of azlactone-functionalized gels for the development of hydrogel actuators. *Soft Matter* **16**, 6044–6049 (2020).
68. Ruskowitz, E. R. et al. Spatiotemporal functional assembly of split protein pairs through a light-activated SpyLigation. *Nat. Chem.* **15**, 694–704 (2023).
69. Furuta, T., Torigai, H., Sugimoto, M. & Iwamura, M. Photochemical properties of new photolabile cAMP derivatives in a physiological saline solution. *J. Org. Chem.* **60**, 3953–3956 (1995).
70. Furuta, T. et al. Brominated 7-hydroxycoumarin-4-ylmethyls: photolabile protecting groups with biologically useful cross-sections for two photon photolysis. *Proc. Natl Acad. Sci. USA* **96**, 1193–1200 (1999).
71. Bojtár, M., Kormos, A., Kis-Petik, K., Kellermayer, M. & Kele, P. Green-light activatable, water-soluble red-shifted coumarin photocages. *Org. Lett.* **21**, 9410–9414 (2019).
72. Rahman, N., Purpura, K. A., Wylie, R. G., Zandstra, P. W. & Shoichet, M. S. The use of vascular endothelial growth factor functionalized agarose to guide pluripotent stem cell aggregates toward blood progenitor cells. *Biomaterials* **31**, 8262–8270 (2010).
73. Wylie, R. G. et al. Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. *Nat. Mater.* **10**, 799–806 (2011).
74. Wylie, R. G. & Shoichet, M. S. Two-photon micropatterning of amines within an agarose hydrogel. *J. Mater. Chem.* **18**, 2716–2721 (2008).
75. Bailey, S. J. et al. Design, synthesis, and application of a water-soluble photocage for aqueous cyclopentadiene-based Diels-Alder photoclick chemistry in hydrogels. *Angew. Chem. Int. Ed.* **62**, e202301157 (2023).
76. Bailey, S. J. et al. Diels–Alder photoclick patterning of extracellular matrix for spatially controlled cell behaviors. *Adv. Mater.* **35**, 2303453 (2023).
77. Poloukhtine, A. A., Mbua, N. E., Wolfert, M. A., Boons, G.-J. & Popik, V. V. Selective labeling of living cells by a photo-triggered click reaction. *J. Am. Chem. Soc.* **131**, 15769–15776 (2009).
78. Bjerknes, M., Cheng, H., McNitt, C. D. & Popik, V. V. Facile quenching and spatial patterning of cylooctynes via strain-promoted alkyne–azide cycloaddition of inorganic azides. *Bioconjugate Chem.* **28**, 1560–1565 (2017).
79. McNitt, C. D., Cheng, H., Ullrich, S., Popik, V. V. & Bjerknes, M. Multiphoton activation of photo-strain-promoted azide alkyne cycloaddition "click" reagents enables in situ labeling with submicrometer resolution. *J. Am. Chem. Soc.* **139**, 14029–14032 (2017).
80. Zhang, H. et al. Rapid bioorthogonal chemistry turn-on through enzymatic or long wavelength photocatalytic activation of tetrazine ligation. *J. Am. Chem. Soc.* **138**, 5978–5983 (2016).
81. Truong, V. X., Tsang, K. M., Ercole, F. & Forsythe, J. S. Red light activation of tetrazine–norbornene conjugation for bioorthogonal polymer cross-linking across tissue. *Chem. Mater.* **29**, 3678–3685 (2017).
82. Truong, V. X. & Barner-Kowollik, C. Red-light driven photocatalytic oxime ligation for bioorthogonal hydrogel design. *ACS Macro Lett.* **10**, 78–83 (2021).
83. Goswami, P. P. et al. BODIPY-derived photoremovable protecting groups unmasked with green light. *J. Am. Chem. Soc.* **137**, 3783–3786 (2015).
84. Peterson, J. A. et al. Family of BODIPY photocages cleaved by single photons of visible/near-infrared light. *J. Am. Chem. Soc.* **140**, 7343–7346 (2018).
85. Kand, D. et al. Water-soluble BODIPY photocages with tunable cellular localization. *J. Am. Chem. Soc.* **142**, 4970–4974 (2020).
86. Li, M., Dove, A. P. & Truong, V. X. Additive-free green light-induced ligation using BODIPY triggers. *Angew. Chem.* **132**, 2304–2308 (2020).
87. Chung, K.-Y. et al. Rapid hydrogel formation via tandem visible light photouncaging and bioorthogonal ligation. *Cell Rep. Phys. Sci.* **3**, 101185 (2022).
This article is an in-depth demonstration of highly red-shifted BODIPY photocages for amine, hydrazine and aminooxy groups on starPEG macromers to create biocompatible hydrogels.
88. Jia, S. & Sletten, E. M. Spatiotemporal control of biology: synthetic photochemistry toolbox with far-red and near-infrared light. *ACS Chem. Biol.* **17**, 3255–3269 (2022).
89. Shieh, P., Hill, M. R., Zhang, W., Kristufek, S. L. & Johnson, J. A. Clip chemistry: diverse (bio)(macro)molecular and material function through breaking covalent bonds. *Chem. Rev.* **121**, 7059–7121 (2021).
90. Arakawa, C. K., Badeau, B. A., Zheng, Y. & DeForest, C. A. Multicellular vascularized engineered tissues through user-programmable biomaterial photodegradation. *Adv. Mater.* **29**, 1703156 (2017).
91. Tsurkan, M. V. et al. Photopatterning of multifunctional hydrogels to direct adult neural precursor cells. *Adv. Healthc. Mater.* **4**, 516–521 (2015).
92. Kloxin, A. M., Kasko, A. M., Salinas, C. N. & Anseth, K. S. Photodegradable hydrogels for dynamic tuning of physical and chemical properties. *Science* **324**, 59–63 (2009).
93. Norris, S. C. P., Soto, J., Kasko, A. M. & Li, S. Photodegradable polyacrylamide gels for dynamic control of cell functions. *ACS Appl. Mater. Interfaces* **13**, 5929–5944 (2021).
94. Badeau, B. A., Comerford, M. P., Arakawa, C. K., Shadish, J. A. & DeForest, C. A. Engineered modular biomaterial logic gates for environmentally triggered therapeutic delivery. *Nat. Chem.* **10**, 251–258 (2018).
95. Griffin, D. R. & Kasko, A. M. Photodegradable macromers and hydrogels for live cell encapsulation and release. *J. Am. Chem. Soc.* **134**, 13103–13107 (2012).
96. Griffin, D. R. & Kasko, A. M. Photoselective delivery of model therapeutics from hydrogels. *ACS Macro Lett.* **1**, 1330–1334 (2012).
97. Gawade, P. M., Shadish, J. A., Badeau, B. A. & DeForest, C. A. Logic-based delivery of site-specifically modified proteins from environmentally responsive hydrogel biomaterials. *Adv. Mater.* **31**, 1902462 (2019).
98. Shadish, J. A., Benuska, G. M. & DeForest, C. A. Bioactive site-specifically modified proteins for 4D patterning of gel biomaterials. *Nat. Mater.* **18**, 1005–1014 (2019).
99. Norris, S. C. P., Tseng, P. & Kasko, A. M. Direct gradient photolithography of photodegradable hydrogels with patterned stiffness control with submicrometer resolution. *ACS Biomater. Sci. Eng.* **2**, 1309–1318 (2016).
100. Batalov, I. et al. 4D grayscale biomaterial customization at high resolution and scale. Preprint at *bioRxiv* https://doi.org/10.1101/2024.01.31.578280 (2024).
This article introduces GIZMO, enabling high-resolution nonbinary material modulation at scale.
101. Lunzer, M. et al. A modular approach to sensitized two-photon patterning of photodegradable hydrogels. *Angew. Chem.* **130**, 15342–15347 (2018).
102. Azagarsamy, M. A., McKinnon, D. D., Alge, D. L. & Anseth, K. S. Coumarin-based photodegradable hydrogel: design, synthesis, gelation, and degradation kinetics. *ACS Macro Lett.* **3**, 515–519 (2014).

103. de Gracia Lux, C. et al. Short soluble coumarin crosslinkers for light-controlled release of cells and proteins from hydrogels. *Biomacromolecules* **16**, 3286–3296 (2015).
104. LeValley, P. J. et al. On-demand and tunable dual wavelength release of antibodies using light-responsive hydrogels. *ACS Appl. Bio Mater.* **3**, 6944–6958 (2020).
105. Iturmendi, A., Theis, S., Maderegger, D., Monkowius, U. & Teasdale, I. Coumarin-caged polyphosphazenes with a visible-light driven on-demand degradation. *Macromol. Rapid Commun.* **39**, 1800377 (2018).
106. Rapp, T. L. & DeForest, C. A. Targeting drug delivery with light: a highly focused approach. *Adv. Drug Deliv. Rev.* **171**, 94–107 (2021).
107. Rapp, T. L. & DeForest, C. A. Tricolor visible wavelength-selective photodegradable hydrogel biomaterials. *Nat. Commun.* **14**, 5250 (2023).
108. Rapp, T. L., Highley, C. B., Manor, B. C., Burdick, J. A. & Dmochowski, I. J. Ruthenium-crosslinked hydrogels with rapid, visible-light degradation. *Chem. Eur. J.* **24**, 2328–2333 (2018).
109. Theis, S. et al. Metallo-supramolecular gels that are photocleavable with visible and near-infrared irradiation. *Angew. Chem. Int. Ed.* **56**, 15857–15860 (2017).
110. Teasdale, I. et al. Dynamic supramolecular ruthenium-based gels responsive to visible/NIR light and heat. *Chem. Eur. J.* **25**, 9851–9855 (2019).
111. Zakeri, B. et al. Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. *Proc. Natl Acad. Sci. USA* **109**, E690–E697 (2012).
112. Veggiani, G. et al. Programmable polyproteams built using twin peptide superglues. *Proc. Natl Acad. Sci. USA* **113**, 1202–1207 (2016).
113. Yang, Z. et al. B12-induced reassembly of split photoreceptor protein enables photoresponsive hydrogels with tunable mechanics. *Sci. Adv.* **8**, eabm5482 (2022).
114. Jiang, B. et al. Injectable, photoresponsive hydrogels for delivering neuroprotective proteins enabled by metal-directed protein assembly. *Sci. Adv.* **6**, eabc4824 (2020).
115. Shadish, J. A., Strange, A. C. & DeForest, C. A. Genetically encoded photocleavable linkers for patterned protein release from biomaterials. *J. Am. Chem. Soc.* **141**, 15619–15625 (2019).
This article demonstrates the first application of a genetically encoded PhoCl fusion to selectively release bioactive proteins from a hydrogel.
116. Chapla, R., Hammer, J. A. & West, J. L. Adding dynamic biomolecule signaling to hydrogel systems via tethered photolabile cell-adhesive proteins. *ACS Biomater. Sci. Eng.* **8**, 208–217 (2022).
117. DeForest, C. A. & Anseth, K. S. Photoreversible patterning of biomolecules within click-based hydrogels. *Angew. Chem. Int. Ed.* **51**, 1816–1819 (2012).
118. Wiley, K. L., Sutherland, B. P., Ogunnaike, B. A. & Kloxin, A. M. Rational design of hydrogel networks with dynamic mechanical properties to mimic matrix remodeling. *Adv. Healthc. Mater.* **11**, 2101947 (2022).
119. Lu, P. et al. Wavelength-selective light-matter interactions in polymer science. *Matter* **4**, 2172–2229 (2021).
120. Zhao, F., Bonasera, A., Nöchel, U., Behl, M. & Bléger, D. Reversible modulation of elasticity in fluoroazobenzene-containing hydrogels using green and blue light. *Macromol. Rapid Commun.* **39**, 1700527 (2018).
121. Konrad, D. B. et al. Computational design and synthesis of a deeply red-shifted and bistable azobenzene. *J. Am. Chem. Soc.* **142**, 6538–6547 (2020).
122. Zhao, Y.-L. & Stoddart, J. F. Azobenzene-based light-responsive hydrogel system. *Langmuir* **25**, 8442–8446 (2009).
123. Kuenstler, A. S. et al. Reconfiguring Gaussian curvature of hydrogel sheets with photoswitchable host–guest interactions. *ACS Macro Lett.* **9**, 1172–1177 (2020).
124. Liubimtsev, N., Zagradska-Paromova, Z., Appelhans, D., Gaitzsch, J. & Voit, B. Photoresponsive double cross-linked supramolecular hydrogels based on a-cyclodextrin/azobenzene host–guest complex. *Macromol. Chem. Phys.* **224**, 2200372 (2023).
125. Accardo, J. V. & Kalow, J. A. Reversibly tuning hydrogel stiffness through photocontrolled dynamic covalent crosslinks. *Chem. Sci.* **9**, 5987–5993 (2018).
126. Accardo, J. V., McClure, E. R., Mosquera, M. A. & Kalow, J. A. Using visible light to tune boronic acid–ester equilibria. *J. Am. Chem. Soc.* **142**, 19969–19979 (2020).
127. Ludwanowski, S. et al. Wavelength-gated adaptation of hydrogel properties via photo-dynamic multivalency in associative star polymers. *Angew. Chem. Int. Ed.* **60**, 4358–4367 (2021).
128. Brown, T. E., Marozas, I. A. & Anseth, K. S. Amplified photodegradation of cell-laden hydrogels via an addition–fragmentation chain transfer reaction. *Adv. Mater.* **29**, 1605001 (2017).
129. Gandavarapu, N. R., Azagarsamy, M. A. & Anseth, K. S. Photo-click living strategy for controlled, reversible exchange of biochemical ligands. *Adv. Mater.* **26**, 2521–2526 (2014).
130. Grim, J. C. et al. A reversible and repeatable thiol–ene bioconjugation for dynamic patterning of signaling proteins in hydrogels. *ACS Cent. Sci.* **4**, 909–916 (2018).
131. Yavitt, F. M. et al. In situ modulation of intestinal organoid epithelial curvature through photoinduced viscoelasticity directs crypt morphogenesis. *Sci. Adv.* **9**, eadd5668 (2023).
132. Hushka, E. A., Yavitt, F. M., Brown, T. E., Dempsey, P. J. & Anseth, K. S. Relaxation of extracellular matrix forces directs crypt formation and architecture in intestinal organoids. *Adv. Healthc. Mater.* **9**, 1901214 (2020).
133. Killaars, A. R. et al. Extended exposure to stiff microenvironments leads to persistent chromatin remodeling in human mesenchymal stem cells. *Adv. Sci.* **6**, 1801483 (2019).
134. Yavitt, F. M. et al. The effect of thiol structure on allyl sulfide photodegradable hydrogels and their application as a degradable scaffold for organoid passaging. *Adv. Mater.* **32**, 1905366 (2020).
135. Wang, H. et al. LOVTRAP: an optogenetic system for photoinduced protein dissociation. *Nat. Methods* **13**, 755–758 (2016).
136. Duan, T., Bian, Q. & Li, H. Light-responsive dynamic protein hydrogels based on LOVTRAP. *Langmuir* **37**, 10214–10222 (2021).
137. Duan, T., Bian, Q. & Li, H. Protein hydrogels with reversibly patterned multidimensional fluorescent images for information storage. *Biomacromolecules* **23**, 3009–3016 (2022).
138. Hammer, J. A., Ruta, A. & West, J. L. Using tools from optogenetics to create light-responsive biomaterials: LOVTRAP-PEG hydrogels for dynamic peptide immobilization. *Ann. Biomed. Eng.* **48**, 1885–1894 (2020).
139. Liu, L. et al. Cyclic stiffness modulation of cell-laden protein–polymer hydrogels in response to user-specified stimuli including light. *Adv. Biosyst.* **2**, 1800240 (2018).
140. Hopkins, E. et al. An optogenetic platform to dynamically control the stiffness of collagen hydrogels. *ACS Biomater. Sci. Eng.* **7**, 408–414 (2021).
141. Hörner, M. et al. Phytochrome-based extracellular matrix with reversibly tunable mechanical properties. *Adv. Mater.* **31**, 1806727 (2019).
142. Emig, R. et al. Benchmarking of Cph1 mutants and *Dr*BphP for light-responsive phytochrome-based hydrogels with reversibly adjustable mechanical properties. *Adv. Biol.* **6**, 2000337 (2022).
143. Wu, X. et al. Reversible hydrogels with tunable mechanical properties for optically controlling cell migration. *Nano Res.* **11**, 5556–5565 (2018).
144. Lyu, S. et al. Optically controlled reversible protein hydrogels based on photoswitchable fluorescent protein Dronpa. *Chem. Commun.* **53**, 13375–13378 (2017).
145. Zandrini, T., Florczak, S., Levato, R. & Ovsianikov, A. Breaking the resolution limits of 3D bioprinting: future opportunities and present challenges. *Trends Biotechnol.* **41**, 604–614 (2023).
146. Levato, R. et al. Light-based vat-polymerization bioprinting. *Nat. Rev. Methods Primers* **3**, 47 (2023).
147. Li, W. et al. Stereolithography apparatus and digital light processing-based 3D bioprinting for tissue fabrication. *iScience* **26**, 106039 (2023).
148. Ma, X. et al. Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting. *Proc. Natl Acad. Sci. USA* **113**, 2206–2211 (2016).
149. Dhariwala, B., Hunt, E. & Boland, T. Rapid prototyping of tissue-engineering constructs, using photopolymerizable hydrogels and stereolithography. *Tissue Eng.* **10**, 1316–1322 (2004).
150. Lu, Y., Mapili, G., Suhali, G., Chen, S. & Roy, K. A digital micro-mirror device-based system for the microfabrication of complex, spatially patterned tissue engineering scaffolds. *J. Biomed. Mater. Res. A* **77A**, 396–405 (2006).
151. Wang, Z. et al. A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks. *Biofabrication* **7**, 045009 (2015).
152. Grigoryan, B. et al. Multivascular networks and functional intravascular topologies within biocompatible hydrogels. *Science* **364**, 458–464 (2019).
153. You, S. et al. High cell density and high-resolution 3D bioprinting for fabricating vascularized tissues. *Sci. Adv.* **9**, eade7923 (2023).
154. Duong, V. T. & Lin, C. Digital light processing 3D bioprinting of gelatin-norbornene hydrogel for enhanced vascularization. *Macromol. Biosci.* **23**, 2300213 (2023).
This article reports DLP with norbornene chemistry to generate vascularized constructs and demonstrate secondary functionalization.
155. Raman, R. et al. High-resolution projection microstereolithography for patterning of neovasculature. *Adv. Healthc. Mater.* **5**, 610–619 (2016).
156. Wang, M. et al. Digital light processing based bioprinting with composable gradients. *Adv. Mater.* **34**, 2107038 (2022).
157. Grigoryan, B. et al. Development, characterization, and applications of multi-material stereolithography bioprinting. *Sci. Rep.* **11**, 3171 (2021).
158. Kunwar, P. et al. Meniscus-enabled projection stereolithography (MAPS). Preprint at *bioRxiv* https://doi.org/10.1101/2023.06.12.544584 (2023).
159. Peng, X. et al. Integrating digital light processing with direct ink writing for hybrid 3D printing of functional structures and devices. *Addit. Manuf.* **40**, 101911 (2021).
160. Hahn, V. et al. Light-sheet 3D microprinting via two-colour two-step absorption. *Nat. Photonics* **16**, 784–791 (2022).
161. Regehly, M. et al. Xolography for linear volumetric 3D printing. *Nature* **588**, 620–624 (2020).
162. Shusteff, M. et al. One-step volumetric additive manufacturing of complex polymer structures. *Sci. Adv.* **3**, eaao5496 (2017).
163. Kelly, B. E. et al. Volumetric additive manufacturing via tomographic reconstruction. *Science* **363**, 1075–1079 (2019).
164. Bernal, P. N. et al. Volumetric bioprinting of complex living-tissue constructs within seconds. *Adv. Mater.* **31**, 1904209 (2019).
This article is the first demonstration of volumetric printing for cellularized constructs using a GelMA bioink.
165. Ribezzi, D. et al. Shaping synthetic multicellular and complex multimaterial tissues via embedded extrusion-volumetric printing of microgels. *Adv. Mater.* **35**, 2301673 (2023).
166. Bernal, P. N. et al. Volumetric bioprinting of organoids and optically tuned hydrogels to build liver-like metabolic biofactories. *Adv. Mater.* **34**, 2110054 (2022).
167. Falandt, M. et al. Spatial-selective volumetric 4D printing and single-photon grafting of biomolecules within centimeter-scale hydrogels via tomographic manufacturing. *Adv. Mater. Technol.* **8**, 2300026 (2023).
168. Chansoria, P. et al. Synergizing algorithmic design, photoclick chemistry and multi-material volumetric printing for accelerating complex shape engineering. *Adv. Sci.* **10**, 2300912 (2023).
169. Rizzo, R., Ruetsche, D., Liu, H. & Zenobi-Wong, M. Optimized photoclick (bio)resins for fast volumetric bioprinting. *Adv. Mater.* **33**, 2102900 (2021).

170. Hahn, M. S., Miller, J. S. & West, J. L. Three-dimensional biochemical and biomechanical patterning of hydrogels for guiding cell behavior. *Adv. Mater.* **18**, 2679–2684 (2006).
171. Ovsianikov, A. et al. Laser printing of cells into 3D scaffolds. *Biofabrication* **2**, 014104 (2010).
172. Gao, L. et al. Myocardial tissue engineering with cells derived from human-induced pluripotent stem cells and a native-like, high-resolution, 3-dimensionally printed scaffold. *Circ. Res.* **120**, 1318–1325 (2017).
173. Richter, B. et al. Guiding cell attachment in 3D microscaffolds selectively functionalized with two distinct adhesion proteins. *Adv. Mater.* **29**, 1604342 (2017).
174. Dobos, A. et al. Thiol–gelatin–norbornene bioink for laser-based high-definition bioprinting. *Adv. Healthc. Mater.* **9**, 1900752 (2020).
175. Dobos, A. et al. On-chip high-definition bioprinting of microvascular structures. *Biofabrication* **13**, 015016 (2021).
176. Tromayer, M. et al. A biocompatible diazosulfonate initiator for direct encapsulation of human stem cells via two-photon polymerization. *Polym. Chem.* **9**, 3108–3117 (2018).
This study reports the first cytocompatible two-photon initiator based on a diazosulfonate (4,4′-(1,2-ethenediyl)bis[2-(3-sulfophenyl)diazenesulfonate), allowing for improved cell viability during multiphoton biofabrication.
177. Urciuolo, A. et al. Hydrogel-in-hydrogel live bioprinting for guidance and control of organoids and organotypic cultures. *Nat. Commun.* **14**, 3128 (2023).
178. Urciuolo, A. et al. Intravital three-dimensional bioprinting. *Nat. Biomed. Eng.* **4**, 901–915 (2020).
179. Chen, Y. et al. Noninvasive in vivo 3D bioprinting. *Sci. Adv.* **6**, eaba7406 (2020).
180. Pradhan, S., Keller, K. A., Sperduto, J. L. & Slater, J. H. Fundamentals of laser-based hydrogel degradation and applications in cell and tissue engineering. *Adv. Healthc. Mater.* **6**, 1700681 (2017).
181. Sarig-Nadir, O., Livnat, N., Zajdman, R., Shoham, S. & Seliktar, D. Laser photoablation of guidance microchannels into hydrogels directs cell growth in three dimensions. *Biophys. J.* **96**, 4743–4752 (2009).
182. Arakawa, C. et al. Biophysical and biomolecular interactions of malaria-infected erythrocytes in engineered human capillaries. *Sci. Adv.* **6**, eaay7243 (2020).
183. Rayner, S. G. et al. Multiphoton-guided creation of complex organ-specific microvasculature. *Adv. Healthc. Mater.* **10**, 2100031 (2021).
184. Gjorevski, N. et al. Tissue geometry drives deterministic organoid patterning. *Science* **375**, eaaw9021 (2022).
This study develops an approach for reproducibly patterning intestinal organoid geometry with high precision using oNB chemistry.
185. DeForest, C. A., Polizzotti, B. D. & Anseth, K. S. Sequential click reactions for synthesizing and patterning three-dimensional cell microenvironments. *Nat. Mater.* **8**, 659–664 (2009).
186. Munoz-Robles, B. G., Kopyeva, I. & DeForest, C. A. Surface patterning of hydrogel biomaterials to probe and direct cell–matrix interactions. *Adv. Mater. Interfaces* **7**, 2001198 (2020).
187. Humphrey, J. D., Dufresne, E. R. & Schwartz, M. A. Mechanotransduction and extracellular matrix homeostasis. *Nat. Rev. Mol. Cell Biol.* **15**, 802–812 (2014).
188. Lampi, M. C. & Reinhart-King, C. A. Targeting extracellular matrix stiffness to attenuate disease: from molecular mechanisms to clinical trials. *Sci. Transl Med.* **10**, eaao0475 (2018).
189. Mabry, K. M., Lawrence, R. L. & Anseth, K. S. Dynamic stiffening of poly(ethylene glycol)-based hydrogels to direct valvular interstitial cell phenotype in a three-dimensional environment. *Biomaterials* **49**, 47–56 (2015).
190. Yeh, Y.-C. et al. Mechanically dynamic PDMS substrates to investigate changing cell environments. *Biomaterials* **145**, 23–32 (2017).
191. Killaars, A. R., Walker, C. J. & Anseth, K. S. Nuclear mechanosensing controls MSC osteogenic potential through HDAC epigenetic remodeling. *Proc. Natl Acad. Sci. USA* **117**, 21258–21266 (2020).
192. Yang, C., Tibbitt, M. W., Basta, L. & Anseth, K. S. Mechanical memory and dosing influence stem cell fate. *Nat. Mater.* **13**, 645–652 (2014).
This article demonstrates for the first time that mesenchymal stem cells possess mechanical memory based on duration spent on a stiff substrate via the YAP–TAZ and RUNX2 signalling pathways using photodegradable substrates.
193. Walker, C. J. et al. Nuclear mechanosensing drives chromatin remodelling in persistently activated fibroblasts. *Nat. Biomed. Eng.* **5**, 1485–1499 (2021).
194. Lee, I.-N. et al. Photoresponsive hydrogels with photoswitchable mechanical properties allow time-resolved analysis of cellular responses to matrix stiffening. *ACS Appl. Mater. Interfaces* **10**, 7765–7776 (2018).
195. Homma, K. et al. Design of azobenzene-bearing hydrogel with photoswitchable mechanics driven by photo-induced phase transition for in vitro disease modeling. *Acta Biomater.* **132**, 103–113 (2021).
196. Yavitt, F. M., Kirkpatrick, B. E., Blatchley, M. R. & Anseth, K. S. 4D materials with photoadaptable properties instruct and enhance intestinal organoid development. *ACS Biomater. Sci. Eng.* **8**, 4634–4638 (2022).
197. Marozas, I. A., Cooper-White, J. J. & Anseth, K. S. Photo-induced viscoelasticity in cytocompatible hydrogel substrates. *New J. Phys.* **21**, 045004 (2019).
198. Carberry, B. J., Rao, V. V. & Anseth, K. S. Phototunable viscoelasticity in hydrogels through thioester exchange. *Ann. Biomed. Eng.* **48**, 2053–2063 (2020).
199. Luo, Y. & Shoichet, M. S. A photolabile hydrogel for guided three-dimensional cell growth and migration. *Nat. Mater.* **3**, 249–253 (2004).
200. Aizawa, Y., Wylie, R. & Shoichet, M. Endothelial cell guidance in 3D patterned scaffolds. *Adv. Mater.* **22**, 4831–4835 (2010).
201. Wade, R. J., Bassin, E. J., Gramlich, W. M. & Burdick, J. A. Nanofibrous hydrogels with spatially patterned biochemical signals to control cell behavior. *Adv. Mater.* **27**, 1356–1362 (2015).
202. Lee, T. T. et al. Light-triggered in vivo activation of adhesive peptides regulates cell adhesion, inflammation and vascularization of biomaterials. *Nat. Mater.* **14**, 352–360 (2015).
203. Farrukh, A., Paez, J. I. & del Campo, A. 4D biomaterials for light-guided angiogenesis. *Adv. Funct. Mater.* **29**, 1807734 (2019).
204. Nair, R. V., Farrukh, A. & del Campo, A. Light-regulated angiogenesis via a phototriggerable VEGF peptidomimetic. *Adv. Healthc. Mater.* **10**, 2100488 (2021).
205. Boekhoven, J., Rubert Pérez, C. M., Sur, S., Worthy, A. & Stupp, S. I. Dynamic display of bioactivity through host–guest chemistry. *Angew. Chem. Int. Ed.* **52**, 12077–12080 (2013).
206. Ricken, J., Medda, R. & Wegner, S. V. Photo-ECM: a blue light photoswitchable synthetic extracellular matrix protein for reversible control over cell–matrix adhesion. *Adv. Biosyst.* **3**, 1800302 (2019).
207. Ma, D. et al. Photoresponsive smart hydrogel microsphere via host-guest interaction for 3D cell culture. *Colloids Surf. A Physicochem. Eng. Asp.* **522**, 97–104 (2017).
208. Kim, Y. et al. Photoswitchable microgels for dynamic macrophage modulation. *Adv. Mater.* **34**, 2205498 (2022).
209. Shadish, J. A. & DeForest, C. A. Site-selective protein modification: from functionalized proteins to functional biomaterials. *Matter* **2**, 50–77 (2020).
210. Broguiere, N. et al. Morphogenesis guided by 3D patterning of growth factors in biological matrices. *Adv. Mater.* **32**, 1908299 (2020).
211. Ma, H., Caldwell, A. S., Azagarsamy, M. A., Gonzalez Rodriguez, A. & Anseth, K. S. Bioorthogonal click chemistries enable simultaneous spatial patterning of multiple proteins to probe synergistic protein effects on fibroblast function. *Biomaterials* **255**, 120205 (2020).
212. Jeon, O., Lee, K. & Alsberg, E. Spatial micropatterning of growth factors in 3D hydrogels for location-specific regulation of cellular behaviors. *Small* **14**, e1800579 (2018).
213. Fisher, S. A. et al. Photo-immobilized EGF chemical gradients differentially impact breast cancer cell invasion and drug response in defined 3D hydrogels. *Biomaterials* **178**, 751–766 (2018).
214. Ming, Z. et al. Photogenerated aldehydes for protein patterns on hydrogels and guidance of cell behavior. *Adv. Funct. Mater.* **28**, 1706918 (2018).
215. Rizwan, M. et al. Photochemically activated Notch signaling hydrogel preferentially differentiates human derived hepatoblasts to cholangiocytes. *Adv. Funct. Mater.* **31**, 2006116 (2021).
216. Lu, Y. H. et al. Temporally controlled photouncaged epidermal growth factor influences cell fate in hydrogels. *ACS Biomater. Sci. Eng.* **8**, 185–195 (2022).
217. Sankaran, S., Zhao, S., Muth, C., Paez, J. & del Campo, A. Toward light-regulated living biomaterials. *Adv. Sci.* **5**, 1800383 (2018).
218. Ravetz, B. D. et al. Photoredox catalysis using infrared light via triplet fusion upconversion. *Nature* **565**, 343–346 (2019).
219. Pearson, S., Feng, J. & del Campo, A. Lighting the path: light delivery strategies to activate photoresponsive biomaterials in vivo. *Adv. Funct. Mater.* **31**, 2105989 (2021).
220. Fast, D. E. et al. Wavelength-dependent photochemistry of oxime ester photoinitiators. *Macromolecules* **50**, 1815–1823 (2017).
221. Irshadeen, I. M. et al. Action plots in action: in-depth insights into photochemical reactivity. *J. Am. Chem. Soc.* **143**, 21113–21126 (2021).
222. Pelloth, J. L. et al. Wavelength-selective softening of hydrogel networks. *Adv. Mater.* **33**, 2102184 (2021).
This is the first example of multiplexed stepwise softening of a hydrogel with three distinct photodegradable crosslinkers.
223. Tong, X., Jiang, J., Zhu, D. & Yang, F. Hydrogels with dual gradients of mechanical and biochemical cues for deciphering cell-niche interactions. *ACS Biomater. Sci. Eng.* **2**, 845–852 (2016).
224. Rape, A. D., Zibinsky, M., Murthy, N. & Kumar, S. A synthetic hydrogel for the high-throughput study of cell–ECM interactions. *Nat. Commun.* **6**, 8129 (2015).
225. Vega, S. L. et al. Combinatorial hydrogels with biochemical gradients for screening 3D cellular microenvironments. *Nat. Commun.* **9**, 614 (2018).
226. Munoz-Robles, B. G. & DeForest, C. A. Irreversible light-activated SpyLigation mediates split-protein assembly in 4D. *Nat. Protoc.* **19**, 1015–1052 (2024).
227. Fairbanks, B. D., Schwartz, M. P., Bowman, C. N. & Anseth, K. S. Photoinitiated polymerization of PEG-diacrylate with lithium phenyl-2,4,6-trimethylbenzoylphosphinate: polymerization rate and cytocompatibility. *Biomaterials* **30**, 6702–6707 (2009).
228. Bryant, S. J., Nuttelman, C. R. & Anseth, K. S. Cytocompatibility of UV and visible light photoinitiating systems on cultured NIH/3T3 fibroblasts in vitro. *J. Biomater. Sci. Polym. Ed.* **11**, 439–457 (2000).
229. Hillel, A. T. et al. Photoactivated composite biomaterial for soft tissue restoration in rodents and in humans. *Sci. Transl Med.* **3**, 93ra67 (2011).
230. Shih, H. & Lin, C.-C. Visible-light-mediated thiol-ene hydrogelation using eosin-Y as the only photoinitiator. *Macromol. Rapid Commun.* **34**, 269–273 (2013).
231. Fancy, D. A. & Kodadek, T. Chemistry for the analysis of protein–protein interactions: rapid and efficient cross-linking triggered by long wavelength light. *Proc. Natl Acad. Sci. USA* **96**, 6020–6024 (1999).
232. Nizamoglu, M. et al. An in vitro model of fibrosis using crosslinked native extracellular matrix-derived hydrogels to modulate biomechanics without changing composition. *Acta Biomater.* **147**, 50–62 (2022).

233. Fu, L. et al. Cartilage-like protein hydrogels engineered via entanglement. *Nature* **618**, 740–747 (2023).
234. Smith, P. T. et al. Additive manufacturing of bovine serum albumin-based hydrogels and bioplastics. *Biomacromolecules* **21**, 484–492 (2020).
235. Lim, K. S. et al. New visible-light photoinitiating system for improved print fidelity in gelatin-based bioinks. *ACS Biomater. Sci. Eng.* **2**, 1752–1762 (2016).
236. Soliman, B. G. et al. Stepwise control of crosslinking in a one-pot system for bioprinting of low-density bioinks. *Adv. Healthc. Mater.* **9**, 1901544 (2020).
237. Li, Z. et al. A straightforward synthesis and structure–activity relationship of highly efficient initiators for two-photon polymerization. *Macromolecules* **46**, 352–361 (2013).
238. Ovsianikov, A. et al. Laser photofabrication of cell-containing hydrogel constructs. *Langmuir* **30**, 3787–3794 (2014).

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