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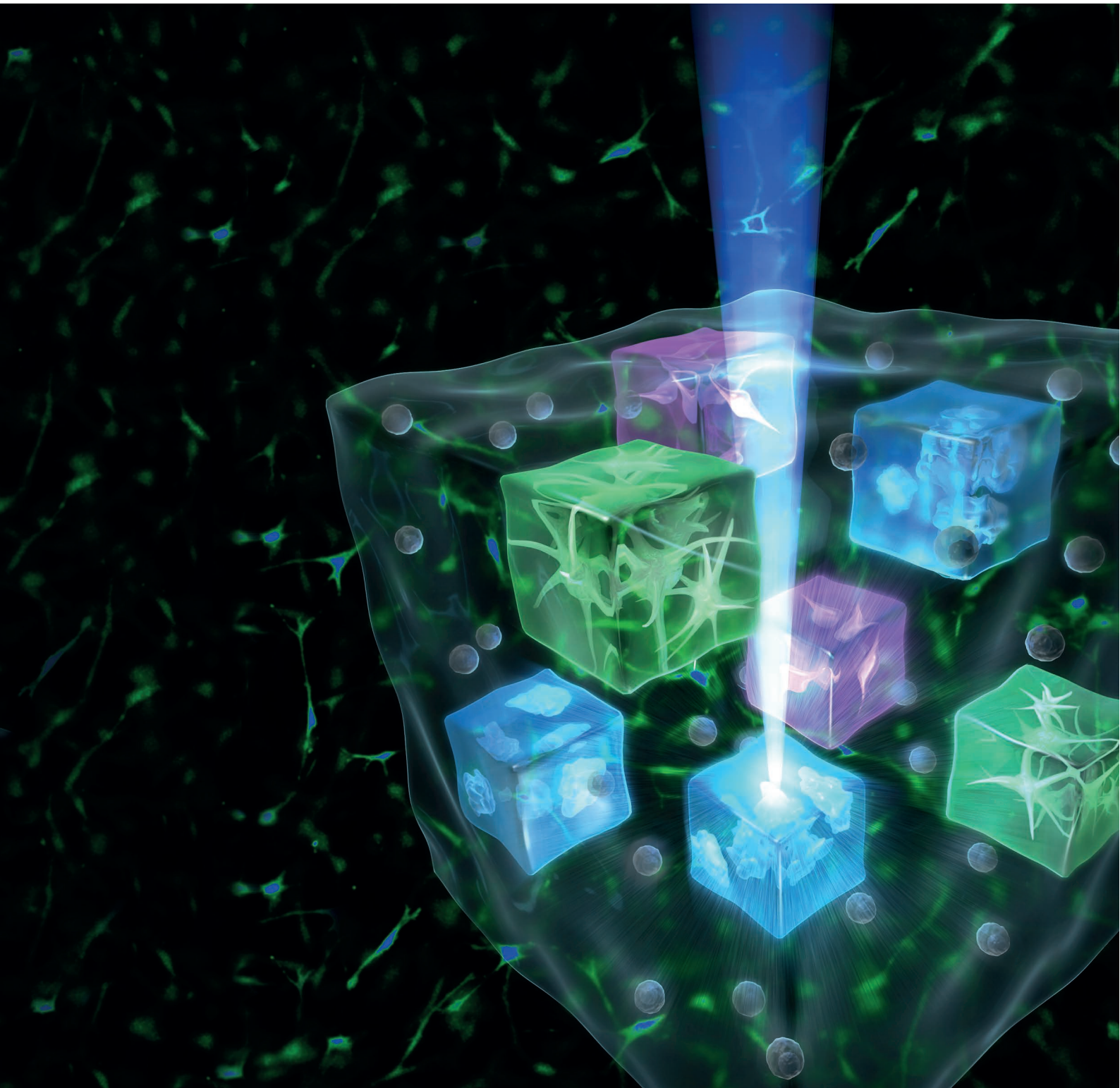
MATERIALS



Photoresponsive biomaterials for targeted drug delivery and 4D cell culture

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Abstract | Biological signalling is regulated through a complex and tightly choreographed interplay between cells and their extracellular matrix. The spatiotemporal control of these interactions is essential for tissue function, and disruptions to this dialogue often result in aberrant cell fate and disease. When disturbances are well understood, correct biological function can be restored through the precise introduction of therapeutics. Moreover, model systems with modifiable physiochemical properties are needed to probe the effects of therapeutic molecules and to investigate cell–matrix interactions. Photoresponsive biomaterials benefit from spatiotemporal tunability, which allows for site-specific therapeutic delivery *in vivo* and 4D modulation of synthetic cell culture platforms to mimic the dynamic heterogeneity of the human body *in vitro*. In this Review, we discuss how light can be exploited to modify different biomaterials in the context of photomediated drug delivery and phototunable cell culture platforms. We survey various photochemistries for their applicability *in vitro* and *in vivo* and for the biochemical and biophysical modification of materials. Finally, we highlight emerging tools and provide an outlook for the field of photoresponsive biomaterials.

Photochemistry plays a fundamental role in many biological processes, including photosynthesis, maintenance of circadian periodicity and sight. Visual interpretation of one's surroundings relies on a photoinduced isomerization reaction that triggers a signalling cascade responsible for vision[1]. Inspired by nature, chemists, biologists and material scientists have long sought to control biological functions through light-driven reactions[2] owing to several advantages they have over chemistries triggered by enzymes, small molecules, temperature, ultrasound or changes in pH. First, light can be directed to specific 3D locations at user-defined times (BOX 1), enabling 4D control over system dynamics. Second, functional tunability can be obtained by varying the administered light dosage. Third, independent control over different biological processes can be achieved in a wavelength-specific manner and finally, the optical tissue window permits regulation *in vivo*. Properly engineered photochemistries open opportunities to examine and alter biological processes with high precision.

Over the past several decades, the library of light-responsive synthetic reactions has vastly expanded with photochemical tools for bond formation, cleavage, isomerization and other molecular rearrangements[3–5] (TABLE 1). Reaction specifics, including excitation wavelength, conversion efficiency and chemical response, must be carefully matched to the desired application. In one of the first applications of photochemistry to control biological activity, the function of ATP was masked with a photolabile nitrobenzyl-based protective 'photocage', which could be removed with UV light to liberate the bioactive species[6]. Photocages represent covalently linked chemical moieties that can be removed through a photoreaction to reveal a biochemical functionality[7]. When incorporated into biomaterials, photochemistries give rise to a variety of photoresponsive constructs[8], which have found widespread application in both drug delivery and tissue engineering (FIG. 1).

The growing interest in smart therapeutic dosing has led to a recent surge in the development of light-based strategies for targeted drug delivery[9,10]. Many therapeutics are tainted with severe off-target effects, limited effectiveness due to short circulation times and the requirement for invasive techniques to administer or monitor their concentration within a patient[11]. In addition to mitigating many of these concerns, photochemical tools enable the therapeutic dosage to be locally controlled and precisely dictated at specific times through external, pulsed light exposure. Therefore, photochemical strategies are an appealing approach to engineer smart delivery vehicles.

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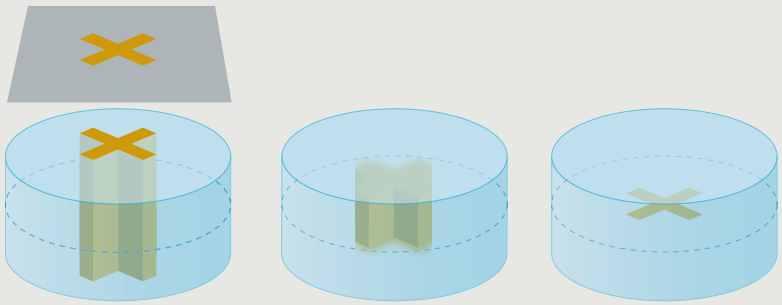
Box 1 | **Techniques for spatially controlled light exposure**

Selective exposure of phototunable biomaterials to light allows for the modification of specific volumes of a material. Directed light exposure can be obtained through lithographic techniques.

Mask-based photolithography. Substrates are exposed to light that is partially obstructed by a designed photomask inserted between the sample and the photonic source. Mask-defined 2D geometric shapes can be applied throughout the thickness of the material. Although limited by the inability to achieve 3D control, traditional photolithography is inexpensive and provides scalable patterning with an x,y-resolution on the order of tens of microns.

Single-photon laser-scanning lithography. Laser light is directed to a specific area within a material to localize photoreactions to regions near the focal point. Programmed laser rastering permits some degree of 3D patterning, although the x,y-resolution (sub-micron) exceeds the z-resolution (typically >25 μm) owing to the unavoidable reaction initiation above and below the focal plane. Despite the longer processing times associated with sample scanning, the technique employs readily accessible equipment and provides some degree of 3D patterning within photoresponsive biomaterials.

Multiphoton laser-scanning lithography. Limitations of single-photon laser-scanning lithography can be addressed through the use of a pulsed near-infrared (NIR) laser source. Near-instantaneous absorption of two low-energy photons confines photoreactions to the focal plane, which improves z-resolution (typically 2–3 μm). NIR light permits deeper sample penetration and avoids cell damage and deterioration of photosensitive molecules surrounding the region of interest. However, this technique is time consuming and relies on specialized, expensive equipment.



Mask-based photolithography | Single-photon laser-scanning lithography | Multiphoton laser-scanning lithography

The spatiotemporal tunability provided by photoreactions for therapeutic delivery has also proved invaluable in the creation of dynamic cell culture platforms. Hydrogels are a class of biomaterials that mimic many properties of native tissue and are commonly used for tissue engineering and 3D cell culture. Although gels can be cast with well-defined initial physiochemical properties, next-generation strategies seek to create biomaterials that capture the dynamic and heterogeneous characteristics of the native extracellular matrix (ECM). Light exposure can be controlled in four dimensions, and therefore, local attributes of photoresponsive biomaterials can be governed on demand and in the presence of live cells. Such dynamic platforms allow for the investigation of disease progression and tissue morphogenesis and, ultimately, the engineering of complex functional tissues.

In this Review, we highlight advances in photoresponsive biomaterial engineering for controlled drug delivery, investigating photoreaction mechanisms and applications of different photochemistries in the targeted release of bioactive therapeutics. We then examine phototunable cell culture platforms with special emphasis on the photochemical reactions that govern 4D material responsiveness, physicochemical tunability and biophysical alterations. Finally, we discuss exciting opportunities for next-generation photoresponsive systems.

Photomediated drug delivery

Controlled drug delivery ideally introduces bioactive compounds to precise locations at defined times, and therapeutic concentrations are maintained by providing protection from biodegradation and clearance *in vivo*. Drug delivery strategies should have high loading efficiency, cytocompatibility and on-demand, dose-controlled release only at desired sites. Among externally controllable stimuli, such as light, magnetic fields or ultrasound, light affords near-instantaneous release with precise on and off spatiotemporal control in a minimally invasive manner. Furthermore, photochemistries are wavelength-specific, offering the opportunity to exploit multiple reactions for on-demand delivery of different therapeutics from a single system. Reactions are being developed that are active in the near-infrared (NIR) range, because they are less damaging to tissue and afford increased penetration depth[12]. Photoresponsive drug delivery vehicles can be categorized on the basis of their underlying photochemical mechanism: an explicit molecular cleavage event, isomerization or rearrangement; or whether light is used in conjunction with additional species to initiate thermal or free-radical processes.

Bond photolysis

Photoscissable covalent bonds can be cleaved with a sufficient dose of wavelength-matched irradiation, forcing molecular dissociation. This photolabile property offers a robust approach to release compounds of interest at a target location through directed light exposure, which is particularly useful for drug delivery. Photosensitive drug delivery vehicles most commonly use nitrobenzyl and coumarin derivatives because of their synthetic tractability, favourable absorbance properties and photokinetics. They can be applied either as a cleavable linker between a therapeutic and a stable delivery vehicle, within the structural support of a photodegradable carrier or as a photocage to inhibit biological activity (FIG. 2a).

Nitrobenzyl derivatives. Nitrobenzyl chemistry was first applied in a biological setting to demonstrate the photoliberation of nitrobenzyl-caged ATP to serve as a substrate for an ATPase upon release[6]. Building on this foundational work, nitrobenzyl derivatives have been extensively employed to release small-molecule therapeutics[13–15], proteins[16,17] and oligonucleotides[18–22] from various materials.

Release profiles are largely dependent on the chemical linkage between the therapeutic and the photoactive nitrobenzyl group. Through the incorporation of a nitrobenzyl ester crosslink in a polyethylene glycol (PEG)-2-aminoethyl methacrylate hydrogel containing small interfering RNA (siRNA), degradation and

Table 1 | **Photochemistries for the control of biomaterial function**

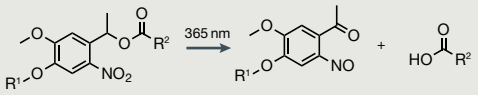
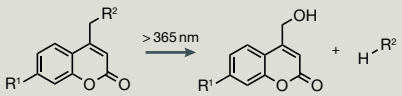
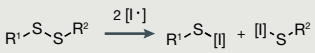
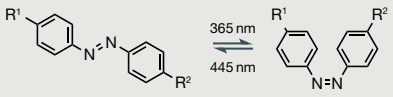
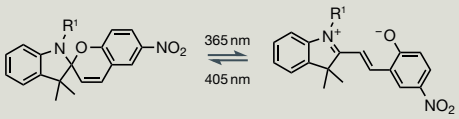
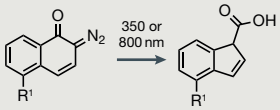
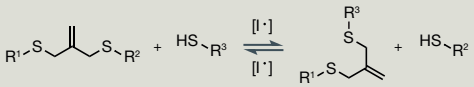
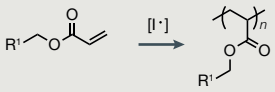
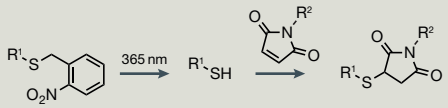
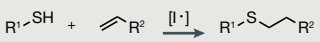
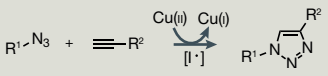
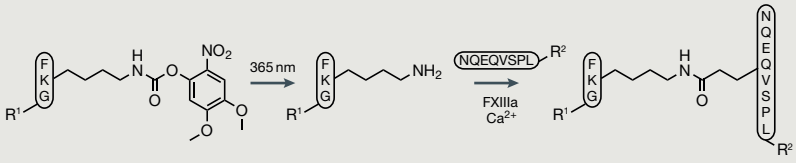
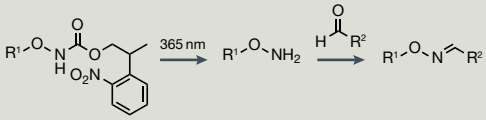
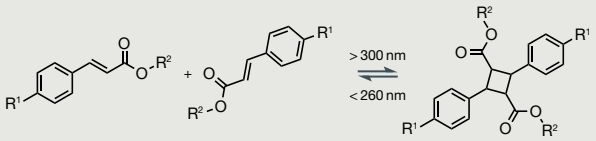
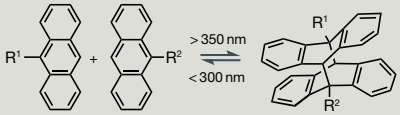
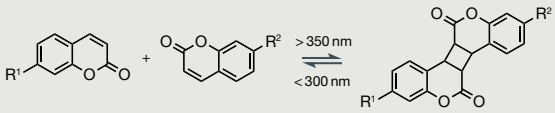
Functional group or reaction	Representative mechanism	Relative rate*	Relative use‡	
			Drug delivery	4D cell culture
Cleavage				
Nitrobenzyl		++	✓✓✓	✓✓✓
Coumarin		+++	✓✓	✓✓
Disulfide		++	–	✓
Isomerization				
Azobenzene		++	✓✓	✓
Spiropyran or merocyanine		++	✓	✓
Rearrangement				
DNQ		++	✓	–
RAFT		+++	–	✓
Addition				
Acrylate		+++	–	✓✓✓
Photocaged Michael addition		++	–	✓✓
Thiol-ene		+++	–	✓✓
CuAAC		+	–	✓
FXIIIa catalysed		+	–	✓
Oxime ligation		++	–	✓

Table 1 (cont.) | **Photochemistries for the control of biomaterial function**

Functional group or reaction	Representative mechanism	Relative rate*	Relative use‡	
			Drug delivery	4D cell culture
Dimerization				
Cinnamate		+	✓	✓
Anthracene		++	✓	✓
Coumarin		++	✓	–

CuAAC, copper-catalysed azide–alkyne cycloaddition; DNQ, 2-diazo-1,2-napthoquinone; FXIIIa, coagulation factor XIII A chain; RAFT, reversible addition–fragmentation chain-transfer. *The relative reaction rate is indicated with plus signs (+). ‡The relative usage of each reaction for drug delivery and 4D cell culture is indicated with check marks.

release of the material were induced upon exposure to UV light[21]. Although nitrobenzyl photolysis enables controlled delivery through light exposure, the lack of long-term stability associated with gradual ester hydrolysis is a potential limitation. In order to minimize hydrolytic degradation upon intradermal injection, therapeutic cargos can be linked through a nitrobenzyl ester derivative to insoluble polymeric microbeads. In this manner, human insulin has been remotely released from polystyrene matrices through short external exposures at 365 nm to regulate blood levels *in vivo*[23]. Replacing the ester with hydrolytically stable linkages (such as amides or carbonate) offers a method to decrease nonspecific release[24]. For example, a nitrobenzyl carbonate linker between a box-like DNA nanostructure and the therapeutic cargo has been developed to release small molecules and large proteins[17]. Nitrobenzyl photocleavage at 302 nm releases biomolecules from the nanostructure, eliciting a biological response within milliseconds. This approach allows for the release of a molecule with intact biological activity. However, the toxicity of the short wavelengths and the limited depth of tissue penetration of the light limit the *in vivo* applicability of this system. Alternatively, hydrophobic therapeutic release under UV irradiation can be achieved using 4,5-dimethoxy-2-nitrobenzyl-containing poly(lactic-co-glycolic acid) nanoparticles[25]. The carbamate linkage employed here increases particle stability and facilitates the controlled release of small-molecule therapeutics 10 weeks after injection in a rat model of choroidal neovascularization[26].

Substantial efforts have been dedicated to improving the photocleavage kinetics and redshifting compound absorbances to increase species cytocompatibility and expand the application of nitrobenzyl-based photochemistry in living systems[27]. Substituent effects can be used to increase nitrobenzyl photocleavage rates at higher wavelengths ($\lambda > 360$ nm)[28]. Three differently substituted nitrobenzyl-based linkers with different wavelength photoresponsiveness (365–436 nm) enable the wavelength-dependent release of three model therapeutics from a single material[29]. Another nitrobenzyl derivative, 2-(2-nitrophenyl)propyloxycarbonyl (NPPOC), can be efficiently cleaved using multiphoton NIR[30,31] and red-light[32] excitation, which makes it applicable for higher wavelength ($\lambda > 360$ nm) drug delivery techniques. Single-photon IR-initiated photocleavage has been achieved through the combination of nitrobenzyl moieties with silicon quantum dots[15] and upconverting nanoparticles[33–37]. The diverse library of nitrobenzyl linkers, which are responsive to a variety of wavelengths, allows for the development of dynamic, smart delivery vehicles for sequential therapeutic release.

Directed release of biological molecules to a specific cell type or tissue within the body by locally concentrating the delivery vehicle can reduce minimum effective therapeutic dosages, increase efficacy and eliminate off-target effects. Specific targeting can be achieved through on-site gelation of photodegradable materials[21] or by exploiting key markers and features of the local microenvironment. For example, cancer cells, which overexpress folate receptors, can be targeted by decorating the vehicle with folic acid residues[38–41]. Micelle carriers modified with folic acid and containing a nitrobenzyl-photocaged camptothecin prodrug demonstrate an improved cellular uptake; chemotherapeutic release into the cytosol is triggered by subsequent light exposure[41]. Similarly, cell-penetrating peptides, that is, amino acid sequences that mediate transport across the cell membrane, can be conjugated to drug delivery vehicles to increase intracellular delivery[42,43] or can be photocaged with nitrobenzyl-derived nitroveratryloxycarbonyls (NVOCs) to control cellular uptake[44–46].

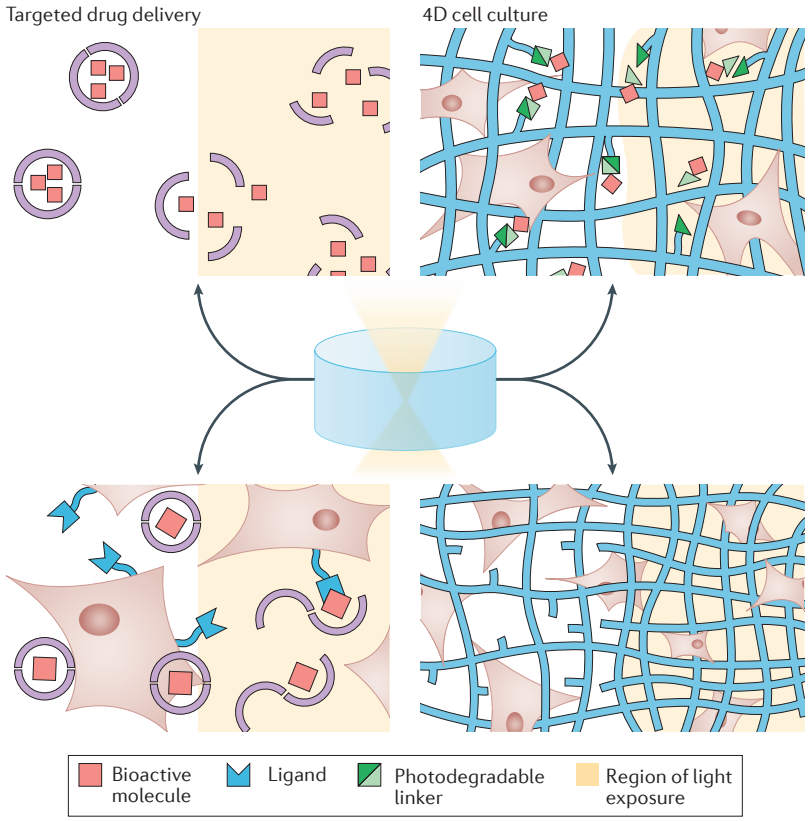


Figure 1 | **Photoresponsive biomaterials as platforms for targeted drug delivery and 4D cell culture.** Localized, on-demand therapeutic release can be controlled through selective light exposure to a photoresponsive delivery vehicle or through photomediated activation of a bioactive molecule, which becomes available for specific-ligand binding. Similarly, photoresponsive hydrogels can be modified with a photodegradable linker, which enables the spatiotemporally defined patterning of biochemical and biophysical cues to recreate the dynamic, heterogeneous cellular microenvironment.

Orthogonal chemistries have been used in conjunction with nitrobenzyl-based linkers to create systems responsive to multiple stimuli[47–51]. For example, a poly(2-hydroxyethyl methacrylate-co-methacrylic acid) microgel containing myoglobin undergoes pH-dependent swelling and de-swelling for slow release and photomediated NVOC-crosslinker cleavage for rapid delivery following vehicle photodegradation[50]. Mesoporous silica nanoparticles (MSNs) have been employed to create a triple-stimuli-responsive vehicle containing a disulfide linkage, which is cleaved under reductive conditions, pH-sensitive poly(2-(diethylamino)-ethyl methacrylate) polymer caps and an *ortho*-nitrobenzyl ester photolinker[51]. Even though these systems represent an important step towards the development of multi-stimuli-sensitive chemistries within a single device, they are limited by leakiness and UV-unresponsiveness. Ideally, delivery vehicles remain fully stable until therapeutic release is required. Hydrogels can be programmed to exhibit Boolean-logic-based degradation of material crosslinks in response to precise combinations of external stimuli for triggered drug delivery[47]. When nitrobenzyl moieties are connected in series with another degradable functionality, the cleavage of either group causes material dissolution (OR gate); when scissile moieties are connected in parallel, cleavage of both is required for degradation (AND gate). Multiple gates hierarchically combined enable complex logic-based delivery in response to light alongside other dynamic stimuli.

Coumarin compounds. Coumarin derivatives have become popular phototriggers since their early demonstration as efficient photocleavable moieties[52]. The high absorption efficiencies, fast cleavage rates, ease of redshifting their absorption profiles and affinity for multiphoton-induced reactions make coumarin derivatives favourable alternatives to nitrobenzyl-based linkers[53,54]. As such, efforts have been dedicated towards the development of new photolabile coumarin compounds, which have been applied to micelles[55], microgels[56] and MSNs[57–59], and used as simple cages[53,60] for photomediated drug delivery. Similar to nitrobenzyl-based delivery vehicles, folic acid residues[59,61,62], complementary mRNA sequences[63] and dual-caging strategies[64] have been used in conjunction with coumarin compounds to target specific cells or tissues.

Minor chemical modifications of coumarin compounds result in large absorption shifts to higher, biocompatible wavelengths without a notable reduction in the quantum yield for cleavage[7]. For example, a 7-amino coumarin compound modified with vinyl groups for radical chain polymerization undergoes photocontrolled swelling and degradation of polystyrene microgels upon exposure to light at 400–450 nm[56]. Similarly, a redshifted, synthetically tractable 7-diethylamino-4-thiocoumarinylmethyl protecting group has a peak absorption above 450 nm and minimal response to UV light, which can be exploited for the wavelength-orthogonal photoinduction of distinct phenotypes in zebrafish with UV and blue light[65]. The coumarinylmethyl backbone can be further modified to create a library of cyan-light-responsive (470–500 nm) cages[66]. These photolabile molecules offer a narrow activation window at various wavelengths and thus represent an important step towards the controlled release of different therapeutics from a common material through wavelength specificity.

In addition to their high quantum yield under single-photon excitation, coumarin moieties are highly sensitive to multiphoton absorption, providing the possibility for therapeutic delivery to deep tissue. 6-Bromo-7-hydroxycoumarin esters and carbamates, which have been used for the controlled uncaging of glutamate in brain tissue[67], require lower light intensities to elicit photocleavage and exhibit a relatively large multiphoton absorption cross section compared with unmodified coumarins. NIR-responsive, coumarin-containing block copolymer micelles have also been used for drug delivery[68,69]. Additionally, the delivery of hydrophilic payloads, such as cells and proteins, has been achieved through the NIR-based degradation of a short, water-soluble coumarin crosslinker[70]. Coumarin-based materials show great promise for *in vivo* drug delivery because of the ease of redshifting the absorption, the high quantum yield and the cytocompatibility.

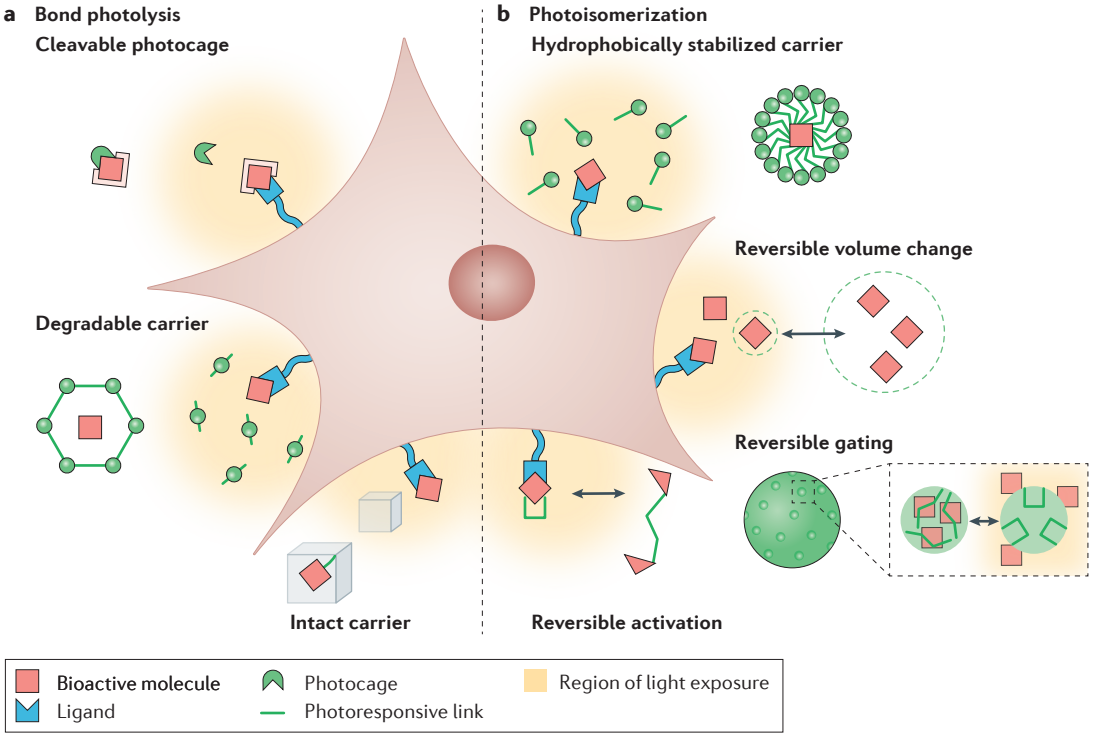


Figure 2 | **Photocontrolled delivery of bioactive molecules. a** | Bond photolysis can be used to confine therapeutic release to specific locations at specific times. Approaches have been developed that rely on the photoliberation of caged species, photodegradation of therapeutic delivery vehicles or photoscission of molecular tethers between a non-degradable carrier and a bioactive molecule. **b** | Photomediated isomerization reactions can be exploited to degrade hydrophobically stabilized delivery vehicles, to trigger changes in payload release rates through the reversible adjustment of the size of the delivery vehicle, for the reversible gating of therapeutics in stable delivery vehicles and for the reversible activation of therapeutic activity through conformational changes.

Photomediated isomerization

Photoexcitation can induce the reversible isomerization of selected organic compounds, such as azobenzenes and spiropyrans, by providing the required energy to reach a π^* state. This conversion initiates a molecular switch from one stereoisomer to the other without producing a molecular by-product. The *trans*-to-*cis* conversion of azobenzene occurs under UV light (365 nm) through a π–π^* transition; the *cis* conformation relaxes to the thermodynamically stable *trans* isomer in the dark or under visible light (445 nm)[71]. Spiropyran undergoes photoinitiated (365 nm) ring-opening isomerization to an unstable zwitterionic merocyanine. The reverse reaction rate is highly dependent on the molecular substituents but can be accelerated by exposure to visible light. These reversible isomerizations can serve as building blocks for the creation of smart drug delivery vehicles (FIG. 2b).

Azobenzene isomerization. Since the first application of an azobenzene photoisomerization for the construction of photoresponsive membrane systems[72], this photoreaction has been used to destabilize micelles and vesicles by disrupting the interactions of the amphiphilic components for burst release[73–76] and as a photoswitch in which drug efficacy is dependent on the conformation[77]. Azobenzene-carrier conjugation has further been applied to create delivery systems[78–81]; for example, vehicles that harness an impeller-type motion induced by repeated *cis*–*trans* isomerizations have been developed to increase diffusion rates out of MSNs[79]. These techniques have been used to release cholesterol[82] and to deliver DNA[83].

Great effort has also been put forth to redshift the absorbance of azobenzene-based photoswitches with the assistance of upconverting nanoparticles[84] and through *ortho*-substitution and *para*-substitution[85–87]. For example, redshifted photoswitchable control can be gained through the tetra-*ortho*-chlorination of a fatty acid azobenzene designed to mimic capsaicin to regulate a nonspecific cation channel[88]. NIR-absorbing azobenzene compounds show promise in controlling biological targets *in vivo*[89,90].

Spiropyran–merocyanine isomerization. Spiropyran photoisomerizes from a hydrophobic closed-ring structure to a hydrophilic, zwitterionic open-ring merocyanine structure. This change in polarity can be exploited for the photoinitiated disruption of hydrophobic and ionic interaction-dependent structures[91–95]. For example, spiropyran–indoline–PEG nanoparticles loaded with the chemotherapeutic docetaxel undergo reversible, UV-initiated (365 nm) shrinkage from 103 nm to 49 nm in diameter[96,97]. Treatment of subcutaneous HT-1080 tumours in nude mice with these nanoparticles using

a single light exposure leads to increased tumour penetration and increased intratumoural vessel diameters through apoptosis-induced vascular decompression, which results in decreased tumour sizes compared with those observed in treatments involving only docetaxel[97]. NIR light (980 nm) can also be used in conjunction with upconverting nanoparticles to release a chemotherapeutic from a spiropyran-containing amphiphilic polymer system *in vitro*[94]. Although only a few applications have been developed so far, reversible isomerization reactions provide the possibility to introduce cyclic on and off control of the activity of a biologic *in vivo*, a desirable trait towards inhibiting off-target activity or to achieve repetitive dosing.

Light-induced rearrangement

Photo-induced molecular rearrangement events can be exploited for therapeutic release. In response to UV light, diazocarbonyl compounds are converted to a ketene intermediate followed by a [2 + 2] cycloaddition adduct or a nucleophilic substituted product in the presence of a weakly or strongly acidic nucleophile — a mechanism referred to as Wolff rearrangement[98]. Such systems can be designed to yield photoproducts with a shift in hydrophobicity strong enough to disrupt amphiphilic systems. 2-Diazo-1,2-naphthoquinone (DNQ) rearranges to a strongly hydrophilic carboxylic acid under UV (350 nm) and multiphoton excitation (800 nm)[99]. The first IR-controlled delivery of a hydrophobic model therapeutic using DNQ was not performed in the presence of cells[100], but a DNQ-containing poly(ethylene oxide)-dendritic polyester micelle affords cytocompatible, triggered release[101]. DNQ coupled with tumour-targeting sugar residues in a dendritic nanoparticle[102] or in the backbone of a brush copolymer[103] exhibits particle degradation owing to NIR-induced shifts in hydrophobicity.

Photothermally induced delivery

The absorption properties and the resulting local heating of conductive nanomaterials have been extensively used to control drug delivery[104]. Although not directly falling into the category of photochemical reactions, such systems can be applied for indirect photomediated therapy. Conductive materials absorb incident photons of the correct wavelength and convert this energy into local heating to increase membrane permeability[105], disrupt endosomes after endocytosis[106,107] and induce phase transitions in temperature-responsive systems[108–111]. Therapeutic release has been demonstrated using light ranging from UV to NIR[112–114] from a variety of carriers, including MSNs[113,115,116], gold nanocages[117], nanorods[114,118], polymer nanoparticles[119–122], nanocrystals[123] and black phosphorous nanosheets[124]. For example, local heating from visible-light-absorbing magnetite nanoparticles dispersed in poly(*N*-isopropylacrylamide-co-vinyl-2-pyrrolidinione) hydrogel beads induces a volume change leading to the release of dexamethasone from a transdermal patch[125]. Thermally conductive nanoparticle-based systems are highly versatile; however, local heating may damage the surrounding tissue and limits the application of these systems to ones in which cell death is desired.

Photosensitization

Photosensitizers produce reactive oxygen species for photodynamic therapy[126,127], prodrug activation[128–130], liposome disruption[131] and drug release from endocytic vesicles through photochemical internalization (PCI)[132–134]. PCI-based gene delivery using a dendrimer complex increases transcription relative to basal levels by 100-fold *in vitro* and *in vivo*[135]. Alternatively, an NIR-absorbing phthalocyanine photosensitizer can be used to locally release an anaesthetic through lipid peroxidation of a phosphocholine liposome carrier[131]. This technique is limited by an initial burst release, in which the area is immediately anaesthetized for 10 h; however, controlled release can be achieved with subsequent exposures. Although photosensitization techniques do not show detectable local temperature changes[136], the produced radical species can induce vascular damage[137]. Applying this effect in a tumour microenvironment to disrupt the endothelium increases the bioavailability of a chemotherapeutic within the tumour. Harsh radical-based chemistry may be appealing to cancer treatment, but side effects and possible tissue damage must be considered for other therapeutic regimes.

Photoresponsive cell culture platforms

There is growing interest in the development of 3D cell culture substrates to investigate fundamental biology, interrogate disease physiology and engineer functional tissue. However, most 3D systems are static with defined physiochemical properties, which cannot capture the dynamics of the ECM. Therefore, tunable 4D biomaterials are being developed that recapitulate the key variable and heterogeneous aspects of native tissues[138]. Hydrogels are crosslinked polymeric networks, which are powerful platforms for 4D cell culture because their chemical and physical properties can be customized to mimic *in vivo* microenvironments. Just as photochemistry has proved beneficial for controlled drug delivery, photoresponsive hydrogels can be used to recreate the spatiotemporal variations of native tissue, including the dynamic presentation of signalling cues and moduli changes that accompany morphogenesis, disease and healing.

Biochemical alteration of biomaterials

The native cellular microenvironment is characterized by the heterogeneous presentation of biochemical cues, that is, small molecules, peptides and proteins, with varying local concentrations. Therefore, efforts have focused on the development of *in vitro* culture platforms that enable spatial and temporal control over biochemical presentation within synthetic materials. One approach is to introduce, remove or reversibly control biochemical functionality by use of photochemistry.

Photomediated introduction of biochemical functionalities into biomaterials. Photochemical addition reactions are essential for the immobilization of bioactive ligands in hydrogels. The gold standard of hydrogel

formation has long been based on the free-radical chain photopolymerization of PEG diacrylate (PEGDA). The hydrophilicity, biocompatibility and bioinertness of PEGDA provide a 'blank slate' in which cells, proteins and small molecules can be encapsulated with high fidelity. Moreover, photopolymerization can be achieved within minutes employing cytocompatible photoinititators[139]. Biochemical cues can be introduced to PEGDA by spatially controlled addition[140–142] (FIG. 3a). Not every acrylate group needs to be consumed for gelation; thus, unreacted moieties remain available for the immobilization of cues. Initially, these free moieties were labelled with an acrylate–PEG–Arg–Gly–Asp (RGD) polymer–peptide conjugate through secondary light exposure at selected volumes within the gel. Local concentrations of the immobilized biomolecule can be tuned through the in-solution precursor concentration and irradiation time[143]. Since then, PEGDA hydrogels have been photochemically modified with multiple cues[142] by both bulk biochemical gradients[144] and tissue-inspired 3D computer-generated patterns to control 3D cellular organization[145].

These techniques have been extensively used to unravel the cellular response to biochemical signals, ranging from short adhesion peptides[146] to full-length proteins[147]. For example, photopatterned hydrogels enable the formation of complex, vascularized systems to overcome diffusion limitations associated with large 3D constructs. Endothelial cells seeded on the surface of a PEGDA hydrogel functionalized with strips (≥50 μm wide) of the adhesion peptide Arg–Gly–Asp–Ser (RGDS) undergo concentration-dependent and width-dependent angiogenesis[146]. Additional patterning with vascular endothelial growth factor (VEGF) initiates the spontaneous formation of endothelial tubules in the gel[147]. The degree of polymerization of PEGDA determines the mechanical properties of the gel; therefore, crosslink formation reduces the finite number of free acrylate groups available for further biochemical functionalization. Additionally, keeping a certain amount of acrylate groups unreacted is difficult to control and characterize. This issue can be addressed by uniformly including photocaged reactive species throughout the material, which can be uncaged for biochemical anchoring. This strategy was first demonstrated in agarose-based gels modified with photocaged thiols[148–151]. Applying UV light to selected volumes of the gel triggers the uncaging of thiols that are subsequently available for Michael-type addition reactions with maleimide-functionalized biomolecules. This method allows for the selective modification of the gel in UV-exposed regions. In contrast to acrylate-based patterning, the mechanical properties of the gel remain the same, because the immobilization of biomolecules is not related to the crosslinking of the gel. This technique has been used to promote dorsal root ganglia cell invasion into RGDS-patterned gels in a concentration-dependent manner[148,152]. The photomediated Michael addition can be extended to the sequential patterning of one protein of a binding pair into a gel, for example, biotin–streptavidin[150], barnase–barstar[150] or human serum albumin–albumin binding domain[151]. Thereby, the gel can be functionalized with a protein of choice by fusing it to the respective binding partner. Although innovative, the limited cytocompatibility of the maleimide-thiol reaction resulting from cross reaction with native thiols as well as the slow reaction rates limit applications in the presence of cells.

'Click chemistry' has also been explored as a bioconjugation strategy for the development of well-defined hydrogels, which can be biochemically functionalized. Click reactions, such as thiol-ene addition or copper-catalysed azide–alkyne cycloaddition (CuAAC), enable one-to-one addition with high reaction yields and specificity[153,154]. The thiol-ene reaction can be photochemically initiated with an appropriate cytocompatible photoinitiator (for example, lithium phenyl-2,4,6-trimethylbenzoylphosphinate[139], Irgacure 2959 (REF. 155) or Eosin Y[156]), resulting in a radical-mediated step-growth reaction between a free thiol and an alkene[157]. Unlike chain reactions, step-growth polymerizations generally evolve into homogeneous networks, forming a uniform material for photopatterning after gelation. The first photoinitiated thiol-ene-based biochemical patterning was demonstrated by conjugating cysteine-containing peptides into CuAAC-based PEG networks without modification of the gel mechanics[153]. To circumvent the use of cytotoxic CuAAC, a bio-orthogonal,

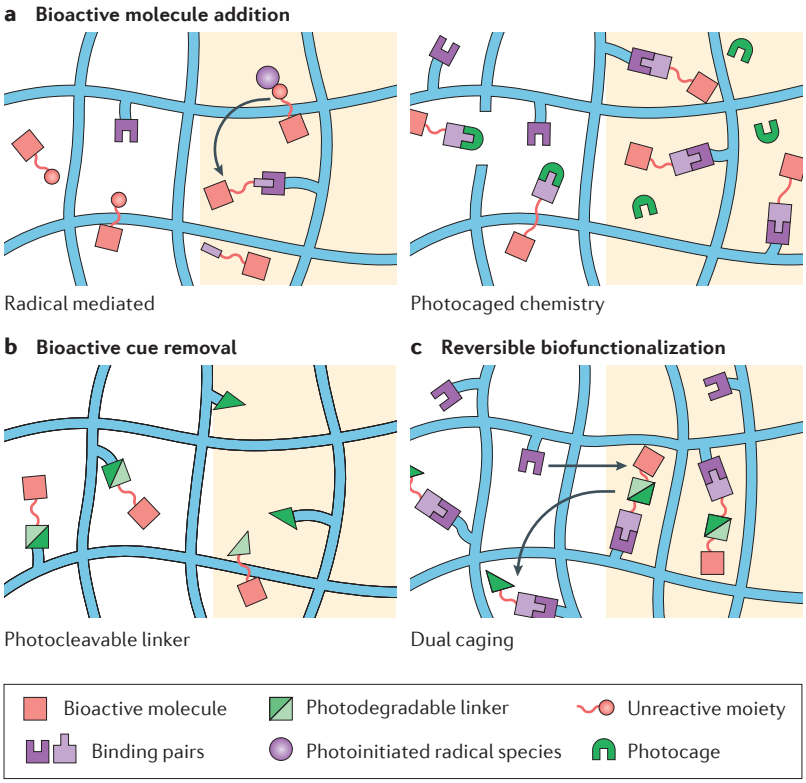


Figure 3 | **Photomediated biochemical alteration of biomaterials. a** | Hydrogels can be biochemically functionalized through photoinitiated, radical-mediated addition reactions and through the selective de-protection of photocaged reactive groups. **b** | Photocleavable linkers allow for the local removal of functional moieties upon light exposure. **c** | Dual caging involves a combination of photoaddition and photoremoval techniques to reversibly control the presentation of biochemical cues using sequential, often orthogonal, light exposures.

cytocompatible polymerization, strain-promoted azide–alkyne cycloaddition (SPAAC) can be used, which is compatible with thiol-ene-based photopatterning[158]. The degree of photopatterning can be precisely controlled by the light dosage. Furthermore, this reaction can be iteratively performed to create complex biochemical gradients and to functionalize a single hydrogel with multiple bioactive peptides[158,159]. Such materials can be used to control 3D cell spreading in gels patterned with islands of RGDS, representing true photo-directed control over 3D cell migration.

Further improvements to thiol-ene-based modification include patterning with allyloxycarbonyl (alloc)-functionalized peptides of gels formed between thiol-functionalized multi-arm PEGs and di-alloc end-functionalized peptide crosslinks[160]. Moreover, strained norbornene functionalities show improved reactivity for the immobilization of cues in PEG hydrogels[161,162]. Likewise, gel stiffness can be increased through further thiol-norbornene-based crosslinking of electrospun coil-like nanofibrous hyaluronic acid (HA) gels, which closely mimic natural ECM[163,164]. Cell protrusion and alignment can be directed by patterns of RGD on the surface of the hydrophilic networks[164].

Ideally, photochemistries retain reactivity in complex medium and in the presence of live cells. Photomodulated enzymatic reactions fulfil these requirements owing to their high selectivity towards target sequences, fast reaction rates and inherent biocompatibility; for example, coagulation factor XIII A chain (FXIIIa) catalyses an addition reaction between a free amine and a carboxamide residue of glutamine[165,166]. 6-Nitroveratryloxycarbonyl (NVOC)-photocaged, lysine-containing peptides can be covalently incorporated in a PEG-based hydrogel and, after photocleavage with laser light (405 nm), a glutamine-labelled biomolecule and the FXIIIa enzyme are swollen into the network, resulting in a stable amide linkage between the two species[165]. Such a patterning strategy can be used to direct cell invasion from human mesenchymal stem cell (hMSC) microclusters into a gel modified with RGD, recombinant fibronectin fragments (FN_{9-10}) or platelet-derived growth factor B (PDGFB). Similarly, photoinitiated, FXIIIa-catalysed immobilization can be applied to a HA-based hydrogel containing *ortho*-nitrobenzyl-photocaged, lysine-containing peptides for the *in situ* control of cell spreading and proliferation[166]. Enzyme-based patterning schemes will prove useful for the conjugation of small molecules, peptides and full-length proteins into 3D tissue constructs.

Light can also be explored for the spatial control of bioactivities through the photoactivation of biologics, which are uniformly present throughout a material. RGD, photocaged at its critical aspartic acid residue, can be activated to selectively promote cell attachment, spreading and migration[167–169]. For example, transdermal irradiation of a photoactivatable 3-(4,5-dimethoxy-2-nitrophenyl)-2-butyl ester-caged RGD moiety has been applied to regulate cell adhesion and to reduce fibrous capsule formation associated with the foreign body response following subcutaneous implantation of PEG hydrogels in mice[170], marking the first demonstration of how photopatterning can be used for the biochemical alteration of a hydrogel material *in vivo*. Photo-uncaging of synthetic peptides has also been shown to promote increased cell attachment and proliferation; however, applying this strategy for the activation of bioactive proteins that regulate more complex cellular functions has yet to be demonstrated.

Photomediated removal of biochemical functionalities from biomaterials. 3D cell culture systems can be made photoresponsive to specifically remove biochemical cues, addressing the biological importance of ligand dynamics in cellular plasticity. Such systems are used to activate or deactivate signalling pathways, to deliver soluble species to encapsulated cells or to create biochemically patterned substrates. For example, chondrogenic differentiation of MSCs is accompanied by fluctuating fibronectin production that influences differentiation signalling pathways. Fibronectin-derived RGDS peptides, which are covalently linked to PEGDA hydrogels through a nitrobenzyl-based photolabile linker, can be stochastically incorporated into the gel during polymerization[171], representing a simple and robust methodology to photorelease bioactive species from mechanically stable materials. Encapsulated MSCs interact with the bioactive peptide and remain in an undifferentiated state in the gel. After UV-initiated photocleavage, the cells exhibit increased signs of chondrogenesis owing to the removal of RGDS peptides from the microenvironment (FIG. 3b).

An individual compound can greatly influence cell function, but controlling several chemical signals within the same system better mimics *in vivo* signalling. The wavelength-selective release of multiple signals was first demonstrated through the selective and simultaneous, or independent release of three model therapeutics from a hydrogel to create multistage release profiles[29]. Two nitrobenzyl-based crosslinkers can be used for wavelength-specific, but not fully orthogonal, cell release from materials[172]. Coupling a coumarin-based photodegradable linker (405 nm) with a classic nitrobenzyl compound (365 nm) allows staggered release of bone morphogenic protein (BMP) 2 and BMP7 from a PEG-based hydrogel to investigate hMSC osteogenesis[173]. Using this approach, the relative rate of protein photorelease can be preferentially influenced by wavelength; however, full orthogonality has not been achieved. Strategies that offer true wavelength-dependent control of biomaterial properties remain elusive.

Reversible control of hydrogel functionality. Advanced biomaterial platforms afford the reversible control of biofunctionalities (FIG. 3c). The first reversible biochemical patterning of PEG-based hydrogels exploited a visible-light-initiated thiol-ene reaction to immobilize thiol-functionalized peptides. UV exposure then triggers cleavage of the nitrobenzyl linkage, resulting in the release of the peptides[174]. Although reversible immobilization has been demonstrated using both photolithographic and multiphoton laser-scanning lithography, the approach does not provide repeatable reversibility, because reactive alkenes are continuously

consumed throughout each immobilization event. Fully dynamic functionalization can be achieved by applying a reversible addition–fragmentation chain-transfer (RAFT) reaction for the iteratively repeatable immobilization of thiol-containing peptides. Using this technique, a PEG-based hydrogel can be functionalized with complex, dynamic patterns of multiple fluorescent molecules[175]. However, this approach relies on free-radical chemistry, which hinders its application for complex, sensitive molecules, such as proteins. Alternatively, a bio-orthogonal photomediated oxime ligation can be used for the immobilization and subsequent removal of full-length proteins through nitrobenzyl photocleavage[176,177]. For example, patterned tethering and release of vitronectin allow for the reversible and spatially controlled osteogenesis of hMSCs[176]. This method requires repeated patterning cycles, which are limited by the finite number of available reactive groups. Thus, strategies for the fully reversible immobilization of biomolecules remain of prime interest for recreating the complexity of the cellular microenvironment.

Biophysical alteration of biomaterials

Tissue biomechanics are crucial for tissue function, and physical forces establish an intimate relationship between cells and their microenvironment. Focal adhesions are protein complexes that connect the cell cytoskeleton to the proteins of the ECM, thereby translating biophysical signals. The stiffness and elasticity of the ECM influence cell adhesion, spreading and morphogenesis, and thus cell fate[178,179]. The mechanical properties of the cell microenvironment undergo drastic changes throughout tissue homeostasis, development, disease progression and healing[180], reflected in the dynamic interactions between cells and their ECM. 4D control of phototunable materials offers a way to recapitulate the biophysical dynamics that occur *in vivo*. The fast kinetics of photochemistry and the high resolution of exposure techniques allow for the spatial and temporal control of hydrogel elasticity and degradation.

Photomediated increase in biomaterial stiffness. In a covalently crosslinked network, the elastic modulus (G') or stiffness is dictated by the crosslink density and thus can be increased with spatiotemporal control through the formation of secondary crosslinks after gelation (FIG. 4). For example, PEGDA gels can be formed without the consumption of all acrylates; chain-growth extension of the remaining reactive groups through patterned secondary photopolymerization of additional PEGDA results in localized increases in elastic moduli from 3 to 7 kPa (REF. 140). This approach allows for the examination of stiffness-dependent biological processes within one material. Macrophages seeded on mechanically graded hydrogels ($G' \approx 5$–100 kPa) preferentially migrate towards stiffer substrate regions[181] — a process known as durotaxis. Hepatic stellate cells cultured on the surface of a photopatterned HA-based hydrogel differentiate into myofibroblasts in stiffer areas of the gel, mimicking a key event in liver fibrosis, which is characterized by matrix stiffening[182]. The formation of myofibroblasts is dependent on the size of the stiff region, and reseeding of the cells onto a 3D substrate of original stiffness initiates a reversion to quiescence. Such photomediated gel-stiffening strategies are not limited to synthetic systems; the crosslinking of artificial proteins containing a photosensitive, non-canonical amino acid, *p*-azidophenylalanine, which undergoes nonspecific photoaddition upon exposure to UV light, leads to the selective stiffening of elastin-based hydrogels[183]. Crosslink formation occurs in a dose-dependent manner such that patterning can be finely tuned across a wide range of moduli (~0.3–1.0 MPa) through variation of the exposure duration.

Photostiffening also proves valuable for examining cell–ECM interactions in nonpolarizing, native-like 3D environments. Methacrylated HA (MeHA) gels can be formed through Michael addition with bis(cysteine)-containing, enzymatically degradable peptide crosslinkers. Some acrylates can be left unreacted for the spatiotemporally controlled photo-addition of non-degradable crosslinks[184,185]. These non-degradable bonds prevent cellular matrix remodelling, thus restricting cell shape changes and differentiation. Human MSCs forced into a spherical morphology undergo adipogenesis, whereas cells permitted to spread preferentially differentiate into osteoblasts. Cell traction forces, which are established through cell-mediated matrix remodelling, are responsible for directed hMSC differentiation[186].

In addition to photo-addition reactions, the photoliberation of reactive functionalities can be used to form new crosslinks after gelation[187,188]. Using photocaged thiols, the crosslinking density of PEG-based biomaterials can be increased through a photo-driven Michael addition[188]. The exposure-dependent nature of the chemistry enables the formation of substrates with different stiffness gradients (3–8 kPa). Human MSCs seeded on top of materials with $G' \geq 5.5$ kPa exhibit durotaxis, suggesting a mechanical threshold to induce a cellular response. A photomediated oxime ligation has also been exploited for gel stiffening, enabling localized material alterations in a fully bio-orthogonal manner[177]. Alternatively, the ionic crosslinking of alginate gels can be controlled through the phototriggered release of a divalent cation, such as calcium[189,190], or a calcium chelator[190] to stiffen or soften the gels, respectively.

Photomediated decrease in biomaterial stiffness. Light can be applied to reduce the crosslink density in a hydrogel, softening the material in a time-independent, controlled manner. High-intensity focused laser light can photoablate transparent materials to create channels and voids[191,192]. Cells can sense these topographical features as demonstrated by axonal regeneration and neurite outgrowth from dorsal root ganglia into photoablated microchannels[193]. However, material photoablation applies indiscriminate bond photolysis and therefore leaves behind unpredictable degradation products. Photocleavable linkers, which dissociate in a predictable manner, offer a low-intensity and cytocompatible alternative to photoablation[171,194–199] (FIG. 4). PEGDA-based hydrogel networks containing nitrobenzyl groups formed through redox-initiated radical chain polymerization

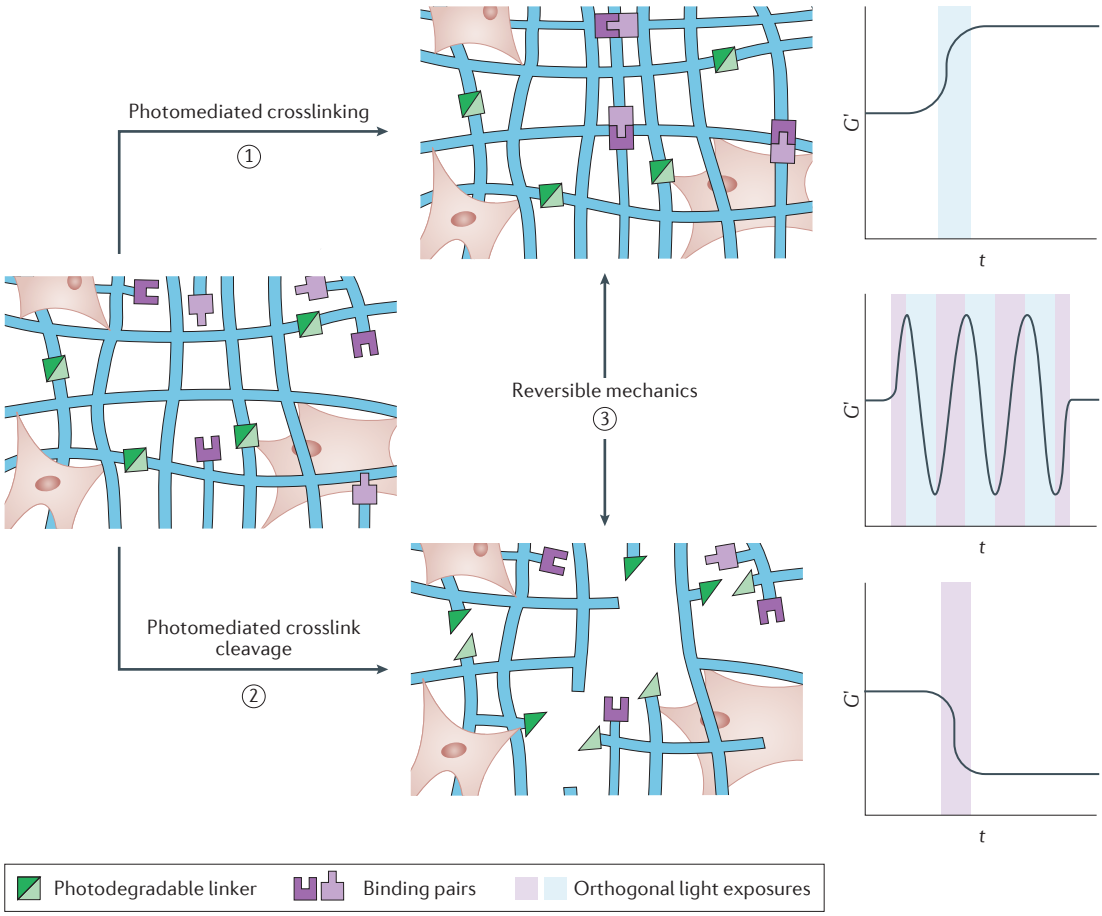


Figure 4 | **Photomediated biophysical alteration of biomaterials.** The biophysical properties of a hydrogel can be controlled through the photomediated crosslinking and cleavage of photodegradable linkers. Photomediated crosslinking can be exploited to increase the elastic modulus (*G′*) of a hydrogel at specific times (*t*) through the formation of additional crosslinks. Softening of a hydrogel can be achieved through the selective degradation of photolabile crosslinks within the gel network. Combining both strategies allows for reversible and spatiotemporal control over hydrogel mechanics, mimicking extracellular matrix dynamics *in vivo*.

degrade upon UV-light exposure[171]. These materials have been extensively used to examine how bulk changes of material properties can influence cell migration[171], how microtopographies affect cell morphology and alignment[200], how elastic gradients influence cell function[201] and how erosion of adhesive ligands induces subcellular detachment[202]. For example, linear gradients of *G′* (7–32 kPa) can be created through graded photodegradation of PEGDA hydrogels to probe the influence of biomechanics on cell phenotype[203] and to control the dedifferentiation of myofibroblasts into quiescent fibroblasts[204]. The reversion into fibroblasts occurs within 6 h of gel softening, mimicking the tissue-healing process. Such photodegradable hydrogels have also been used to investigate whether cells possess a mechanical memory of substrate conditions. By modifying the elastic properties of the substrate, it has been demonstrated that the influence of the mechanical characteristics on cell fate is dynamic and reversible[205].

Bio-orthogonal, step-growth polymerization chemistries offer a homogeneous backbone with improved mechanical integrity and rapid erosion compared with that of chain-growth polymerized gels[206]. Independent control over biochemical and biophysical properties has been achieved using photodegradable SPAAC-based networks through the inclusion of a vinyl moiety for thiol-ene photoconjugation, which is initiated by visible light[156]. In this system, physical channels can be eroded and decorated with RGD to direct fibroblast motility in three dimensions. Similarly, channels can be created through the photodegradation of nitrobenzyl-containing SPAAC networks to encourage encapsulated motor neurons derived from embryonic stem cells to form neuronal axons[207]. Fully eroded channels offer the opportunity to engineer complex cellular networks and to mimic complex biological features, for example, vasculature[208] and multicellular aggregation[209–211], by directing cell migration.

Alternative to nitrobenzyl cleavage, disulfide-crosslinked networks can be degraded through a radical-mediated disulfide fragmentation reaction[212]. In the presence of a photoinitiator, light exposure generates chemical radicals that propagate and cause multiple crosslink degradation events within seconds. This classic degradation chemistry can be applied for the rapid release of encapsulated cells from biomaterials, which

is used for harvesting specific cell populations from heterogeneous cultures *ex vivo* for a downstream analysis of single cell types[156,213,214].

Redshifted chemistries can have a unique absorption spectrum and therefore enable the precise control of multiple functionalities at the same location in a single system. For example, a thiol-ene hydrogel polymerized with visible light undergoes UV-mediated photodegradation through a nitrophenylalanine photoscission reaction, which facilitates dual-wavelength control over network mechanics[215]. Photochemistries that extend material degradation further into the visible range are also being developed; for example, coumarin-based photodegradable units can be included, which are cleaved under exposure to visible light (405 nm)[216,217].

Reversible control over physical properties. The functional control of most of the techniques discussed above is unidirectional. However, to capture the dynamic nature of cell–ECM interactions, materials with photoreversible stiffness are being engineered (FIG. 4). PEG macromers can be decorated with moieties that reversibly dimerize to sequentially stiffen or soften materials. For example, cinnamylidene acetyl moieties dimerize when exposed to UV light (>300 nm)[218–220], which can be reversed to a certain degree upon exposure to higher-energy UV light (254 nm). However, this strategy suffers from photocleavage inefficiencies, undesired side reactions and poor molecular stability, and requires extended periods (0.5–1 h) of cytotoxic UV light to modulate the material[220]. Substituting nitrocinnamate for cinnamylidene acetyl moieties increases the photoreactivity and storage stability[221]. The photodimerization of anthracene[222,223] and coumarin[224,225] has also been explored for the reversible alteration of network mechanics, although undesired photocleavage reactions limit their cyclability. Despite their ability to offer nearly reversible mechanical modulation, these systems have found only limited application in tissue engineering owing to the long exposures to cytotoxic light (<300 nm).

By contrast, azobenzene undergoes efficient photoisomerization under cytocompatible exposure conditions and can be used to disrupt host–guest interactions in a reversible, wavelength-specific manner[226]. An azobenzene moiety can act as a junction between a cyclodextrin–polymer complex, thus exhibiting cyclic gel-to-sol transitions. Azobenzene can also be incorporated within PEG gel backbones, resulting in the reversible modulation of the mechanical properties of the gel following exposure to UV (365 nm) or visible (400–500 nm) light[227]. The hydrogel stiffness decreases by ~200 Pa upon photoisomerization of *trans*-azobenzene to *cis*-azobenzene, attributed to disruption of the hydrogen bond. Reversion to a stiffer substrate is induced by exposure to visible light or thermal relaxation ($t_{1/2}$ ~9 h at 37 °C). Primary porcine aortic valvular interstitial cells can be encapsulated in such a material before either light treatments, demonstrating cytocompatibility. The development of effective strategies to reversibly and repetitively modulate changes in gel mechanics in the presence of live cells remains an ongoing effort in the field.

Independent physiochemical tunability

Biochemical and biophysical matrix cues usually act cooperatively to influence cell function. Therefore, design principles need to be developed to simultaneously and precisely control both aspects within synthetic cell culture systems (FIG. 5). So far, only a few strategies have enabled the recapitulation of the physiochemical heterogeneity of native ECM. The combination of different wavelength-orthogonal photochemistries allows for the independent control of material stiffness and biochemical cues[156]. Distinct wavelengths of light can be used to dictate local substrate mechanics and the presentation of fibronectin to regulate cell function in a MeHA hydrogel[228]. Stiffness gradients can be created by a two-step Michael addition and a visible-light-based crosslinking procedure, and biochemical patterning of a gel can be accomplished through nitrobenzyl-photocage removal, liberating a thiol for a maleimide-based reaction with biomolecules. Dual patterning constitutes a high-throughput technique to probe cell–ECM interactions; however, the cytotoxicity of thiol–maleimide chemistry prevents translation to 3D cell studies. Although each of these strategies represents an important step towards the independent control of physicochemical properties, capturing the entire physiochemical aspects of native ECM remains elusive.

Outlook

Photochemistry can be near-instantaneously controlled in four dimensions, providing a powerful tool for targeted drug delivery and advanced cell culture. Many photoresponsive biomaterial systems have already been designed that provide high precision for probing and directing living processes, and the field continues to explore photochemistry for a variety of applications, especially for the dynamic photomodulation of material properties *in vivo*[170,229,230]. Future prospects exist in optically activating reactions at deeper locations within living human tissue; currently, the limited tissue penetration of commonly utilized light sources restricts the application of photoresponsive biomaterials to transdermal patches, the eye or sites just a few millimetres below the surface of the skin. While substantial effort has been dedicated to redshifting reactive moieties, chemistries that are hyperactive within the NIR optical window of biological tissue ($\lambda = 600–1200$ nm) are needed. A critical design constraint is to create reactive components that are responsive to the low energy supplied by high-wavelength light. Additionally, the species must remain stable and photochemically inert in dark and physiological conditions. Reactants with enhanced quantum yields and tunable absorbance properties will enable biocompatible photochemistries, which can be performed fast and in deep tissue.

Another area of active development addresses the request to independently conduct several different photoreactions within the same material system. Fluorescent microscopes now permit the simultaneous visualization of four or more fluorophores within one sample and even more when spectral deconvolution is employed; however, independent photomodulation of even two biomaterial

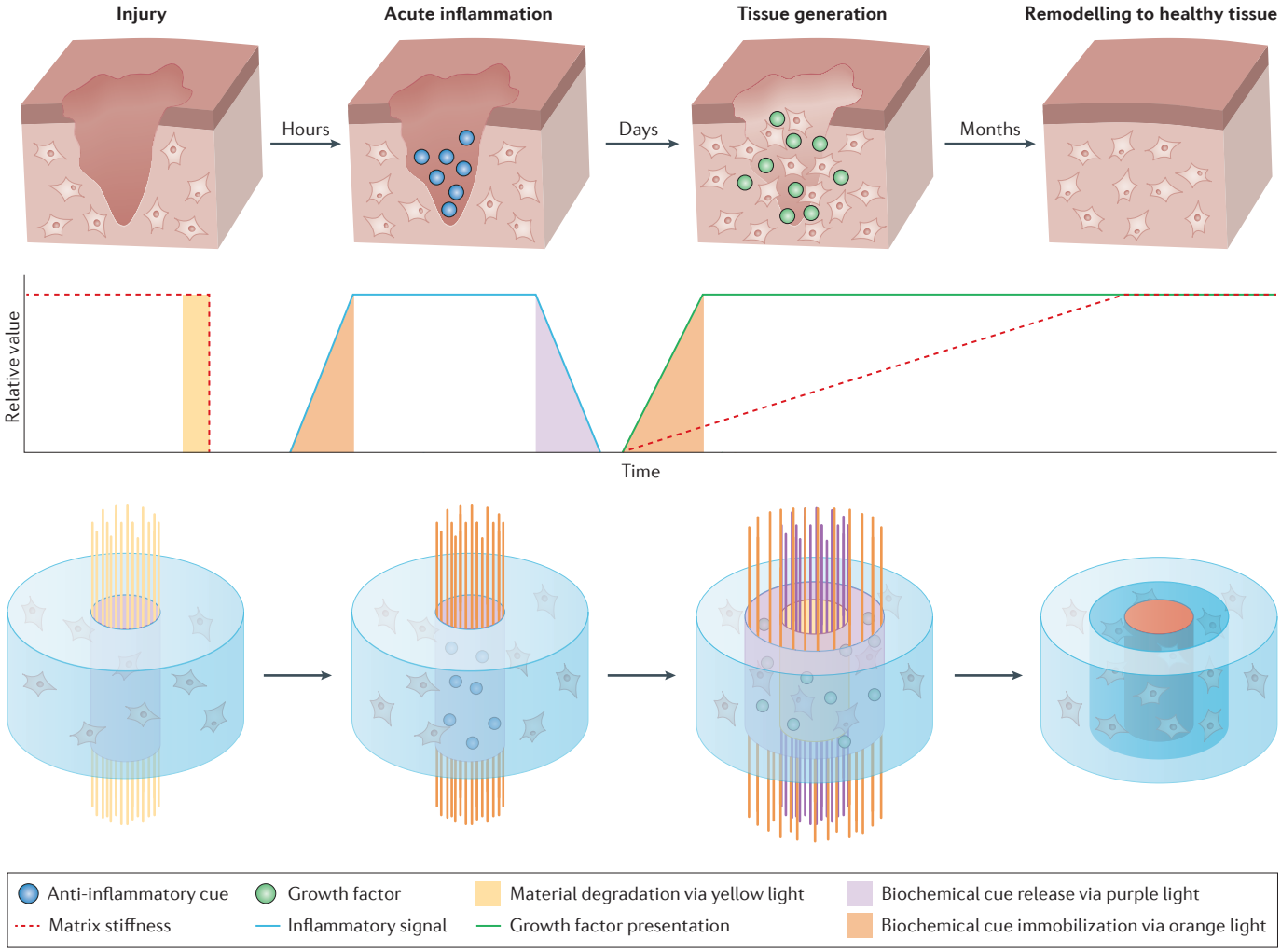


Figure 5 | **Independent physiochemical tuning of biomaterials to mimic *in vivo* processes.** Dynamic biological processes, such as wound healing, can be recapitulated by applying appropriately designed photochemistries, which allow for complete orthogonal control over the physiochemical properties of a cell culture substrate *in vitro*. Treatment of the three physiochemically and temporally distinct stages of the tissue repair process can be modelled through the reversible, local presentation of inflammatory signals and growth factors and through variations in the matrix stiffness. After injury resulting in the complete elimination of local cells and their extracellular matrix, damaged tissue activates an acute inflammatory response marked by the infiltration of key inflammatory cues from the blood plasma. Cells then proliferate at an increased rate to aid tissue generation promoted through the differential presentation of growth factors. Finally, the extracellular matrix is remodelled to restore tissue mechanics. Sequential exposure to different wavelengths of light triggers specific and orthogonal modifications of the material: yellow represents mechanical degradation, orange represents biochemical cue immobilization, and purple represents biochemical cue release. Thereby, an engineered tissue can be guided by light through the different stages of wound healing *in vitro*.

properties remains challenging. Continued effort must be made towards generating libraries of photoactive species for reactions that can be initiated in a wavelength-orthogonal manner. Components must have not only well-separated maximum absorbances but also narrow absorption peak widths to ensure minimal spectral overlap and independent activation[231]. This will enable the design of combinatorial drug delivery systems that release therapeutics in a medically relevant manner and to determine the biological importance of distinct physiochemical cues that are independently regulated within a common material.

The majority of photoresponsive biomaterials engineered to date rely on reactions that proceed only in one direction. The establishment of photoreactions that can be reversibly triggered using cytocompatible wavelengths remains of prime importance to control therapeutic activities within the body and to dynamically manipulate the physiochemical properties of synthetic cell culture platforms. Incorporation of allyl sulfides, originally developed as agents for RAFT polymerization, into materials has shown promise in creating reversible constructs[175,232], as has azobenzene-mediated host–guest chemistry[233,234]. Borrowing from biology, reaction schemes using photoresponsive proteins, which can undergo reversible dimerization or conformational changes, may greatly improve the dynamic control over local material properties.

Many photoreactions commonly employed for biomaterial alteration, for example, thiol-ene, methacrylate-based and acrylate-based chain polymerization, are driven by free radicals, which can elicit damaging and nonspecific reactions with cells and tissues. Other reactions, such as photocaged Michael additions, rely on moieties that exhibit undesired cross reactions with functional groups found in biomacromolecules, including DNA or proteins. Many reactions proceed exothermally or operate through a photothermal effect, generating heat, which can damage the surrounding tissue. Reaction bio-orthogonality has to be considered to ensure that phototools can be safely and effectively used within living systems. The development of novel bio-orthogonal chemistries extends well past the field of photochemistry, and therefore, the establishment of general strategies to photoregulate reactions will prove invaluable.

Technological advances are also needed to direct light exposure onto and within materials. Multiphoton lithography enables material control in three dimensions, but the high equipment costs and slow processing speeds limit its application and scale-up. This issue can be addressed by the use of low-cost light sources with narrow emission spectra of tunable wavelengths over a broad range of power. Next-generation optical fibres and endoscopic techniques may further extend the reach of photomodulation techniques to light-impenetrable regions of the body. Collaborations with physicists and machinists specializing in optics will be crucial in achieving these goals.

Emerging optogenetic tools, combining aspects of both genetics and optics, facilitate precise control over intracellular processes. In addition to governing specific signalling events using light, strategies involving photoactivated Cre recombinase[235,236] (Cre–Lox recombination) and CRISPR–Cas9 (REFS 237,238) are providing opportunities to optically guide targeted genome editing. Through the combination of optogenetics and photoresponsive biomaterial platforms, independent manipulation of signalling events both within and around cells could be achieved. Such hybrid systems would provide complete control over probing and directing 4D cell fate.

Photochemistry will continue to enrich the development of next-generation biomaterials to meet clinical needs and further our understanding of dynamic biological processes. Clearly, the future for photoresponsive biomaterials remains bright.

1. Palczewski, K. Chemistry and biology of vision. *J. Biol. Chem.* **287**, 1612–1619 (2012).
2. Brieke, C., Rohrbach, F., Gottschalk, A., Mayer, G. & Heckel, A. Light-controlled tools. *Angew. Chem. Int. Ed.* **51**, 8446–8476 (2012).
3. Yu, H., Li, J., Wu, D., Qiu, Z. & Zhang, Y. Chemistry and biological applications of photo-labile organic molecules. *Chem. Soc. Rev.* **39**, 464–473 (2010).
4. Zhu, C., Ninh, C. & Bettinger, C. J. Photoreconfigurable polymers for biomedical applications: chemistry and macromolecular engineering. *Biomacromolecules* **15**, 3474–3494 (2014).
5. Cui, J., Miguel, V. S. & del Campo, A. Light-triggered multifunctionality at surfaces mediated by photolabile protecting groups. *Macromol. Rapid Commun.* **34**, 310–329 (2013).
6. Kaplan, J. H., Forbush, B. & Hoffman, J. F. Rapid photolytic release of adenosine 5′-triphosphate from a protected analog: utilization by the sodium:potassium pump of human red blood cell ghosts. *Biochemistry* **17**, 1929–1935 (1978).
7. Klán, P. *et al.* Photoremovable protecting groups in chemistry and biology: reaction mechanisms and efficacy. *Chem. Rev.* **113**, 119–191 (2013).
8. Ercole, F., Davis, T. P. & Evans, R. A. Photo-responsive systems and biomaterials: photochromic polymers, light-triggered self-assembly, surface modification, fluorescence modulation and beyond. *Polym. Chem.* **1**, 37–54 (2010).
9. Wang, Y., Shim, M. S., Levinson, N. S., Sung, H.-W. & Xia, Y. Stimuli-responsive materials for controlled release of theranostic agents. *Adv. Funct. Mater.* **24**, 4206–4220 (2014).
10. Barhoumi, A., Liu, Q. & Kohane, D. S. Ultraviolet light-mediated drug delivery: principles, applications, and challenges. *J. Control. Release* **219**, 31–42 (2015).
11. Velema, W. A., Szymanski, W. & Feringa, B. L. Photopharmacology: beyond proof of principle. *J. Am. Chem. Soc.* **136**, 2178–2191 (2014).
12. Olejniczak, J., Carling, C.-J. & Almutairi, A. Photocontrolled release using one-photon absorption of visible or NIR light. *J. Control. Release* **219**, 18–30 (2015).
13. Breitinger, H.-G. A., Wieboldt, R., Ramesh, D., Carpenter, B. K. & Hess, G. P. Synthesis and characterization of photolabile derivatives of serotonin for chemical kinetic investigations of the serotonin 5-HT 3 receptor. *Biochemistry* **39**, 5500–5508 (2000).
14. Shi, Y. *et al.* Light-triggered release of ciprofloxacin from an *in situ* forming click hydrogel for antibacterial wound dressings. *J. Mater. Chem. B* **3**, 8771–8774 (2015).
15. Paul, A. *et al.* Photoresponsive real time monitoring silicon quantum dots for regulated delivery of anticancer drugs. *J. Mater. Chem. B* **4**, 521–528 (2016).
16. Cabane, E., Malinova, V., Menon, S., Palivan, C. G. & Meier, W. Photoresponsive polymersomes as smart, triggerable nanocarriers. *Soft Matter* **7**, 9167–9176 (2011).
17. Kohman, R. E., Cha, S. S., Man, H.-Y. & Han, X. Light-triggered release of bioactive molecules from DNA nanostructures. *Nano Lett.* **16**, 2781–2785 (2016).
18. Shestopalov, I. A., Sinha, S. & Chen, J. K. Light-controlled gene silencing in zebrafish embryos. *Nat. Chem. Biol.* **3**, 650–651 (2007).
19. Inlay, M. A. *et al.* Synthesis of a photocaged tamoxifen for light-dependent activation of Cre-ER recombinase-driven gene modification. *Chem. Commun.* **49**, 4971–4973 (2013).
20. Li, L. *et al.* Aptamer photoregulation *in vivo*. *Proc. Natl Acad. Sci. USA* **111**, 17099–17103 (2014).
21. Huynh, C. T. *et al.* Photocleavable hydrogels for light-triggered siRNA release. *Adv. Healthc. Mater.* **5**, 305–310 (2015).
22. Faal, T. *et al.* 4-Hydroxytamoxifen probes for light-dependent spatiotemporal control of Cre-ER mediated reporter gene expression. *Mol. Biosyst.* **11**, 783–790 (2015).
23. Sarode, B. R., Kover, K., Tong, P. Y., Zhang, C. & Friedman, S. H. Light control of insulin release and blood glucose using an injectable photoactivated depot. *Mol. Pharm.* **13**, 3835–3841 (2016).
24. D'Souza, A. J. M. & Topp, E. M. Release from polymeric prodrugs: linkages and their degradation. *J. Pharm. Sci.* **93**, 1962–1979 (2004).
25. Fomina, N., McFearin, C., Sermsakdi, M., Edigin, O. & Almutairi, A. UV and near-IR triggered release from polymeric nanoparticles. *J. Am. Chem. Soc.* **132**, 9540–9542 (2010).
26. Huu, V. A. N. *et al.* Light-responsive nanoparticle depot to control release of a small molecule angiogenesis inhibitor in the posterior segment of the eye. *J. Control. Release* **200**, 71–77 (2015).
This study reports the first *in vivo* therapeutic release from photodegradable polymeric nanoparticles in the eye.
27. Aujard, I. *et al. o*-Nitrobenzyl photolabile protecting groups with red-shifted absorption: syntheses and uncaging cross-sections for one- and two-photon excitation. *Chem. Eur. J.* **12**, 6865–6879 (2006).
28. Holmes, C. P. Model studies for new *o*-nitrobenzyl photolabile linkers: substituent effects on the rates of photochemical cleavage. *J. Org. Chem.* **62**, 2370–2380 (1997).
29. Griffin, D. R. & Kasko, A. M. Photoselective delivery of model therapeutics from hydrogels. *ACS Macro Lett.* **1**, 1330–1334 (2012).
This study describes the wavelength-selective release of multiple therapeutics from a single biomaterial.
30. Donato, L. *et al.* Water-soluble, donor-acceptor biphenyl derivatives in the 2-(o-nitrophenyl)propyl series: highly efficient two-photon uncaging of the neurotransmitter γ-aminobutyric acid at λ = 800 nm. *Angew. Chem. Int. Ed.* **51**, 1840–1843 (2012).
31. Olejniczak, J., Sankaranarayanan, J., Viger, M. L. & Almutairi, A. Highest efficiency two-photon degradable copolymer for remote controlled release. *ACS Macro Lett.* **2**, 683–687 (2013).
32. Carling, C.-J. *et al.* Efficient red light photo-uncaging of active molecules in water upon assembly into nanoparticles. *Chem. Sci.* **7**, 2392–2398 (2015).
33. Yang, Y. *et al.* NIR light controlled photorelease of siRNA and its targeted intracellular delivery based on upconversion nanoparticles. *Nanoscale* **5**, 231–238 (2013).
34. Yang, Y., Velmurugan, B., Liu, X. & Xing, B. NIR photoresponsive crosslinked upconverting nanocarriers toward selective intracellular drug release. *Small* **9**, 2937–2944 (2013).
35. Wang, W. *et al.* Efficient triplet-triplet annihilation-based upconversion for nanoparticle phototargeting. *Nano Lett.* **15**, 6332–6338 (2015).
36. Yan, B., Boyer, J.-C., Branda, N. R. & Zhao, Y. Near-infrared light-triggered dissociation of block copolymer micelles using upconverting nanoparticles. *J. Am. Chem. Soc.* **133**, 19714–19717 (2011).
37. Yan, B., Boyer, J.-C., Habault, D., Branda, N. R. & Zhao, Y. Near infrared light triggered release of biomacromolecules from hydrogels loaded with upconversion nanoparticles. *J. Am. Chem. Soc.* **134**, 16558–16561 (2012).
38. Sudimack, J. & Lee, R. J. Targeted drug delivery via the folate receptor. *Adv. Drug Deliv. Rev.* **41**, 147–162 (2000).
39. Fan, N.-C., Cheng, F.-Y., Ho, J. A. & Yeh, C.-S. Photocontrolled targeted drug delivery: photocaged biologically active folic acid as a light-responsive tumor-targeting molecule. *Angew. Chem. Int. Ed.* **51**, 8806–8810 (2012).
40. Choi, S. K. *et al.* Light-controlled release of caged doxorubicin from folate receptor-targeting PAMAM dendrimer nanoconjugate. *Chem. Commun.* **46**, 2632–2634 (2010).

41. Hu, X., Tian, J., Liu, T., Zhang, G. & Liu, S. Photo-triggered release of caged camptothecin prodrugs from dually responsive shell cross-linked micelles. *Macromolecules* **46**, 6243–6256 (2013).
42. Azagarsamy, M. A., Alge, D. L., Radhakrishnan, S. J., Tibbitt, M. W. & Anseth, K. S. Photocontrolled nanoparticles for on-demand release of proteins. *Biomacromolecules* **13**, 2219–2224 (2012).
43. Koren, E. & Torchilin, V. P. Cell-penetrating peptides: breaking through to the other side. *Trends Mol. Med.* **18**, 385–393 (2012).
44. Shamay, Y., Adar, L., Ashkenasy, G. & David, A. Light induced drug delivery into cancer cells. *Biomaterials* **32**, 1377–1386 (2011).
45. Yang, Y. Y., Yang, Y. Y., Xie, X., Cai, X. & Mei, X. Preparation and characterization of photo-responsive cell-penetrating peptide-mediated nanostructured lipid carrier. *J. Drug Target.* **22**, 891–900 (2014).
46. Lin, W. *et al.* Enhanced small interfering RNA delivery into cells by exploiting the additive effect between photo-sensitive peptides and targeting ligands. *J. Pharm. Pharmacol.* **67**, 1215–1231 (2015).
47. 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.* http://dx.doi.org/10.1038/nchem.2917 (2018).
48. Lee, J. S., Deng, X., Han, P. & Cheng, J. Dual stimuli-responsive poly(β-amino ester) nanoparticles for on-demand burst release. *Macromol. Biosci.* **15**, 1314–1322 (2015).
49. Liu, G., Zhou, L., Guan, Y., Su, Y. & Dong, C.-M. Multi-responsive polypeptidosome: characterization, morphology transformation, and triggered drug delivery. *Macromol. Rapid Commun.* **35**, 1673–1678 (2014).
50. Klinger, D. & Landfester, K. Dual stimuli-responsive poly(2-hydroxyethyl methacrylate-co-methacrylic acid) microgels based on photo-cleavable cross-linkers: pH-dependent swelling and light-induced degradation. *Macromolecules* **44**, 9758–9772 (2011).
This study describes multi-stimuli-responsive drug delivery for differential release profiles of a single therapeutic.
51. Zhang, Y. *et al.* Polymer-coated hollow mesoporous silica nanoparticles for triple-responsive drug delivery. *ACS Appl. Mater. Interfaces* **7**, 18179–18187 (2015).
52. Givens, R. S. & Matuszewski, B. Photochemistry of phosphate esters: an efficient method for the generation of electrophiles. *J. Am. Chem. Soc.* **106**, 6860–6861 (1984).
53. Suzuki, A. Z. *et al.* Coumarin-4-ylmethoxycarbonyls as phototriggers for alcohols and phenols. *Org. Lett.* **5**, 4867–4870 (2003).
54. Geissler, D. *et al.* (Coumarin-4-yl)methyl esters as highly efficient, ultrafast phototriggers for protons and their application to acidifying membrane surfaces. *Angew. Chem. Int. Ed.* **44**, 1195–1198 (2005).
55. Jin, Q., Mitschang, F. & Agarwal, S. Biocompatible drug delivery system for photo-triggered controlled release of 5-fluorouracil. *Biomacromolecules* **12**, 3684–3691 (2011).
56. Huang, Q., Bao, C., Ji, W., Wang, Q. & Zhu, L. Photocleavable coumarin crosslinkers based polystyrene microgels: phototriggered swelling and release. *J. Mater. Chem.* **22**, 18275 (2012).
57. Mal, N. K., Fujiwara, M., Tanaka, Y., Taguchi, T. & Matsukata, M. Photo-switched storage and release of guest molecules in the pore void of coumarin-modified MCM-41. *Chem. Mater.* **15**, 3385–3394 (2003).
58. Lin, Q. *et al.* Anticancer drug release from a mesoporous silica based nanophotocage regulated by either a one- or two-photon process. *J. Am. Chem. Soc.* **132**, 10645–10647 (2010).
59. Ji, W. *et al.* Coumarin-containing photo-responsive nanocomposites for NIR light-triggered controlled drug release via a two-photon process. *J. Mater. Chem. B* **1**, 5942 (2013).
60. Ando, H., Furuta, T., Tsien, R. Y. & Okamoto, H. Photo-mediated gene activation using caged RNA/DNA in zebrafish embryos. *Nat. Genet.* **28**, 317–325 (2001).
61. Seo, H. J. & Kim, J.-C. 7-Acetoxycoumarin dimer-incorporated and folate-decorated liposomes: photoresponsive release and *in vitro* targeting and efficacy. *Bioconjug. Chem.* **25**, 533–542 (2014).
62. Long, Y.-B., Gu, W.-X., Pang, C., Ma, J. & Gao, H. Construction of coumarin-based cross-linked micelles with pH responsive hydrazone bond and tumor targeting moiety. *J. Mater. Chem. B* **4**, 1480–1488 (2016).
63. Ohtsuki, T. *et al.* Phototriggered protein syntheses by using (7-diethylaminocoumarin-4-yl)methoxycarbonyl-caged aminoacyl tRNAs. *Nat. Commun.* **7**, 12501 (2016).
64. Lin, Q. *et al.* Highly discriminating photorelease of anticancer drugs based on hypoxia activatable phototrigger conjugated chitosan nanoparticles. *Adv. Mater.* **25**, 1981–1986 (2013).
65. Fournier, L. *et al.* A blue-absorbing photolabile protecting group for *in vivo* chromatically orthogonal photoactivation. *ACS Chem. Biol.* **8**, 1528–1536 (2013).
66. Fournier, L. *et al.* Coumarinylmethyl caging groups with redshifted absorption. *Chem. Eur. J.* **19**, 17494–17507 (2013).
67. 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).
68. Babin, J. *et al.* A new two-photon-sensitive block copolymer nanocarrier. *Angew. Chem. Int. Ed.* **48**, 3329–3332 (2009).
69. Kumar, S. *et al.* Near-infrared light sensitive polypeptide block copolymer micelles for drug delivery. *J. Mater. Chem.* **22**, 7252 (2012).
70. Lux, C. d. G *et al.* Short soluble coumarin crosslinkers for light-controlled release of cells and proteins from hydrogels. *Biomacromolecules* **16**, 3286–3296 (2015).
71. Bandara, H. M. D. & Burdette, S. C. Photoisomerization in different classes of azobenzene. *Chem. Soc. Rev.* **41**, 1809–1825 (2012).
72. Kano, K. *et al.* Photoresponsive membranes. Regulation of membrane properties by photoreversible *cis-trans* isomerization of azobenzenes. *Chem. Lett.* **9**, 421–424 (1980).
73. Bisby, R. H., Mead, C. & Morgan, C. G. Active uptake of drugs into photosensitive liposomes and rapid release on UV photolysis. *Photochem. Photobiol.* **72**, 57–61 (2000).
74. Wang, Y. *et al.* Photocontrolled self-assembly and disassembly of block ionomer complex vesicles: a facile approach toward supramolecular polymer nanocontainers. *Langmuir* **26**, 709–715 (2010).
75. Zhang, H. *et al.* Reversible morphology transitions of supramolecular polymer self-assemblies for switch-controlled drug release. *Chem. Commun.* **51**, 15366–15369 (2015).
76. Liu, D., Wang, S., Xu, S. & Liu, H. Photocontrollable intermittent release of doxorubicin hydrochloride from liposomes embedded by azobenzene-contained glycolipid. *Langmuir* **33**, 1004–1012 (2017).
77. Sheldon, J. E., Dcona, M. M., Lyons, C. E., Hackett, J. C. & Hartman, M. C. T. Photoswitchable anticancer activity via *trans-cis* isomerization of a combretastatin A-4 analog. *Org. Biomol. Chem.* **14**, 40–49 (2015).
78. Angelos, S., Choi, E., Vogtle, F., DeCola, L. & Zink, J. I. Photo-driven expulsion of molecules from mesostructured silica nanoparticles. *J. Phys. Chem. C* **111**, 6589–6592 (2007).
79. Lu, J., Choi, E., Tamanoi, F. & Zink, J. I. Light-activated nanoimpeller-controlled drug release in cancer cells. *Small* **4**, 421–426 (2008).
80. Guardado-Alvarez, T. M., Sudha Devi, L., Russell, M. M., Schwartz, B. J. & Zink, J. I. Activation of snap-top capped mesoporous silica nanocontainers using two near-infrared photons. *J. Am. Chem. Soc.* **135**, 14000–14003 (2013).
81. Tarn, D. *et al.* A reversible light-operated nanovalve on mesoporous silica nanoparticles. *Nanoscale* **6**, 3335–3343 (2014).
82. Zhu, Y. & Fujiwara, M. Installing dynamic molecular photomechanics in mesopores: a multifunctional controlled-release nanosystem. *Angew. Chem. Int. Ed.* **46**, 2241–2244 (2007).
83. Yuan, Q. *et al.* Photon-manipulated drug release from a mesoporous nanocontainer controlled by azobenzene-modified nucleic acid. *ACS Nano* **6**, 6337–6344 (2012).
84. Liu, J., Bu, W., Pan, L. & Shi, J. NIR-triggered anticancer drug delivery by upconverting nanoparticles with integrated azobenzene-modified mesoporous silica. *Angew. Chem. Int. Ed.* **52**, 4375–4379 (2013).
85. Bléger, D., Schwarz, J., Brouwer, A. M. & Hecht, S. *o*-Fluoroazobenzenes as readily synthesized photoswitches offering nearly quantitative two-way isomerization with visible light. *J. Am. Chem. Soc.* **134**, 20597–20600 (2012).
86. Knie, C. *et al. ortho*-fluoroazobenzenes: visible light switches with very long-lived *Z* isomers. *Chem. Eur. J.* **20**, 16492–16501 (2014).
87. Konrad, D. B., Frank, J. A. & Trauner, D. Synthesis of redshifted azobenzene photoswitches by late-stage functionalization. *Chem. Eur. J.* **22**, 4364–4368 (2016).
88. Frank, J. A. *et al.* Photoswitchable fatty acids enable optical control of TRPV1. *Nat. Commun.* **6**, 7118 (2015).
89. Broichhagen, J., Frank, J. A. & Trauner, D. A. Roadmap to success in photopharmacology. *Acc. Chem. Res.* **48**, 1947–1960 (2015).
90. Dong, M., Babalhavaeji, A., Samanta, S., Beharry, A. A. & Woolley, G. A. Red-shifting azobenzene photoswitches for *in vivo* use. *Acc. Chem. Res.* **48**, 2662–2670 (2015).
91. Achilleos, D. S., Hatton, T. A. & Vamvakaki, M. Light-regulated supramolecular engineering of polymeric nanocapsules. *J. Am. Chem. Soc.* **134**, 5726–5729 (2012).
92. Son, S., Shin, E. & Kim, B.-S. Light-responsive micelles of spiropyran initiated hyperbranched polyglycerol for smart drug delivery. *Biomacromolecules* **15**, 628–634 (2014).
93. Chen, S., Jiang, F., Cao, Z., Wang, G. & Dang, Z.-M. Photo, pH, and thermo triple-responsive spiropyran-based copolymer nanoparticles for controlled release. *Chem. Commun.* **51**, 12633–12636 (2015).
94. Chen, S. *et al.* Nanocomposites of spiropyran-functionalized polymers and upconversion nanoparticles for controlled release stimulated by near-infrared light and pH. *Macromolecules* **49**, 7490–7496 (2016).
95. Chang, D., Yan, W., Yang, Y., Wang, Q. & Zou, L. Reversible light-controllable intelligent gel based on simple spiropyran-doped with biocompatible lecithin. *Dye. Pigment.* **134**, 186–189 (2016).
96. Tong, R., Hemmati, H. D., Langer, R. & Kohane, D. S. Photoswitchable nanoparticles for triggered tissue penetration and drug delivery. *J. Am. Chem. Soc.* **134**, 8848–8855 (2012).
This study describes photoisomerization-directed release for repetitive dosing deep within tissue.
97. Tong, R., Chiang, H. H. & Kohane, D. S. Photoswitchable nanoparticles for *in vivo* cancer chemotherapy. *Proc. Natl Acad. Sci. USA* **110**, 19048–19053 (2013).
98. Wolff, L. About diazoanhydrides. *Liebigs Ann. Chemie* **325**, 129–195 (1902).
99. Urdabayev, N. K. & Popik, V. V. Wolff rearrangement of 2-diazo-1(2H)-naphthalenone induced by nonresonant two-photon absorption of NIR radiation. *J. Am. Chem. Soc.* **126**, 4058–4059 (2004).
100. Goodwin, A. P., Mynar, J. L., Ma, Y., Fleming, G. R. & Frêchet, J. M. J. Synthetic micelle sensitive to IR light via a two-photon process. *J. Am. Chem. Soc.* **127**, 9952–9953 (2005).
101. Mynar, J. L. *et al.* Two-photon degradable supramolecular assemblies of linear-dendritic copolymers. *Chem. Commun.* 2081–2082 (2007).
102. Sun, L., Yang, Y., Dong, C.-M. & Wei, Y. Two-photon-sensitive and sugar-targeted nanocarriers from degradable and dendritic amphiphiles. *Small* **7**, 401–406 (2011).
103. Yuan, Y. *et al.* Conjugated polymer and drug co-encapsulated nanoparticles for chemo- and photo-thermal combination therapy with two-photon regulated fast drug release. *Nanoscale* **7**, 3067–3076 (2015).
104. Ahmad, R., Fu, J., He, N. & Li, S. Advanced gold nanomaterials for photothermal therapy of cancer. *J. Nanosci. Nanotechnol.* **15**, 1–14 (2015).
105. Gu, L., Koymen, A. R. & Mohanty, S. K. Crystalline magnetic carbon nanoparticle assisted photothermal delivery into cells using CW near-infrared laser beam. *Sci. Rep.* **4**, 5106 (2014).
106. Febvay, S., Marini, D. M., Belcher, A. M. & Clapham, D. E. Targeted cytosolic delivery of cell-impermeable compounds by nanoparticle-mediated, light-triggered endosome disruption. *Nano Lett.* **10**, 2211–2219 (2010).
107. Vivero-Escoto, J. L., Slowing, I. I., Wu, C.-W. & Lin, V. S.-Y. Photoinduced intracellular controlled release drug delivery in human cells by gold-capped mesoporous silica nanosphere. *J. Am. Chem. Soc.* **131**, 3462–3463 (2009).
108. Troutman, T. S., Leung, S. J. & Romanowski, M. Light-induced content release from plasmon resonant liposomes. *Adv. Mater.* **21**, 2334–2338 (2009).

109. Yavuz, M. S. *et al.* Gold nanocages covered by smart polymers for controlled release with near-infrared light. *Nat. Mater.* **8**, 935–939 (2009).
110. Rengan, A. K., Jagtap, M., De, A., Banerjee, R. & Srivastava, R. Multifunctional gold coated thermo-sensitive liposomes for multimodal imaging and photo-thermal therapy of breast cancer cells. *Nanoscale* **6**, 916–923 (2014).
111. Basuki, J. S. *et al.* Photo-modulated therapeutic protein release from a hydrogel depot using visible light. *Angew. Chem. Int. Ed.* **56**, 966–971 (2017).
112. Ghosh, P., Han, G., De, M., Kim, C. K. & Rotello, V. M. Gold nanoparticles in delivery applications. *Adv. Drug Deliv. Rev.* **60**, 1307–1315 (2008).
113. Aznar, E. *et al.* pH- and photo-switched release of guest molecules from mesoporous silica supports. *J. Am. Chem. Soc.* **131**, 6833–6843 (2009).
114. Kang, H. *et al.* Near-infrared light-responsive core-shell nanogels for targeted drug delivery. *ACS Nano* **5**, 5094–5099 (2011).
115. Lee, J., Park, H. & Kim, W. J. Nano 'chocolate waffle' for near-IR responsive drug releasing system. *Small* **11**, 5315–5323 (2015).
116. Tang, Y. *et al.* An aptamer-targeting photoresponsive drug delivery system using 'off–on' graphene oxide wrapped mesoporous silica nanoparticles. *Nanoscale* **7**, 6304–6310 (2015).
117. Cobley, C. M., Au, L., Chen, J. & Xia, Y. Targeting gold nanocages to cancer cells for photothermal destruction and drug delivery. *Expert Opin. Drug Deliv.* **7**, 577–587 (2010).
118. Hribar, K. C., Lee, M. H., Lee, D. & Burdick, J. A. Enhanced release of small molecules from near-infrared light responsive polymer-nanorod composites. *ACS Nano* **5**, 2948–2956 (2011).
119. Niidome, T. *et al.* PEG-modified gold nanorods with a stealth character for *in vivo* applications. *J. Control. Release* **114**, 343–347 (2006).
120. MacLeod, M. J. & Johnson, J. A. PEGylated N-heterocyclic carbene anchors designed to stabilize gold nanoparticles in biologically relevant media. *J. Am. Chem. Soc.* **137**, 7974–7977 (2015).
121. Li, W. *et al.* Remote modulation of neural activities via near-infrared triggered release of biomolecules. *Biomaterials* **65**, 76–85 (2015).
122. Yu, H. *et al.* pH- and NIR light-responsive micelles with hyperthermia-triggered tumor penetration and cytoplasm drug release to reverse doxorubicin resistance in breast cancer. *Adv. Funct. Mater.* **25**, 2489–2500 (2015).
123. Sherlock, S. P., Tabakman, S. M., Xie, L. & Dai, H. Photothermally enhanced drug delivery by ultrasmall multifunctional FeCo/graphitic shell nanocrystals. *ACS Nano* **5**, 1505–1512 (2011).
124. Chen, W. *et al.* Black phosphorus nanosheet-based drug delivery system for synergistic photodynamic/photothermal/chemotherapy of cancer. *Adv. Mater.* **29**, 1603864 (2017).
125. Kim, H. *et al.* Visible light-triggered on-demand drug release from hybrid hydrogels and its application in transdermal patches. *Adv. Healthc. Mater.* **4**, 2071–2077 (2015).
126. Bonnett, R. Photosensitizers of the porphyrin and phthalocyanine series for photodynamic therapy. *Chem. Soc. Rev.* **24**, 19 (1995).
127. Ackroyd, R., Kelty, C., Brown, N. & Reed, M. The history of photodetection and photodynamic therapy. *Photochem. Photobiol.* **74**, 656 (2001).
128. Bio, M. *et al.* Site-specific and far-red-light-activatable prodrug of combretastatin A-4 using photo-unclick chemistry. *J. Med. Chem.* **56**, 3936–3942 (2013).
129. Hossion, A. M. L., Bio, M., Nkepang, G., Awuah, S. G. & You, Y. Visible light controlled release of anticancer drug through double activation of prodrug. *ACS Med. Chem. Lett.* **4**, 124–127 (2013).
130. Ke, M.-R. *et al.* A tumor-targeted activatable phthalocyanine-tetrapeptide-doxorubicin conjugate for synergistic chemo-photodynamic therapy. *Eur. J. Med. Chem.* **127**, 200–209 (2017).
131. Rwei, A. Y. *et al.* Repeatable and adjustable on-demand sciatic nerve block with phototriggerable liposomes. *Proc. Natl Acad. Sci. USA* **112**, 15719–15724 (2015).
132. Berg, K. & Moan, J. Lysosomes as photochemical targets. *Int. J. Cancer* **59**, 814–822 (1994).
133. Berg, K. *et al.* Photochemical Internalization: a novel technique for delivery of macromolecules into cytosol. *Cancer Res.* **59**, 1180–1183 (1999).
134. Selbo, P. K. *et al.* Photochemical internalization provides time- and space-controlled endolysosomal escape of therapeutic molecules. *J. Control. Release* **148**, 2–12 (2010).
135. Nishiyama, N. *et al.* Light-induced gene transfer from packaged DNA enveloped in a dendrimeric photosensitizer. *Nat. Mater.* **4**, 934–941 (2005).
136. Carter, K. A. *et al.* Porphyrin-phospholipid liposomes permeabilized by near-infrared light. *Nat. Commun.* **5**, 3546 (2014).
137. Luo, D. *et al.* Porphyrin-phospholipid liposomes with tunable leakiness. *J. Control. Release* **220**, 484–494 (2015).
138. DeForest, C. A. & Anseth, K. S. Advances in bioactive hydrogels to probe and direct cell fate. *Annu. Rev. Chem. Biomol. Eng.* **3**, 421–444 (2012).
139. 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).
140. 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).
This study demonstrates physiochemical photopatterning within hydrogel biomaterials.
141. Lee, S.-H. H., Moon, J. J. & West, J. L. Three-dimensional micropatterning of bioactive hydrogels via two-photon laser scanning photolithography for guided 3D cell migration. *Biomaterials* **29**, 2962–2968 (2008).
142. Hoffmann, J. C. & West, J. L. Three-dimensional photolithographic patterning of multiple bioactive ligands in poly(ethylene glycol) hydrogels. *Soft Matter* **6**, 5056–5063 (2010).
143. Hahn, M. S. *et al.* Photolithographic patterning of polyethylene glycol hydrogels. *Biomaterials* **27**, 2519–2524 (2006).
144. DeLong, S. A., Moon, J. J. & West, J. L. Covalently immobilized gradients of bFGF on hydrogel scaffolds for directed cell migration. *Biomaterials* **26**, 3227–3234 (2005).
145. Culver, J. C. *et al.* Three-dimensional biomimetic patterning in hydrogels to guide cellular organization. *Adv. Mater.* **24**, 2344–2348 (2012).
146. Moon, J. J., Hahn, M. S., Kim, I., Nsiah, B. A. & West, J. L. Micropatterning of poly(ethylene glycol) diacrylate hydrogels with biomolecules to regulate and guide endothelial morphogenesis. *Tissue Eng. Part A* **15**, 579–585 (2009).
147. Leslie-Barbick, J. E., Shen, C., Chen, C. & West, J. L. Micron-scale spatially patterned, covalently immobilized vascular endothelial growth factor on hydrogels accelerates endothelial tubulogenesis and increases cellular angiogenic responses. *Tissue Eng. Part A* **17**, 221–229 (2011).
148. Luo, Y. & Shoichet, M. S. A photolabile hydrogel for guided three-dimensional cell growth and migration. *Nat. Mater.* **3**, 249–253 (2004).
149. Wosnick, J. H. & Shoichet, M. S. Three-dimensional chemical patterning of transparent hydrogels. *Chem. Mater.* **20**, 55–60 (2008).
150. Wylie, R. G. *et al.* Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. *Nat. Mater.* **10**, 799–806 (2011).
This study describes the strategy to simultaneously immobilize multiple proteins within a synthetic cell culture platform.
151. Wylie, R. G. & Shoichet, M. S. Three-dimensional spatial patterning of proteins in hydrogels. *Biomacromolecules* **12**, 3789–3796 (2011).
152. Luo, Y. & Shoichet, M. S. Light-activated immobilization of biomolecules to agarose hydrogels for controlled cellular response. *Biomacromolecules* **5**, 2315–2323 (2004).
153. Polizzotti, B. D., Fairbanks, B. D. & Anseth, K. S. Three-dimensional biochemical patterning of click-based composite hydrogels via thiolene photopolymerization. *Biomacromolecules* **9**, 1084–1087 (2008).
154. Adzima, B. J. *et al.* Spatial and temporal control of the alkyne-azide cycloaddition by photoinitiated Cu(II) reduction. *Nat. Chem.* **3**, 258–261 (2011).
155. 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. Ed.* **11**, 439–457 (2000).
156. DeForest, C. A. & Anseth, K. S. Cytocompatible click-based hydrogels with dynamically tunable properties through orthogonal photocoupling and photodegradation reactions. *Nat. Chem.* **3**, 925–931 (2011).
157. Hoyle, C. E. & Bowman, C. N. Thiol-ene click chemistry. *Angew. Chem. Int. Ed.* **49**, 1540–1573 (2010).
158. 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).
159. DeForest, C. A., Sims, E. A. & Anseth, K. S. Peptide-functionalized click hydrogels with independently tunable mechanics and chemical functionality for 3D cell culture. *Chem. Mater.* **22**, 4783–4790 (2010).
160. 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).
161. Fairbanks, B. D. *et al.* A versatile synthetic extracellular matrix mimic via thiol-norbornene photopolymerization. *Adv. Mater.* **21**, 5005–5010 (2009).
162. Alge, D. L., Azagarsamy, M. A., Donohue, D. F. & Anseth, K. S. Synthetically tractable click hydrogels for three-dimensional cell culture formed using tetrazine-norbornene chemistry. *Biomacromolecules* **14**, 949–953 (2013).
163. 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).
164. 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).
165. Mosiewicz, K. A. *et al. In situ* cell manipulation through enzymatic hydrogel photopatterning. *Nat. Mater.* **12**, 1071–1077 (2013).
166. Griffin, D. R. *et al.* Hybrid photopatterned enzymatic reaction (HyPER) for *in situ* cell manipulation. *ChemBioChem* **15**, 233–242 (2014).
167. Petersen, S. *et al.* Phototriggering of cell adhesion by caged cyclic RGD peptides. *Angew. Chem. Int. Ed.* **120**, 3236–3239 (2008).
168. Ohmuro-Matsuyama, Y. & Tatsu, Y. Photocontrolled cell adhesion on a surface functionalized with a caged arginine-glycine-aspartate peptide. *Angew. Chem. Int. Ed.* **47**, 7527–7529 (2008).
169. Weis, S., Lee, T. T., del Campo, A. & García, A. J. Dynamic cell-adhesive microenvironments and their effect on myogenic differentiation. *Acta Biomater.* **9**, 8059–8066 (2013).
170. 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).
This study describes a powerful example in which biomaterial photomodification, including spatial patterning, is performed *in vivo*.
171. 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).
A demonstration of cytocompatible biomaterial photodegradation.
172. Griffin, D. R. & Kasko, A. M. Photodegradable macromers and hydrogels for live cell encapsulation and release. *J. Am. Chem. Soc.* **134**, 13103–13107 (2012).
173. Azagarsamy, M. A. & Anseth, K. S. Wavelength-controlled photocleavage for the orthogonal and sequential release of multiple proteins. *Angew. Chem. Int. Ed.* **52**, 13803–13807 (2013).
174. DeForest, C. A. & Anseth, K. S. Photoreversible patterning of biomolecules within click-based hydrogels. *Angew. Chem. Int. Ed.* **51**, 1816–1819 (2011).
175. 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).
176. 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).
This study depicts a powerful approach to reversibly modify hydrogels with full-length proteins in the presence of live cells.

177. 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).
178. Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677–689 (2006).
179. Reilly, G. C. & Engler, A. J. Intrinsic extracellular matrix properties regulate stem cell differentiation. *J. Biomech.* **43**, 55–62 (201§0).
180. Janmey, P. A. & Miller, R. T. Mechanisms of mechanical signaling in development and disease. *J. Cell Sci.* **124**, 9–18 (2011).
181. Nemir, S., Hayenga, H. N. & West, J. L. PEGDA hydrogels with patterned elasticity: novel tools for the study of cell response to substrate rigidity. *Biotechnol. Bioeng.* **105**, 636–644 (2010).
182. Guvendiren, M., Perepelyuk, M., Wells, R. G. & Burdick, J. A. Hydrogels with differential and patterned mechanics to study stiffness-mediated myofibroblastic differentiation of hepatic stellate cells. *J. Mech. Behav. Biomed. Mater.* **38**, 198–208 (2014).
183. Nowatzki, P. J., Franck, C., Maskarinec, S. A., Ravichandran, G. & Tirrell, D. A. Mechanically tunable thin films of photosensitive artificial proteins: preparation and characterization by nanoindentation. *Macromolecules* **41**, 1839–1845 (2008).
184. Khetan, S., Katz, J. S. & Burdick, J. A. Sequential crosslinking to control cellular spreading in 3-dimensional hydrogels. *Soft Matter* **5**, 1601–1606 (2009).
185. Khetan, S. & Burdick, J. A. Patterning network structure to spatially control cellular remodeling and stem cell fate within 3-dimensional hydrogels. *Biomaterials* **31**, 8228–8234 (2010).
186. Khetan, S. *et al.* Degradation-mediated cellular traction directs stem cell fate in covalently crosslinked three-dimensional hydrogels. *Nat. Mater.* **12**, 458–465 (2013).
This study describes the on-demand material crosslinking used to reveal the importance of matrix interactions for stem cell differentiation.
187. Liu, Z. *et al.* Spatiotemporally controllable and cytocompatible approach builds 3D cell culture matrix by photo-uncaged-thiol michael addition reaction. *Adv. Mater.* **26**, 3912–3917 (2014).
188. Mosiewicz, K. A., Kolb, L., Van Der Vlies, A. J. & Lutolf, M. P. Microscale patterning of hydrogel stiffness through light-triggered uncaging of thiols. *Biomater. Sci.* **2**, 1640–1651 (2014).
189. Cui, J., Wang, M., Zheng, Y., Rodríguez Muñiz, G. M. & del Campo, A. Light-triggered cross-linking of alginates with caged Ca^{2+}. *Biomacromolecules* **14**, 1251–1256 (2013).
190. Stowers, R. S., Allen, S. C. & Suggs, L. J. Dynamic phototuning of 3D hydrogel stiffness. *Proc. Natl Acad. Sci. USA* **112**, 1953–1958 (2015).
191. Brandenberg, N. & Lutolf, M. P. In situ patterning of microfluidic networks in 3D cell-laden hydrogels. *Adv. Mater.* **28**, 7450–7456 (2016).
192. Heintz, K. A. *et al.* Fabrication of 3D biomimetic microfluidic networks in hydrogels. *Adv. Healthc. Mater.* **5**, 2153–2160 (2016).
193. Berkovitch, Y., Yelin, D. & Seliktar, D. Photo-patterning PEG-based hydrogels for neuronal engineering. *Eur. Polym. J.* **72**, 473–483 (2015).
194. Johnson, J. A., Finn, M. G., Koberstein, J. T. & Turro, N. J. Synthesis of photocleavable linear macromonomers by ATRP and star macromonomers by a tandem ATRP-click reaction: precursors to photodegradable model networks. *Macromolecules* **40**, 3589–3598 (2007).
195. Kloxin, A. M., Tibbitt, M. W. & Anseth, K. S. Synthesis of photodegradable hydrogels as dynamically tunable cell culture platforms. *Nat. Protoc.* **5**, 1867–1887 (2010).
196. Johnson, J. A., Baskin, J. M., Bertozzi, C. R., Koberstein, J. T. & Turro, N. J. Copper-free click chemistry for the *in situ* crosslinking of photodegradable star polymers. *Chem. Commun.* **0**, 3064–3066 (2008).
197. Wong, D. Y., Griffin, D. R., Reed, J. & Kasko, A. M. Photodegradable hydrogels to generate positive and negative features over multiple length scales. *Macromolecules* **43**, 2824–2831 (2010).
198. Frey, M. T. & Wang, Y. L. A photo-modulatable material for probing cellular responses to substrate rigidity. *Soft Matter* **5**, 1918–1924 (2009).
199. Tsang, K. M. C. *et al.* Facile one-step micropatterning using photodegradable gelatin hydrogels for improved cardiomyocyte organization and alignment. *Adv. Funct. Mater.* **25**, 977–986 (2015).
200. Kirschner, C. M. & Anseth, K. S. In situ control of cell substrate microtopographies using photolabile hydrogels. *Small* **9**, 578–584 (2013).
201. Kloxin, A. M., Tibbitt, M. W., Kasko, A. M., Fairbairn, J. a & Anseth, K. S. Tunable hydrogels for external manipulation of cellular microenvironments through controlled photodegradation. *Adv. Mater.* **22**, 61–66 (2010).
202. Tibbitt, M. W., Kloxin, A. M., Dyamenahalli, K. U. & Anseth, K. S. Controlled two-photon photodegradation of PEG hydrogels to study and manipulate subcellular interactions on soft materials. *Soft Matter* **6**, 5100–5108 (2010).
203. Kloxin, A. M., Benton, J. A. & Anseth, K. S. In situ elasticity modulation with dynamic substrates to direct cell phenotype. *Biomaterials* **31**, 1–8 (2010).
204. Wang, H., Haeger, S. M., Kloxin, A. M., Leinwand, L. A. & Anseth, K. S. Redirecting valvular myofibroblasts into dormant fibroblasts through light-mediated reduction in substrate modulus. *PLoS ONE* **7**, e39969 (2012).
205. Yang, C., Tibbitt, M. W., Basta, L. & Anseth, K. S. Mechanical memory and dosing influence stem cell fate. *Nat. Mater.* **13**, 645–652 (2014).
206. 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).
207. McKinnon, D. D., Brown, T. E., Kyburz, K. A., Kiyotake, E. & Anseth, K. S. Design and characterization of a synthetically accessible, photodegradable hydrogel for user-directed formation of neural networks. *Biomacromolecules* **15**, 2808–2816 (2014).
208. 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).
209. Bernard, A. B., Lin, C.-C. C. & Anseth, K. S. A microwell cell culture platform for the aggregation of pancreatic beta-cells. *Tissue Eng. Part C Methods* **18**, 583–592 (2012).
210. Lewis, K. J. R. *et al.* In vitro model alveoli from photodegradable microsphere templates. *Biomater. Sci.* **3**, 821–832 (2015).
211. Kloxin, A. M. *et al.* Responsive culture platform to examine the influence of microenvironmental geometry on cell function in 3D. *Integr. Biol.* **4**, 1540–1549 (2012).
212. Fairbanks, B. D., Singh, S. P., Bowman, C. N. & Anseth, K. S. Photodegradable, photoadaptable hydrogels via radical-mediated disulfide fragmentation reaction. *Macromolecules* **44**, 2444–2450 (2011).
213. Tamura, M. *et al.* Optical cell separation from three-dimensional environment in photodegradable hydrogels for pure culture techniques. *Sci. Rep.* **4**, 4793 (2014).
214. Truong, V. X. *et al.* Photodegradable gelatin-based hydrogels prepared by bioorthogonal click chemistry for cell encapsulation and release. *Biomacromolecules* **16**, 2246–2253 (2015).
215. Ki, C. S., Shih, H. & Lin, C. C. Facile preparation of photodegradable hydrogels by photopolymerization. *Polymer* **54**, 2115–2122 (2013).
216. Zhu, C. C. & Bettinger, C. J. Light-induced remodeling of physically crosslinked hydrogels using near-IR wavelengths. *J. Mater. Chem. B* **2**, 1613–1618 (2014).
217. Azagarsamy, M. A., McKinnon, D. D., Age, D. L. & Anseth, K. S. Coumarin-based photodegradable hydrogel: design, synthesis, gelation, and degradation kinetics. *ACS Macro Lett.* **3**, 515–519 (2014).
218. Andreopoulos, F. M. *et al.* Photoscissable hydrogel synthesis via rapid photopolymerization of novel PEG-based polymers in the absence of photoinitiators. *J. Am. Chem. Soc.* **118**, 6235–6240 (1996).
219. Andreopoulos, F. M., Beckman, E. J. & Russell, A. J. Light-induced tailoring of PEG-hydrogel properties. *Biomaterials* **19**, 1343–1352 (1998).
220. Andreopoulos, F. M., Beckman, E. J. & Russell, A. J. Photoswitchable PEG-CA hydrogels and factors that affect their photosensitivity. *J. Polym. Sci. Part A Polym. Chem.* **38**, 1466–1476 (2000).
221. Zheng, Y. *et al.* A novel photoscissile poly(ethylene glycol)-based hydrogel. *Adv. Funct. Mater.* **11**, 37–40 (2001).
222. Zheng, Y. J. *et al.* PEG-based hydrogel synthesis via the photodimerization of anthracene groups. *Macromolecules* **35**, 5228–5234 (2002).
223. Sako, Y. & Takaguchi, Y. A photo-responsive hydrogelator having gluconamides at its peripheral branches. *Org. Biomol. Chem.* **6**, 3843–3847 (2008).
224. Chen, Y. & Geh, J. L. Copolymers derived from 7-acryloyloxy-4-methylcoumarin and acrylates: 2. Reversible photocrosslinking and photocleavage. *Polymer* **37**, 4481–4486 (1996).
225. Maddipatla, M. V. S. N. *et al.* Photoresponsive coumarin polyesters that exhibit cross-linking and chain scission properties. *Macromolecules* **46**, 5133–5140 (2013).
226. Tamesue, S., Takashima, Y., Yamaguchi, H., Shinkai, S. & Harada, A. Photoswitchable supramolecular hydrogels formed by cyclodextrins and azobenzene polymers. *Angew. Chem. Int. Ed.* **49**, 7461–7464 (2010).
227. Rosales, A. M., Mabry, K. M., Nehls, E. M. & Anseth, K. S. Photoresponsive elastic properties of azobenzene-containing poly(ethylene-glycol)-based hydrogels. *Biomacromolecules* **16**, 798–806 (2015).
228. 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).
229. Smith, D. J. *et al.* A multiphase transitioning peptide hydrogel for suturing ultrasmall vessels. *Nat. Nanotechnol.* **11**, 95–102 (2016).
230. Sharma, B. *et al.* Human cartilage repair with a photoreactive adhesive-hydrogel composite. *Sci. Transl. Med.* **5**, 167ra6 (2013).
231. San Miguel, V., Bochet, C. G. & del Campo, A. Wavelength-selective caged surfaces: how many functional levels are possible? *J. Am. Chem. Soc.* **133**, 5380–5388 (2011).
232. 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).
233. Zhao, Y.-L. & Stoddart, J. F. Azobenzene-based light-responsive hydrogel system. *Langmuir* **25**, 8442–8446 (2009).
234. Wang, D., Wagner, M., Butt, H.-J. & Wu, S. Supramolecular hydrogels constructed by red-light-responsive host–guest interactions for photo-controlled protein release in deep tissue. *Soft Matter* **11**, 7656–7662 (2015).
235. Schindler, S. E. *et al.* Photo-activatable Cre recombinase regulates gene expression *in vivo*. *Sci. Rep* **5**, 13627 (2015).
236. Kawano, F., Okazaki, R., Yazawa, M. & Sato, M. A photoactivatable Cre–loxP recombination system for optogenetic genome engineering. *Nat. Chem. Biol.* **12**, 1059–1064 (2016).
237. Nihongaki, Y., Kawano, F., Nakajima, T. & Sato, M. Photoactivatable CRISPR-Cas9 for optogenetic genome editing. *Nat. Biotechnol.* **33**, 755–760 (2015).
238. Nihongaki, Y., Furuhata, Y., Otabe, T., Hasegawa, Saki Yoshimoto, K. & Sato, M. CRISPR-Cas9-based photoactivatable transcription systems to induce neuronal differentiation. *Nat. Methods* **14**, 963–966 (2017).

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