Visible Light-Responsive Dynamic Biomaterials: Going Deeper and Triggering More

Teresa L. Rapp and Cole A. DeForest*

Photoresponsive materials have been widely used in vitro for controlled therapeutic delivery and to direct 4D cell fate. Extension of the approaches into a bodily setting requires use of low-energy, long-wavelength light that penetrates deeper into and through complex tissue. This review details recent reports of photoactive small molecules and proteins that absorb visible and/or near-infrared light, opening the door to exciting new applications in multiplexed and in vivo regulation.

1. Introduction

Over the past decade, light-responsive biomaterials have established and expanded newfound opportunities for cell culture and biomedical research.[1–3] From some of the first reports of directed cell growth in phototunable materials,[4–7] the ability to trigger changes in biomaterial properties on demand and with spatiotemporal control has enabled a variety of research breakthroughs,[8] including the creation of synthetic microvasculature,[9–11] lineage-specified cell differentiation,[12,13] and establishment of the concept of biological mechanical memory.[14] Additionally, photodynamically stiffening materials have proven useful in disease modeling,[15–17] while softening materials can be used to deliver cells and therapeutics in vivo.[3,16] Moreover, biomaterials photopatterned with bioactive peptides and/or proteins have been used to direct cell proliferation, migration, and differentiation,[18–23] most recently with single- and subcellular resolutions.[24] On the benchtop, photosensitive materials have proven versatile and customizable, affording user-defined control over mechanics, signal presentation, and biomolecule release.

Exploiting light-mediated chemistries to modulate material properties gives researchers the ability to tune and control chemical reactions both in time and space.[25] Relying on physiological conditions to trigger a material response can be challenging, as local enzyme concentration, pH, and even reducing environments can vary widely in living samples and among patients.[26,27] Utilizing an external trigger can help to standardize research and clinical outcomes by putting the power to initiate material alteration in the hands of the patient or provider. Light is unique among other possible external triggers for these type of events (e.g., ultrasound, magnetic or electric field, and exogenously administered small molecules) in affording a highly localized material response, the ability to accurately tune the extent of material change, and the potential to modulate different physiochemical properties using different wavelengths.

While light-responsive biomaterials have made waves on the benchtop, their applicability has rarely reached beyond in vitro cell culture. Fundamental limitations imposed by the commonly available chemistries in conjunction with tissue opacity render in vivo applications largely impossible. The photoresponsive molecules most frequently used in materials respond best to near-ultraviolet (near-UV) and blue light, both of which offer minimal penetration through tissue.[28] While some biomaterials modified with these photoresponsive groups have been used in vivo, their activation is confined to transplant locations just beneath the skin.[29] Extension of the approaches into an in vivo setting requires use of low-energy, long-wavelength light capable of penetrating significantly further into complex tissue.

The desire to expand possibilities for in vivo modulation has led to a significant push toward red-shifting the activation wavelengths for such photoresponsive molecules. These chemical advances, coupled with technological developments in optics to administer light locally in vivo, are enabling new and exciting opportunities to photocontrol materials in a living setting. Recognizing that several recent reports have detailed the use of UV- and blue-light responsive species and their material science applications,[1,30,31] here we highlight systems whose photoactivation can be controlled with lower energy light approaching the optical window of mammalian tissue. For the purposes of this review, we will limit discussion to photoactive small molecules and proteins whose single-photon excitation wavelengths lie in the visible and near-infrared (near-IR) regions and can be used to modulate biomaterial properties in vivo with light.

Dr. T. L. Rapp, Prof. C. A. DeForest
Department of Chemical Engineering
University of Washington
3781 Okanogan Lane NE, Seattle, WA 98195, USA
E-mail: ProfCole@uw.edu

Prof. C. A. DeForest
Department of Bioengineering
University of Washington
3720 15th Ave NE, Seattle, WA 98105, USA

Prof. C. A. DeForest
Institute for Stem Cell & Regenerative Medicine
University of Washington
850 Republican Street, Seattle, WA 98109, USA

Prof. C. A. DeForest
Molecular Engineering & Sciences Institute
University of Washington
3946 W Stevens Way NE, Seattle, WA 98195, USA

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adhm.201901553.

ADVANCED
SCIENCE NEWS
www.advancedsciencenews.com

ADVANCED
HEALTHCARE
MATERIALS
www.advhealthmat.de

2. Delivering Light to Deep Tissue: Taking Inspiration from Photodynamic Therapy (PDT)

Light has been exploited in medicine since the 19th century, when UV light was found to successfully treat lupus vulgaris, a discovery that won physician Niels Finsen the Nobel Prize in Physiology and Medicine in 1903.[32] Targeted PDT was developed in the 1960s following observation that hematoporphyrin derivatives exhibit higher toxicity and increased tumor uptake under light irradiation.[33] In the years since these first discoveries and the birth of PDT, several other photosensitizing molecules have been developed with some passing clinical trials.[32] PDT has found utility in treating a wide variety of cancers,[34] as well as actinic keratosis, atherosclerotic plaques, rheumatoid arthritis, prophylaxis of arterial restenosis, and age-associated macular degeneration.[35] Advancements in photosensitizers have come hand-in-hand with advances in optical advances to deliver light within complex tissue. Lasers and clinical light sources have been developed for surgical applications as well as outpatient procedures.[32] Clinical light sources have been engineered that excite photosensitizers at wavelengths across the electromagnetic spectrum, spanning wavelengths between 100 and 1100 nm.[32] With the existence of United States' Food and Drug Administration-approved surgical lasers and light treatment options for patients, it continues to become easier to imagine several in vivo applications of biomaterials that respond to light in a clinical setting.

Despite the many technological advances in optics, the poor penetration depth of high-energy ultraviolet light has limited clinical applications of many biomaterials and photosensitizers. The consensus in the field is clear: new photoresponsive molecules that respond to tissue-penetrating wavelengths of light must be developed to increase the clinical relevance of these therapies.[36–38]

3. Light Penetration through Complex Tissue: The Photodynamic Therapy Window

Optical penetration through complex tissue is hampered by two major processes: 1) absorption and 2) scattering of incident light by cells and the extracellular matrix (ECM). Skin and other complex tissues in the body are comprised of a multitude of cell types and heterogeneous 3D structures that affect light absorption and scattering. Tissue and its composite molecules (e.g., fatty acids, lipids, proteins, sugars) also act to scatter light through elastic scattering principles. This elastic scattering is due to inhomogeneous electric polarization in tissue, whereby incident light interacts with intermolecular charges, causing them to move at the same frequency and become a radiating dipole.[32] This process interferes with each photon's ability to penetrate deeply into tissue, though its extent decreases with increasing wavelength; for example, 400 nm light is scattered nearly 25-fold more than 900 nm light. In addition to the inherent scattering properties of complex tissue, individual components of standard biological tissue (e.g., proteins, lipids, water) absorb different wavelengths of light to differing extents. Though all these components absorb some amount of light, hemoglobin (both in its oxygen-bound and -unbound forms),

Teresa L. Rapp is a Washington Research Foundation Postdoctoral Fellow in the Department of Chemical Engineering at the University of Washington, where she began in 2019. Her interests include expanding the use of light-responsive biomaterials in 4D cell culture and animal models.

Cole A. DeForest is the Dan Evans Career Development assistant professor in the Departments of Chemical Engineering and Bioengineering at the University of Washington, where he began in 2014. His research program focuses on the development and exploitation of user-programmable biomaterials for 4D cell culture and targeted drug delivery.

water, fat, and melanin are considered the strongest absorbers of visible and IR light (**Figure 1**).[39,40]

This unique property of skin and other biological tissue to permit deeper light penetration given specific longer wavelengths has been aptly named the "PDT window." This window is typically reported between 650 and 950 nm, sometimes out to 1100 nm. At these wavelengths, light absorption is minimized (though still significant) and scattering less pronounced, together permitting deeper light penetration.

Though light-responsive therapies show remarkable promise and offer many comparative advantages in controlling material response, they must respond to red and near-IR light to be effective in vivo. Current photoresponsive molecules need to be reimagined alongside development of new classes of IR-sensitive materials. Not only will such development be key in the aid and development of new clinical technologies, red-shifting photoresponsive molecules will also open the field to multiplexed responses within one material, providing newfound levels of control for in vitro study.

4. Wavelength-Dependent Strategies for Introducing Dynamic Material Response

As research progresses toward red-light activation, strategies based on optogenetic proteins and ruthenium complexes have demonstrated substantial promise. Ruthenium polypyridyl complexes have been shown to undergo clean ligand exchange under visible light, with some reported complexes extending
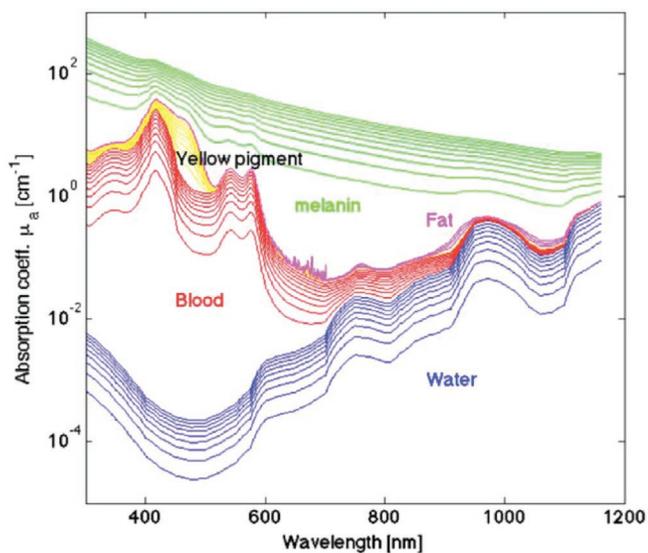
Figure 1. Total absorption coefficient of a generic tissue (μ_a, cm^{-1}) as water (blue), blood at 75% oxygenation (red), yellow pigment (yellow), melanin (green), and fat (pink) is added. While individual tissues will contain varying amounts of these primary absorbers, this figure indicates the presence of the PDT window between 650 and 950 nm. Reproduced with permission.[39] Copyright 2013, IOP.

into the near-IR. Optogenetic proteins exhibiting photo(dis)association or photocleavage have also been recently introduced into the biomaterials space. While these represent some of the most promising options to date, they are complemented by new purely organic photoresponsive molecules that have also broken into the visible spectrum. In this section, we highlight many of these systems, grouping photoresponsive molecules based on their primary activation wavelengths while stepping deeper into the photodynamic window.

4.1. Photoactive Species Responding to 400–500 nm Light

4.1.1. PhoCl: A Photocleavable Protein, 400 nm

Recently evolved by the optogenetics community, PhoCl is a photocleavable 27.8 kDa monomeric protein that undergoes irreversible peptide backbone cleavage under irradiation with 400 nm light.[41] Reported in 2017, PhoCl is one of the first monomeric photocleavable proteins published to date and has been specifically evolved to minimize the distance between the cleavage location and any C-terminal species. Photocleavage occurs between amino acids Phe231 and His232 comprising the mature chromophore, severing the 15 C-terminal amino acid residues from the rest of PhoCl while quenching the innate fluorescence of the parent protein (**Figure 2**A).

In a recent report, Shadish et al. demonstrated expression of chimeric protein fusions in which bioactive species were genetically appended to PhoCl's C-terminus. Taking advantage of a chemoenzymatic strategy to introduce a single reactive azide
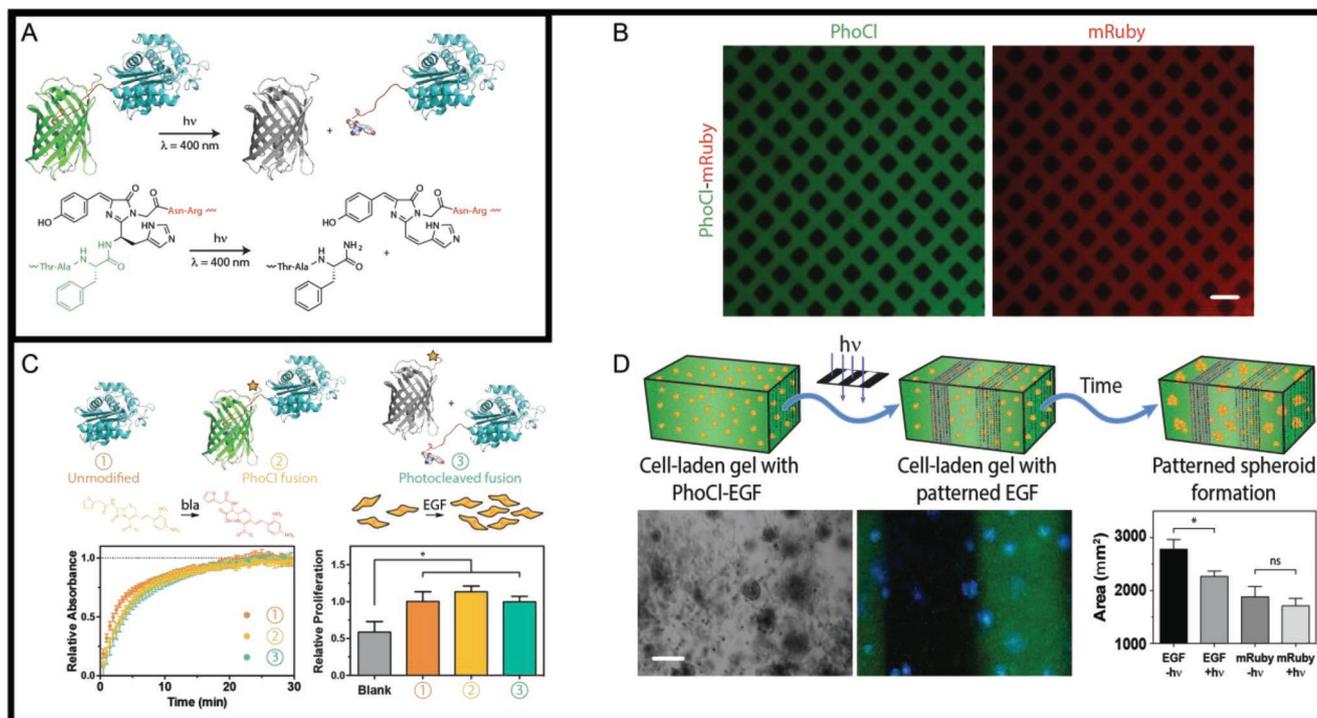

Figure 2. PhoCl as a photoresponsive moiety. A) PhoCl (green) cleaves at the amide bond on the backbone between Phe231 and His232, releasing the C-terminally fused protein cargo (blue). B) Photopatterned cleavage of PhoCl-mRuby yields a protein-patterned hydrogel biomaterial. PhoCl fluoresces green, mRuby red. Scale bar = 200 µm. C) Active bla and EGF PhoCl fusions show modification at the C-terminus does not affect activity, based on bla's ability to enzymatically act upon a chromogenic substrate or EGF's capacity to promote cell proliferation. D) Patterned EGF promotes localized spheroid (blue) growth within 3D cell culture. Scale bar = 200 µm. Adapted with permission.[43] Copyright 2019, American Chemical Society.

ADVANCED
SCIENCE NEWS
www.advancedsciencenews.com

ADVANCED
HEALTHCARE
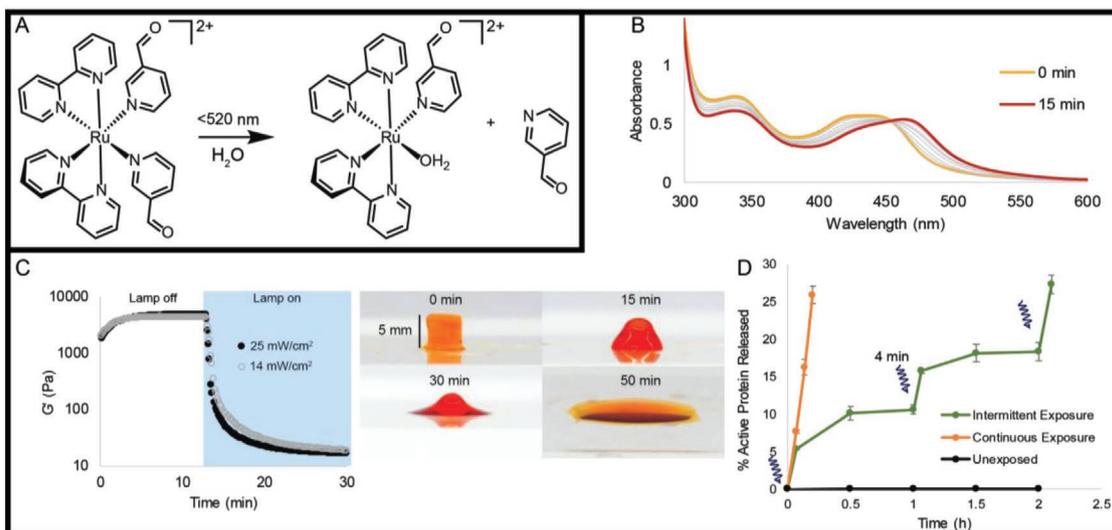MATERIALS
www.advhealthmat.de

Figure 3. RuAldehyde photocleavage and incorporation into hyaluronic acid hydrogel. A) Mechanism of photocleavage of RuAldehyde. B) Photolysis as observed by absorbance spectroscopy for varying light exposures; a red shift in absorbance indicates exchange of a coordinated pyridine ligand for water. C) Photodegradation of an HA-based RuAldehyde-crosslinked hydrogel as observed by photorheometry and on the benchtop. Larger hydrogels required longer light exposures to fully degrade. D) Temporally controlled photorelease of model enzyme bla from the hydrogel using burst light irradiation of a hydrogel, as measured by bla activity in the supernatant. Adapted with permission.[51] Copyright 2018, Wiley–VCH.

at the protein fusion's N-terminus, the photocleavable protein was conjugated to poly(ethylene glycol) (PEG)-based hydrogels formed through strain-promoted azide-alkyne cycloaddition (SPAAC).[42] With this genetically encoded system of protein cargo conjugated to a photoresponsive crosslinker, Shadish et al. demonstrated the incorporation and subsequent photorelease of several different proteins of interest from biomaterials, including red-fluorescent mRuby (Figure 2B), beta-lactamase (bla) as a model enzyme (Figure 2C), and epidermal growth factor (EGF) to direct cell growth (Figure 2D).

Even though this system responds to light falling at the lower end of the visible spectrum, its unique power lies in the fact that the photodegradable species is entirely genetically encodable. Provided proteins of interest can be cloned and recombinantly expressed, this system produces fully modified, active protein ready for incorporation into a hydrogel upon purification. This advance makes photochemistry accessible to those with minimal lab equipment and expertise in organic synthesis. Furthermore, it may be possible to further red-shift PhoCl's absorbance through directed evolution, resulting in a photocleavable protein that can be activated in vivo.

4.1.2. RuAldehyde: A Ruthenium Polypyridyl Crosslinker, 450 nm

Ruthenium polypyridyl complexes have been appropriated from photodynamic therapy for use in biomaterials by redesigning the complex to favor ligand exchange with local solvent over reactive oxygen species (ROS) generation.[44–47] This ligand-exchange process is reported to be relatively clean, exhibiting radical-free cleavage of a metal–ligand bond.[48] While ruthenium complexes have been successfully used in vivo and in vitro as caging groups for bioactive small molecules,[47] recently these types of complexes have found utility in biomaterials settings.[49,50] Ruthenium complexes are also remarkably soluble in aqueous buffers,

making them amenable to use in complex biological systems. The repurposing of ruthenium complexes as crosslinkers for hydrogel materials was pioneered by Rapp et al. in their recent report of an aldehyde-modified ruthenium complex RuAldehyde (Ru(bipyridine)$_2$(3-pyridinaldehyde)$_2$) (**Figure 3**A).[51] Using two monodentate pyridine ligands modified with aldehydes, a photocleavable crosslinker was generated with a λ_{max} absorbance at 450 nm and a significant tail extending out to 520 nm (Figure 3B), permitting photocleavage with light <520 nm.

The photophysical properties of RuAldehyde permitted rapid degradation of hydrogels formed through its reaction with hydrazine-modified hyaluronic acid (HA-HYD). The Ru-HA gel formation proceeded rapidly, yielding a hydrazone-linked hydrogel within the first minute of mixing as measured by rheometry (Figure 3C). In addition to its strong response to blue and green light, RuAldehyde and RuAldehyde-crosslinked hydrogels demonstrated a rapid response to visible light irradiation (400–500 nm, Hg lamp), one of the fastest photodegrading gels reported to date (Figure 3C). The duality of the RuAldehyde-HA-HYD hydrogel system was demonstrated in the use of the Ru crosslinker to both crosslink the hydrogel matrix and covalently attach model enzyme bla to the hydrogel matrix via an amide bond (Figure 3D). Due to the intentionality of leaving the Ru crosslinker attached to the hydrogel matrix after photodegradation, the toxicity of the photoproducts was greatly diminished, demonstrating that ruthenium-based photochemistry can be used in biomaterials intended for tissue culture and in vivo material designs.[51]

4.1.3. Ru(bpy)$_2$(4-(aminomethyl)pyridine)$_2$: Multiphoton-Active Gel Crosslinker, <395 nm

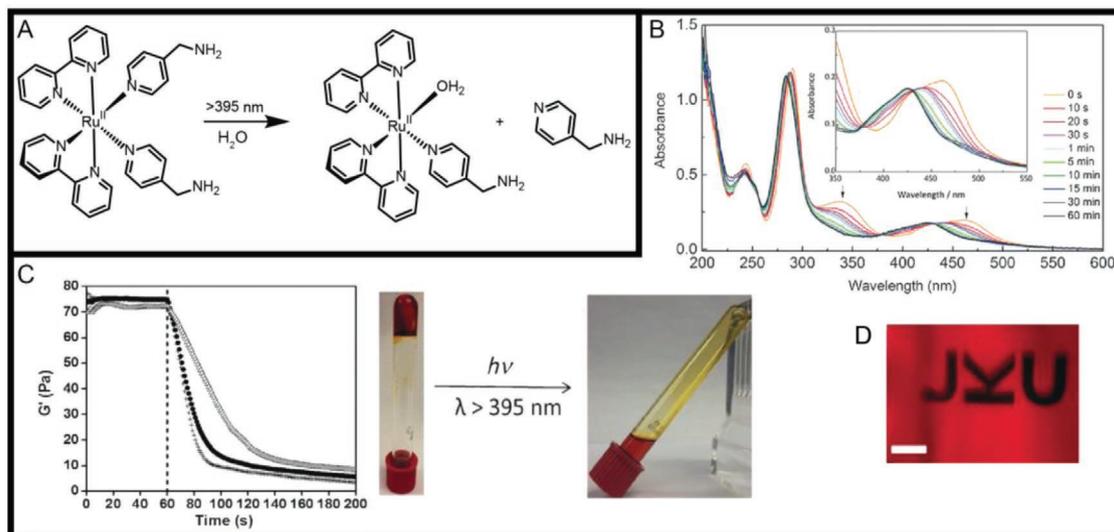In another example using ruthenium bipyridine (bpy) complexes as a backbone crosslinker in hydrogel materials, Theis

**ADVANCED
SCIENCE NEWS**

www.advancedsciencenews.com

**ADVANCED
HEALTHCARE
MATERIALS**

www.advhealthmat.de

Figure 4. Ruthenium-based gel undergoes rapid degradation using visible (>395 nm) light. A) Irradiation in water resulted in the exchange of one pyridine-based ligand for water. B) Extended irradiation in acetonitrile gave dual ligand exchange, as indicated by an intermediate absorption peak at 450 nm. C) Rapid gel degradation under visible light irradiation (400–500 nm, 10, 25, and 35 mW cm^{-2}) observed by photorheology. D) Multiphoton etching of ruthenium-crosslinked gel with 800 nm light. Scale bar = 100 μm. Adapted with permission under the terms of the CC-BY 4.0 license.[52] Copyright 2017, the Authors. Published by Wiley–VCH.

et al. synthesized a Ru complex with pendant amines for future reaction with a diisocyanate polymer (**Figure 4**A). Ru(bpy)$_2$(4-(aminomethyl)pyridine)$_2$ had similar photophysical properties as RuAldehyde, with the added feature of the exchange of both 4-(aminomethyl)pyridine (4AMP) ligands upon irradiation in acetonitrile (Figure 4B). In the same manner as RuAldehyde, Ru(bpy)$_2$(4AMP)$_2$ reacted rapidly and covalently with hexamethylene diisocyanate prepolymer and sequentially extended into a polymer matrix with Jeffamine ED-2003 to form a stable gel. Ru(bpy)$_2$(4AMP)$_2$ was also rapidly photocleaved with near-UV and visible light (>395 nm), yielding the photoproduct Ru(bpy)$_2$(4-AMP)(MeCN) within 1 min at a dilute concentration, with that time increasing for more concentrated samples (Figure 4C).

Ruthenium polypyridyl complexes are also photocleavable under multiphoton irradiation with near-IR high frequency pulsed light. In this case, Theis et al. etched a hydrogel crosslinked by Ru(bpy)$_2$(4-AMP)$_2$ using 800 nm light (Figure 4D). Low-energy light can be highly focused to such a point where the simultaneous absorption of two photons can lead to bond cleavage via the same electronic pathway. This highly focused light is powerfully demonstrated here in crisp edge-patterned soft materials due to the limited background cleavage. Multiphoton irradiation has also been proposed as a method to further red shift the excitation wavelength, though the small activation volume can make this method challenging for triggering larger scale events.

4.1.4. Light-Oxygen-Voltage Sensing-Domain 2 (LOV2): Modulating Material Stiffness, 470 nm

Another photoactive species recently popularized by the optogenetic field is the (LOV2) protein, which binds noncovalently and

high affinity to the small α-helical protein Jα.[53,54] Under mild blue light irradiation (470 nm), LOV2 undergoes a large conformation shift that displaces Jα by an estimated tens of angstroms (**Figure 5**A). Since this process rapidly reverses in the dark, materials crosslinked with LOV2-Jα can undergo iterative softening and stiffening cycles upon pulsed light irradiation (Figure 5B).

Liu et al. recently demonstrated reversible photoreversible stiffening of hydrogel biomaterials crosslinked with a diazide-modified LOV2-Jα fusion using SPAAC.[55] The conformation change of the LOV2-Jα interaction is traceable by UV–vis spectroscopy, with 470 nm irradiation nearly bleaching the visible absorbance, which can be recovered over time in the dark (Figure 5C). Once formed, the hydrogel can be repeatedly softened and stiffened again over time with very brief 470 nm irradiation windows (Figure 5D); this process that can be cycled hundreds of times with no measurable hysteresis.[55]

A powerful application space for on-demand softening materials is the culturing of fibroblasts in cell culture; fibroblasts are activated by stiff substrates, and variable stiffnesses are known to induce changes in cell morphology.[56] Liu et al. demonstrated the efficacy in using their LOV2-Jα material to culture and selectively activate fibroblasts in vitro using an α-smooth muscle actin luciferase (αSMA-Luc) reporter cell line. They cultured 3T3 fibroblast cells within 3D LOV2-Jα hydrogels in three conditions: under continuous light irradiation (470 nm, 1 mW cm^{-2}), in the dark, or with cycled irradiation (470 nm, 1 mW cm^{-2}, 1 min on, 4 min off). The first two conditions yielded expected results, whereby the comparatively soft irradiated hydrogels resulted in less activation than with the stiffer gels kept in the dark. Interestingly, the culture material with cycled compliance yielded the highest fibroblast activation, indicating a more complex pathway to fibroblast activation than previously suggested (Figure 5E).
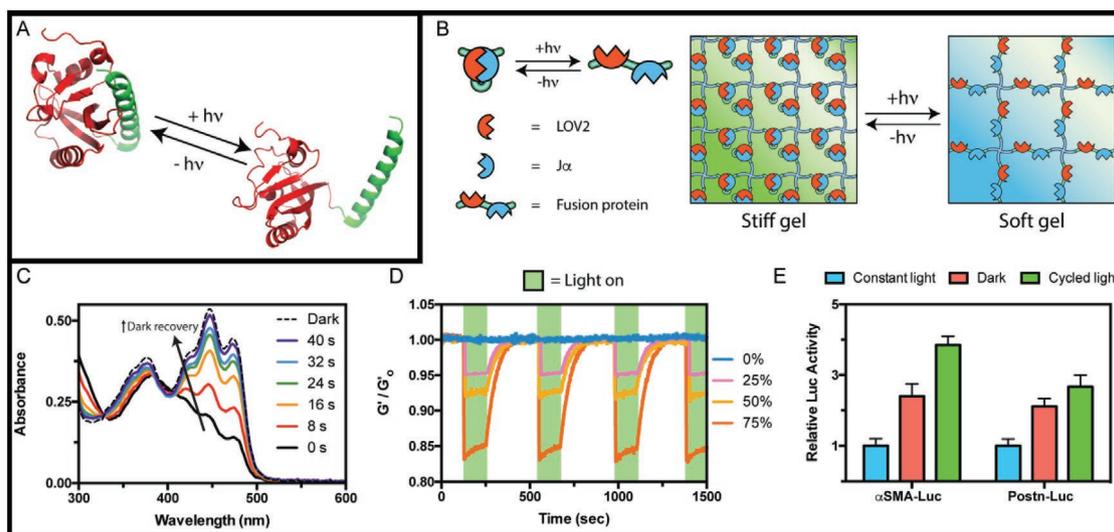
ADVANCED
SCIENCE NEWS
www.advancedsciencenews.com

ADVANCED
HEALTHCARE
MATERIALS
www.advhealthmat.de

Figure 5. LOV2-mediated material softening. A) LOV2 noncovalently binds to Jα, an interaction that can be disrupted by brief 470 nm irradiation. B) Schematic showing material stiffness changes upon irradiation, with recovery in the dark. C) UV–vis spectra tracking the reassociation of LOV2 and Jα. D) PEG-based hydrogel crosslinked with 0–75% of crosslinks formed from N_3-LOV2-Jα-N_3. Increasing percentage of LOV2-Jα-based crosslinks gave a higher dynamic range of gel softening upon irradiation. E) Increased fibroblast activation (as indicated by high αSMA-Luc expression) was observed for both stiff hydrogels and those with cycled stiffness. Adapted with permission.[55] Copyright 2018, Wiley–VCH.

The unusual findings presented by Liu et al. further highlighted the need for additional dynamic biomaterials development, including those that exploit external triggers for material modulation. Photocontrol over material stiffness or presentation of a signaling protein/peptide will enable crucial discoveries in the biological space as our techniques for in vitro cell culture better recapitulate complex biological phenomena.

4.2. Photoactive Species Responding to 500–600 nm Light

4.2.1. Ru(biq)$_2$(L)$_2$: Red Shifting Ruthenium Complexes, 592 nm

In the quest to establish small-molecule crosslinkers that cleave under low-energy irradiation, ruthenium polypyridyl complexes continue to dominate the literature. The relative ease of synthetic modifications for red shifting activation makes ruthenium complexes prime targets for use as visible light-sensitive material crosslinkers. Rapp et al. published one such example, demonstrating the effect of substituting the standard bipyridine ligand for 2,2′-biquinoline (biq). In these complexes, biq is an electron-withdrawing ligand that decreases the energy of the singlet metal-to-ligand charge transfer state responsible for the strong visible absorbance band.[57]

Rapp et al. demonstrated the synthesis of two Ru-based crosslinkers that have high efficiency in the 500–600 nm range: Ru(bpy)(biq)L$_2$ and Ru(biq)$_2$L$_2$, where L is 5-hexynenitrile. Both were stable in the dark, but could be rapidly photodegraded; upon irradiation with visible light (532 nm for both), nitrile-based ligand exchange with water (**Figure 6**A) was confirmed by UV–vis spectroscopy (Figure 6B). The modification of the photolabile ligands with an alkyne permitted incorporation into a PEG-based hydrogel formed via copper-mediated azide-alkyne

cycloaddition (CuAAC). Ru(biq)$_2$(5-hexynenitrile)$_2$-crosslinked hydrogels underwent photolysis using orange light (592 nm), irradiating into the extensive absorbance tail of the complex. This permitted wavelength-selective degradation in the presence of hydrogels crosslinked with the blue-shifted Ru(bpy)$_2$(5-hexynenitrile)$_2$ (Figure 6C).

4.2.2. Ru-H$_2$O: Reconfiguring Surface Properties, 530 nm

Ru(biq)-type polypyridyl complexes have found recent popularity not only as crosslinkers for hydrogel materials but also in the development of surface modulatory molecules designed for in vivo usage. Recently, Xie et al. demonstrated the use of a Ru(tpy-COOH)(biq)(H$_2$O) complex, where tpy-COOH is 6-2,2′:6′,2-″terpyridin-4′-yloxy hexanoic acid, to coordinate thioether ligands reversibly at room temperature (**Figure 7**A).[58] Thioether-modified ligands coordinate to ruthenium centers under relatively mild conditions (10 molar excess, ambient temperature). Once coordinated, the ligand is thermally stable but photolytically active. Upon irradiation with green light (530 nm), the thioether ligand is exchanged with water (Figure 7B).

With such mild coordination conditions, Xie et al. showed the ability to reconfigure a surface modified with Ru(tpyCOOH)(biq)(H$_2$O) complexes via the COOH group on the tpy ligand. Room temperature exchange of one thioether-modified fluorophore was demonstrated with fluorescein (FITC)-isothiocyanate and rhodamine (Rhod)-isothiocyanate (Figure 7C). Soaking the surface in thioether solution (10×10^{-3} M) for 1 h was sufficient to coordinate the fluorophore to the surface, which was released into solution following a 10 min irradiation with 530 nm light. Beyond the decorating of surfaces with fluorophores, Xie et al. also demonstrated the photomodulation of
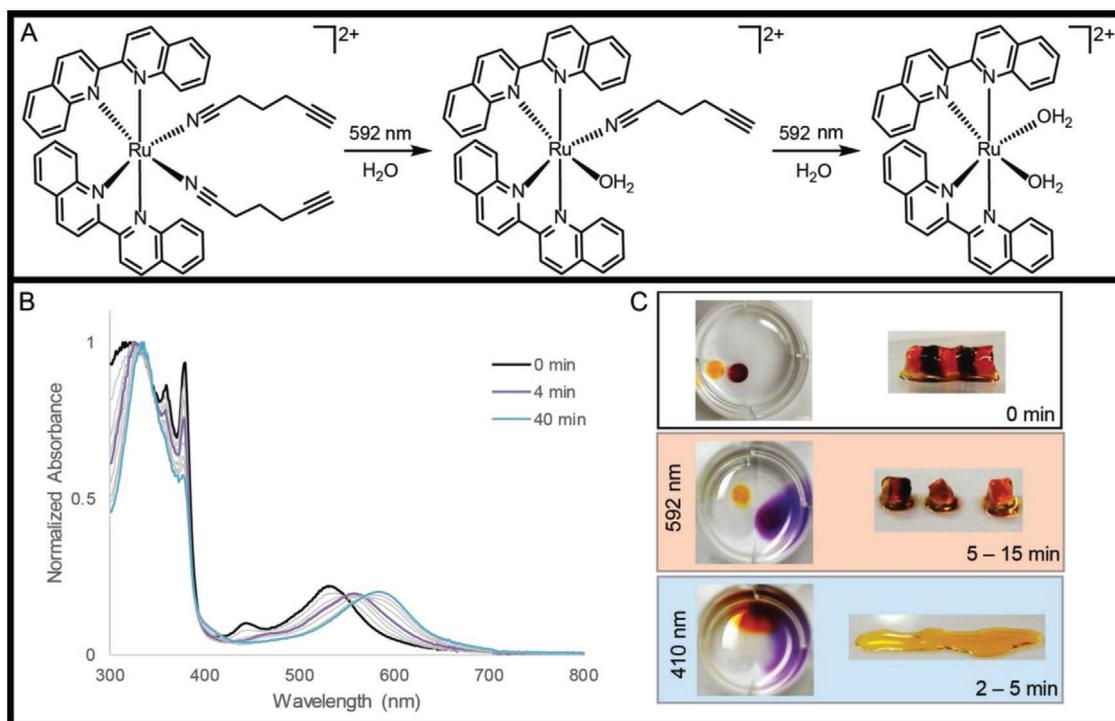
Figure 6. Ruthenium complexes can be redesigned to absorb low-energy light. A) Ru(biq)$_2$L$_2$ complexes exchange both ligands upon irradiation into the visible ^{1}MLCT band. B) UV–vis trace showing the shift in absorbance during Ru(biq)$_2$L$_2$ irradiation under 532 nm irradiation. C) Selective material degradation using Ru(biq)$_2$L$_2$ and Ru(bpy)$_2$L$_2$-crosslinked hydrogel materials. Irradiation with orange light led to degradation of only "red" hydrogels crosslinked with Ru(biq)$_2$L$_2$, followed by blue light irradiation degrading "orange" hydrogels. Adapted with permission under the terms of the CC BY-NC 3.0 license.[57] Copyright 2019, the Authors. Published by The Royal Society of Chemistry.

protein adsorption and the wettability of the surface by substituting alternatively modified thioether ligands. In one example, a thioether-terminated PEG ligand was coordinated to prevent protein adsorption, demonstrating photopatterned protein adsorption with fluorescently labeled bovine serum albumin (BSA) (Figure 7D). As it relates to the foreign body response in vivo, protein adsorption on hard biomaterials remains an area of active study; the use of modified ruthenium complexes to modulate surface properties has excellent promise in future in vivo exploratory studies.[59–62]

4.3. Photoactive Species Responding to 600–900 nm Light

4.3.1. Tetra-Ortho-Methoxy-Substituted Azobenzene: Red Shifting Guest–Host Interactions, 625 nm

Azobenzene–cyclodextrin supramolecular complexes have been used frequently in the past to transiently crosslink hydrogel matrices.[63–65] Azobenzene molecules in their *trans* conformation form a hydrophobic interaction with the core of betacyclodextrin rings, which is interrupted as the azobenzene undergoes a *trans–cis* isomerization under UV light irradiation.[63] The noncovalent nature of the hydrophobic interactions between the azobenzene group and the hydrophobic core of the cyclodextrin leads to the generation of strong yet shearthinning hydrogel matrices which find utility in injectable hydrogels.[64,66] Efforts to red-shift azobenzene's activation have

focused on the addition of electron-withdrawing groups on the benzene rings, reducing the energy of the N=N bond and adding steric strain, as demonstrated recently by Wang et al. in the form of a tetra-ortho-methoxy-substituted azobenzene (mAzo, **Figure 8**A).[67]

In their recent work, Wang et al. synthesized an mAzomodified polymer, which was coupled with a β-cyclodextrinmodified poly(acrylic acid) to form a stiff hydrogel. Their modified mAzo group responded to red-light irradiation (625 nm) and underwent a *trans-cis* isomerization triggering a gel-to-sol transition in the hydrogel material.[68] Under 625 nm irradiation or heat, the mAzo reverted to the *trans* state and restored the initial gel stiffness (Figure 8B). The reversibility of the gel–sol transition was tested by sequentially irradiating and heating the sample, demonstrating near-complete conversion between the *cis* and *trans* isomers of the mAzo, and reversible stiffening/softening of the hydrogel.

On-demand protein release from the mAzo/β-cyclodextrinbased hydrogel was evaluated using model species BSA. Fluorescently labeled BSA was noncovalently loaded into the gel, which was released in vitro upon red light-triggered hydrogel degradation (Figure 7C). This transition was also demonstrated through 2 mm thick porcine tissue placed between the light source and the hydrogel (Figure 8D), highlighting the potential for in vivo regulation. The design of a supramolecular hydrogel to deliver proteins on demand in response to tissuepenetrating red light is a robust one; based on the various published structures of red-shifted azobenzenes, this represents a
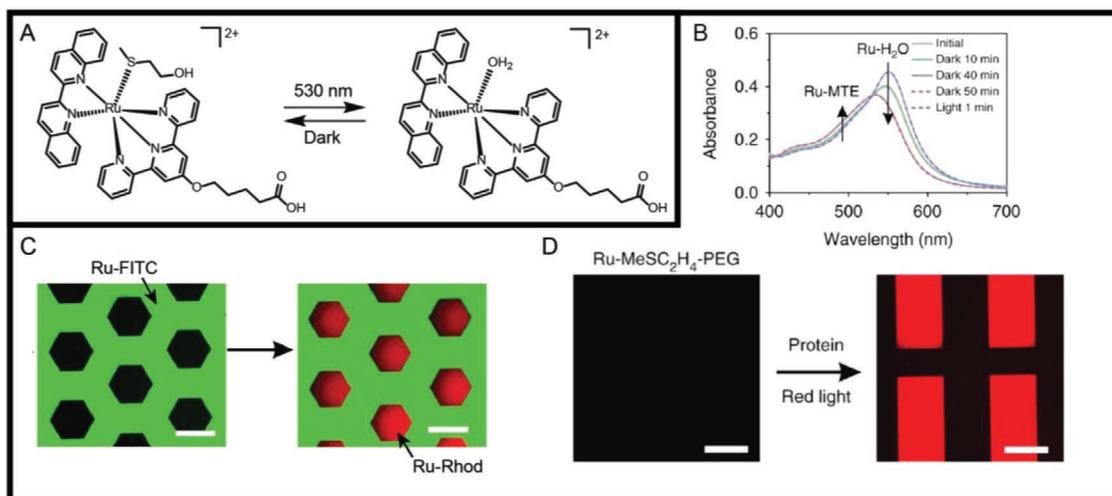
Figure 7. Using ruthenium complexes to modify surface properties such as protein adhesion and wettability. A) Coordination of thioethers is possible at room temperature, generating a material stable for days in aqueous environments until irradiated with visible light. B) Coordination and photolysis can be tracked by UV–vis absorbance spectroscopy. C) Patterning of FITC and Rhod dyes on the surface of a material with green-light irradiation. D) Patterning protein absorption with FITC-tagged BSA on a surface protected with PEG-thioether. PEG bound to Ru was photoreleased upon irradiation with red light, revealing un-PEGylated surfaces for protein binding. Scale bars = 300 µm. Adapted with permission under the terms of the CC-BY license.[58] Copyright 2018, the Authors. Published by Springer Nature.

promising avenue worth further pursuit.[69–72] The possibility for a modulatory hydrogel material for easy redesign with different azobenzene groups is also worth considering with this system, as the same β-cyclodextran-modified polymer can be used to crosslink hydrogel materials with different azobenzene groups, potentially yielding hydrogel materials that respond to different wavelengths of light. As of the time of this review's publication, such a strategy has yet to be reported in the literature.

4.3.2. Cyanobacterial Phytochrome 1: A Reversible Noncovalent Protein–Protein Interaction (740 nm)

Taking inspiration from developments in the optogenetics community toward red light-induced protein–protein interactions, Horner et al. repurposed a recently reported light-responsive protein derived from cyanobacterial phytochrome 1 (Cph1). Cph1 undergoes a secondary structure shift upon irradiation with red (660 nm) leading to protein dimerization;
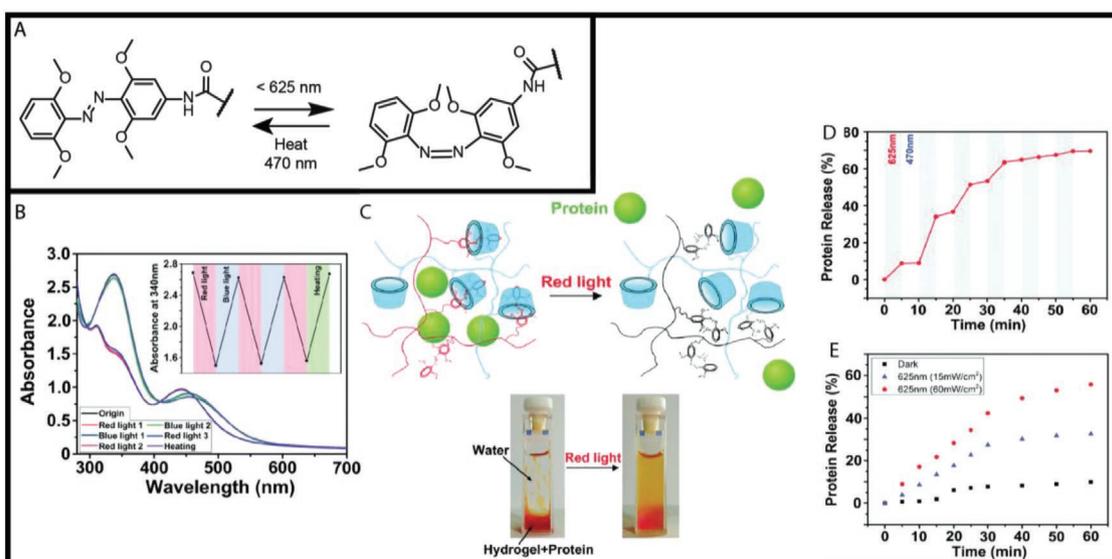

Figure 8. Red-shifted methoxy-azobenzene enables protein release from hydrogels through tissue. A) The reversible cis–trans isomerization of mAzo under red-light irradiation, followed by heating or blue-light irradiation. B) Reversibility of the isomerization process observed by absorbance spectroscopy. C,D) FITC-tagged BSA release from hydrogels upon gel softening with red light (<625 nm). E) BSA release followed by irradiation through 2 mm thick porcine tissue with red light. Adapted with permission under the terms of the CC BY-NC 3.0 license.[67] Copyright 2015, the Authors. Published by The Royal Society of Chemistry.
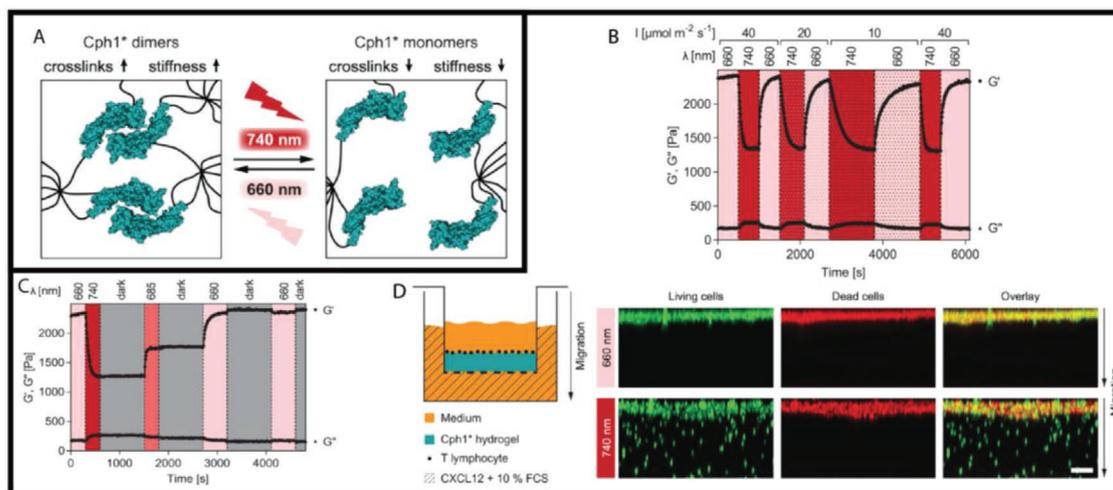
**ADVANCED
SCIENCE NEWS**

www.advancedsciencenews.com

**ADVANCED
HEALTHCARE
MATERIALS**

www.advhealthmat.de

Figure 9. Cph1-crosslinked PEG hydrogels for tissue engineering. A) Cph1 homodimerizes under 660 nm irradiation, and reverts to its monomeric form under 740 nm illumination. B) This process is reversible, leading to softening and stiffening of PEG-based hydrogel. C) Exposure of gels to intermediate wavelengths offers materials with intermediate stiffness. D) T cell migration following a gradient of CXCL12 through a hydrogel material which had been softened with IR light. Scale bar = 100 µm. Adapted with permission.[76] Copyright 2019, Wiley–VCH.

far-red light (740 nm) induces reversion to the monomeric state (**Figure 9**A).

The photocontrolled dimerization/monomerization of Cph1 permits the ability to reversibly soften and stiffen a hydrogel material. This, coupled with the inherent biocompatibility of the genetically encoded system, is a powerful tool for the design of materials to study stem cell differentiation and cell migration. Horner et al. showed the reversible softening and stiffening of their hydrogel material (eight-arm PEG, end-functionalized with Cph1) both rheologically and by measuring the relative pore size of the material (Figure 9B). Hydrogels formed through Cph1 homodimerization can be reversibly softened and stiffened upon iterated exposure to 740 or 660 nm light, opening channels for cell migration while softening the material. Such photocontrollability extends to different wavelengths between 660 and 740 nm, wherein exposure to intermediate wavelengths can also stiffen the hydrogel materials (Figure 9C). Material photomodulation was used to control T cell migration through a gel (Figure 9D).

While this report highlighted the strength of this material in benchtop experiments, with an impressively red-shifted response, a Cph1-crosslinked gel would be especially powerful in studying the effect of changing material dynamics in vivo. Softer materials may interact with the host's immune system differently than stiff materials, prompting the study of varying material stiffness in implanted biomaterials.[73,74] Additionally, modulating material stiffness in vivo could provide researchers with a platform for studying heart disease and fibroblast activation.[15,75]

5. Multiplexing Biomaterial Photoresponse: More Wavelengths Means More Power

With this concerted push to develop crosslinking methodologies that respond to low-energy light, many new opportunities in basic research are made available. Expanding the library of

photolytic molecules across the rainbow of the visible spectrum not only allows researchers to develop clinically relevant technologies, but also permits selective control over multiple events in the same system. Biology is comprised of a series of well-coordinated sequential events spanning many time and length scales; the ability to affect more than one event without disturbing the system (e.g., reopening a wound, sequentially administering several drugs) is a potentially powerful tool that has not yet been realized for in vivo systems. Light is uniquely positioned to trigger events sequentially within a material, but despite the advancement of new photoresponsive molecules that respond to lower energy light, surprisingly few applications of wavelength-selective dynamic biomaterials have been published to date. Here we will review some of the select few combinations that demonstrate the power of a multiplexed photocleavable system, as well as potential future applications where this technology might prove to be useful.

5.1. Nitrobenzyl and Coumarin: 365 and 405 nm

In one of the first examples of wavelength-selective biomaterial response, Azagarsamy et al. used oNB- and coumarin-based crosslinkers (**Figure 10**A) to selectively release both a small molecule pair (i.e., rhodamine and fluorescein) and two different proteins [bone morphogenic protein (BMP)-2 and BMP-7] from PEG-based hydrogels (Figure 10B).[77,78] This early example represented a demonstration of the power of exploiting wavelength-selective photochemistries to control biomolecule presentation to living cells.

Reports suggest the importance of concurrent versus sequential release of BMP-2 and BMP-7 in differentiation of mesenchymal stem cells (MSCs);[79,80] utilizing a single hydrogel-based system that can release BMP-2 or BMP-7 in response to different wavelengths permits quantitative investigation of this phenomena. Using the oNB and coumarin-based photocleavable linkers, Azagarsamy et al. demonstrated
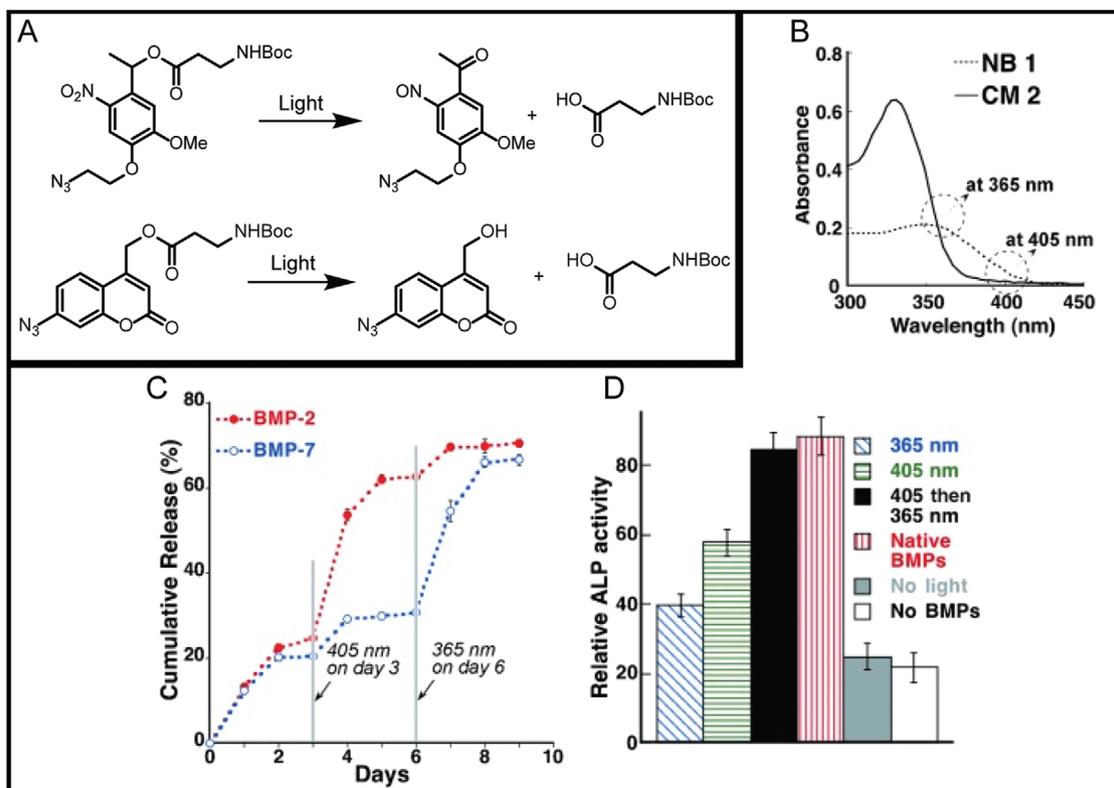
Figure 10. *o*-Nitrobenzyl (*o*NB) and coumarin-based photodegradable crosslinkers used to direct stem cell growth. A) *o*NB- and coumarin-based linkers undergo photoscission. B) Absorbance spectra of *o*NB and coumarin linkers. C) Sequential release of BMP-2 (attached to the hydrogel via *o*NB) and BMP-7 (attached via coumarin). D) hMSCs undergo osteogenic differentiation (indicated by increased alkaline phosphase (ALP) activity) upon release of both proteins. Adapted with permission.[77] Copyright 2013, Wiley–VCH.

sequential delivery of these proteins. In doing so, the authors successfully regulated lineage-specific differentiation of human MSCs (Figure 10D). While these studies represented a powerful technological breakthrough, absorbance wavelengths of *o*NB (activated at the low end of the visible spectrum) and coumarin (activated in the near-UV) limit this design to benchtop applications. Additionally, there were challenges in this combination of crosslinkers in achieving selective protein release due to incomplete photocleavage wavelength orthogonality.

5.2. Nitrobenzyl and Perylene: 365 and 470 nm

Photodegradable hydrogels are powerful tools not only in delivering small molecules or protein-based therapeutics, but also for the release of cells from their matrix and eventual degradation in the body. Multiple studies using rapidly degrading hydrogels to release cells for further sorting or seeding experiments have shown this technique to be invaluable on the benchtop.[3,16,30,81,82] Modifying these hydrogel matrices to respond to individual wavelengths of light could be a powerful tool in its own right, triggering the sequential release of specific cell types into surrounding tissue. Truong et al. recently demonstrated such a strategy, as well as a new synthetic method of easily exchanging one photoresponsive molecule for another.[83]

Using two wavelength-separated photosensitizing molecules based on *o*NB (P2) and perylene (P8) (**Figure 11**A), they

formed two hydrogels that selectively respond to different wavelengths of light (Figure 11B). The authors demonstrate a rheological decrease in hydrogel elasticity upon irradiation with green (530 nm), blue (470 nm), and near-UV (365 nm) light (Figure 11C), choosing photosensitizers P8 for its excellent response to blue and green light, and P2 for its near complete wavelength-separated response from P8.

Cell viability remained high for those encapsulated within and photoreleased from hydrogels modified with P2 and P8 (Figure 11D). Hydrogels crosslinked with P2 and P8 were seeded with human mesenchymal stem cells (hMSCs) and L929 murine fibroblasts, respectively and incubated at 37 °C for several days. Upon irradiation with blue light (20 mW cm^{-2}, 10 min), the P8 hydrogel was completely degraded and L929 fibroblasts released to the media. Subsequent irradiation with near-UV light (10 mW cm^{-2}, 10 min) released the hMSCs (Figure 11D). Cell viability was not significantly decreased during or after irradiation for all light sources, except for extended irradiation with green light (93% viability of hMSCs was observed after 20 min irradiation with 520 nm light).

While this combination of crosslinkers still depends on the UV-responsive *o*NB, the use of green light here, as well as the complete wavelength separation of the two crosslinkers represents a significant advance. One of the strongest aspects of this report is the facile synthesis and incorporation of multiple photosensitizing groups that can be used to control material degradation, as well as their cytocompatibility in 3D cell culture.
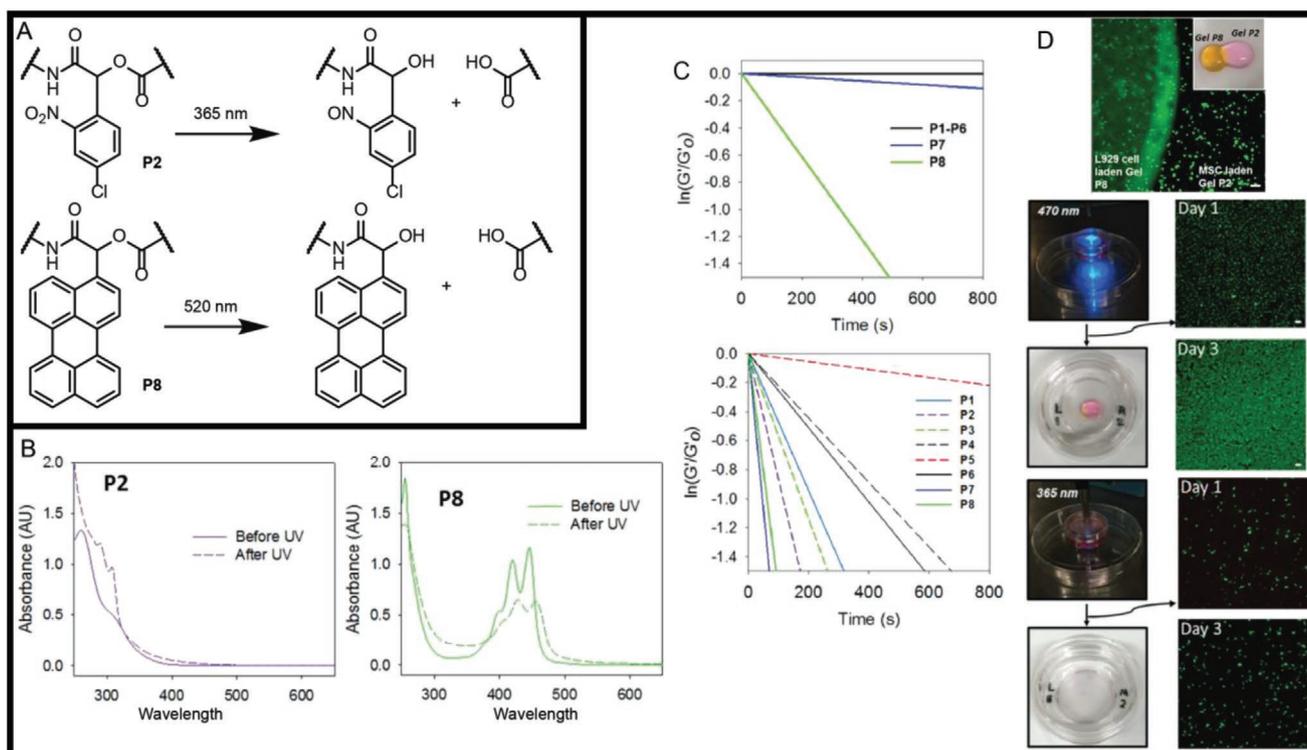
Figure 11. Wavelength-selective hydrogel degradation. A) Photosensitizing molecules P2 and P8 based on *o*NB and perylene respectively are used to create a photolabile bond in the PEG-based hydrogel network. B) UV–vis absorbance traces of P2 and P8 showing excellent wavelength separation of the two chromophores. C) Rheology demonstrating selective degradation of a PEG-based hydrogel crosslinked with P8 and P2 at 520 nm (top) and 365 nm (bottom). Both P8 and P2 degrade at 365 nm, but only P8 shows excellent degradation properties at 520 nm. D) Viable cell release from P8 and P2-modified hydrogels, extending to 3 days post hydrogel release. For these studies, 470 and 365 nm were used to degrade the hydrogel selectively and to release viable cells. Adapted with permission.[83] Copyright 2017, American Chemical Society.

The power of such a simple method for creating a photodegradable hydrogel could be realized in future reports to create a complex hydrogel system with relative synthetic ease, expanding the response wavelength further into the visible spectrum. Future studies should identify and characterize these new photoproducts to confirm their lack of toxicity and the presence of any side products, free radicals, or reactive oxygen species formed.

5.3. Ru(bpy)$_2$L$_2$ and Ru(biq)$_2$L$_2$: 450 and 592 nm

In the first completely visible dual-wavelength responsive system, Rapp et al. used two ruthenium complexes as crosslinkers for a PEG-based hydrogel, inducing hydrogel degradation selectively using orange (592 nm) and blue (450 nm) light.[57] Coordinating nitrile-based ligands modified with an alkyne, they were able to form a hydrogel with azide-modified four-arm PEG and selectively degrade hydrogel sections. The kinetics of bond cleavage only permitted unidirectional degradation, with the red-shifted Ru(biq)$_2$L$_2$ having an efficiency of photocleavage at 520 M^{-1} cm^{-1} compared to blue-shifted Ru(bpy)$_2$L$_2$'s efficiency of 980 M^{-1} cm^{-1}.

The separation of the two complex's maximum absorbance was over 100 nm (420 nm for Ru(bpy)$_2$L$_2$, 535 nm for Ru(biq)$_2$L$_2$), permitting wavelength-selective activation with far less background than has been reported with other photodegradable crosslinker combinations. The system reported by Rapp et al. was not tested in vitro for its biocompatibility, but the exclusive use of low-energy light shows promise for further studies with ruthenium-based crosslinkers.

6. Perspectives

In this review, we have highlighted many research applications where light-responsive biomaterials have and continue to shine. While most dynamic photosensitive biomaterials still depend upon UV-initiated chemistries, we have highlighted recent efforts to create materials that respond to visible and near-IR light. In these early efforts, materials containing transition metal complexes or based on photoresponsive proteins have been reported, each offering great promise toward in vivo modulation and in wavelength-selective biomaterial alteration.

6.1. Application Space for Wavelength-Selective Dynamic Biomaterials

6.1.1. Dynamic Matrix Stiffness

The ability to modulate material stiffness over time offers a powerful handle in mechanobiology. While materials of

ADVANCED
SCIENCE NEWS
www.advancedsciencenews.com

ADVANCED
HEALTHCARE
MATERIALS
www.advhealthmat.de

different stiffness may be implanted in vivo, allowing for study of static stiffness on immune response and cell health, dynamically compliant materials may offer additional insights into cell mechanobiological response. In preliminary work discussed previously, repeated stiffening and softening of hydrogel materials containing encapsulated 3T3 fibroblasts yielded differential activation as compared with statically stiff or soft materials.[55] Extension of this work in vivo would further elucidate the role of dynamic network stiffness on cell fate.[15,75]

As softer materials are thought to interact with the host immune system differently than stiff materials, several studies have been performed using materials of varying static stiffness.[73,74] In one recent example, stiffer hydrogels (323 kPa) were found to polarize THP-1-derived macrophages to a more pro-inflammatory state, while softer hydrogels (11–32 kPa) were more conducive to a pro-healing state.[84] As this phenomenon has been studied primarily in vitro with cultured cell lines on static materials, strategies to study immune activation in response to dynamically stiffening materials both in vitro and in vivo would be of interest.

Dynamic alteration to ECM stiffness also plays a significant role in heart disease, where injury can increase local ECM stiffness from 3 up to 50 kPa.[85] This increase in ECM stiffness in turn activates fibroblasts, which further perpetuates the disease state through factor secretion and matrix remodeling. Understanding and mediating this response to injury is key to development of new cell-based therapies for heart injury repair. Moreover, as fibroblast activation occurs relatively quickly (<10 min),[86] development of materials capable of manipulating this dynamic environment will be key to the discovery of new therapies.[87,88]

6.1.2. Sequential Therapeutic Cargo Release

Sequential or combinatorial release of multiple therapies is incredibly promising, especially in the areas of chemotherapy (usually exploiting small molecule drugs)[89–91] and directing local tissue response to biomaterials (usually proteins).[92,93] In a recent example, combinatorial delivery of neurotrophic factor-3 and glial-cell derived neurotrophic factor was found to improve healing following spinal cord injury.[93] Biomaterials designed for sequential protein therapeutic release have been used in vivo to improve healing following myocardial infarction, bone fractures, and rebuild vascular networks.[94] Virtually all of these reported systems rely on inherent physiological properties, such as hydrolysis or diffusion rates. User-triggered therapeutic release is likely to provide a better foundation for future designs, elucidated proper dose and timing of release to maximize positive outcomes. Delivery strategies taking advantage using low-energy, wavelength-separated light would provide for enhanced release control while informing future autonomous biomaterial design.

6.2. Challenges in Synthesizing NIR-Responsive Molecules

Photoresponsive molecules that cleave under low-energy light irradiation are universally expected to contain at least one "weak" bond, presenting challenges for the synthesis of photocleavable linkers that are dark stable. Molecular design must carefully balance molecular orbital adjustment to yield accessible excited states for bond cleavage, while simultaneously maintaining thermal stability in complex biological environments. Side-stepping this tall order, researchers have sought to render known stable photoresponsive molecules responsive to low-energy light. There are two common techniques currently advocated in the literature: 1) coupling UV-absorbing photoresponsive molecules with upconverting, NIR-absorbing nanoparticles, or 2) using multiphoton irradiation with highly pulsed NIR light to cleave bonds.

6.2.1. Upconverting Nanoparticles and Photothermal Therapy

Coupling photoresponsive molecules with upconverting nanoparticles is a common method for improving the low-energy light absorption of many well-defined photoresponsive molecules. The marriage of upconverting nanoparticles and current photoresponsive technologies is a logical one, taking advantage of the significant research in upconversion borrowed from solar cell research as well as the rich history of near-UV-sensitive oNB groups in biological applications. Much of the research in red-light sensitive materials relies on using upconverting nanoparticles to transform red or near-IR light into higher energy blue or UV light. Coupled with oNB and coumarin photoresponsive molecules, these nanoparticles or nanomaterials can render a material sensitive to low-energy light.

Nanoparticulate hybrid systems can be designed to respond to light in different ways. Upconverting nanoparticles convert low-energy light to high-energy light, enough to cleave a photocleavable group attached nearby. Photothermal nanoparticulate systems convert low-energy light into heat, which has localized effects on the material, degrading or melting the hydrogel material immediately surrounding the nanoparticles. However, both of these methods have significant drawbacks when translating in vivo.

Thermal material degradation often requires prohibitively large photonic flux, far more than the recommended maximum light exposure of 330–350 mW cm^{-2} for near-IR wavelengths between 808 and 980 nm.[95] Though recent advancements have achieved localized heating with lower fluxes of light,[96] the use of metal nanoparticles still presents several challenges for translation into in vivo experiments. Upconverting nanoparticles suffer from similar challenges. Upconversion efficiencies are regularly minimal at best, requiring high light power and flux to achieve an effect in the biomaterial.[97] Upconversion has been used to pattern proteins on surfaces,[98] and release protein cargo through 2 cm thick tissue,[99] but the strategy has not yet been demonstrated in vivo.

6.2.2. Two-Photon Response In Vivo

The other response to the challenges of high-energy irradiation is the use of multiphoton irradiation—most commonly two-photon (2P)—to cleave photoresponsive molecules. 2P microscopy has recently gained ground primarily on the basis of

**ADVANCED
SCIENCE NEWS**

www.advancedsciencenews.com

**ADVANCED
HEALTHCARE
MATERIALS**

www.advhealthmat.de

its use of low-energy laser lines which increase photonic penetration depth. As many common photoresponsive molecules have a non-zero 2P cross-section, they lithographically respond to low-energy 2P irradiation in much the same way they do to high-energy single-photon light. For example, the common *o*NB group can be cleaved with highly pulsed 740 nm light that falls well within the PDT window.[11] Using a 2P-based strategy based on pulsed NIR light eliminates the need to redesign new photosensitive molecules and provides an easier pathway to in vivo regulation of light-responsive biomaterials.

The use of multiphoton light is not without its challenges, however. Since the requirement that each molecule absorbs two photons at nearly the same moment, the focal volume of a 2P microscope is comparatively quite small ($\approx$0.5 μm^3). While such a small focal volume enables precise control over biomaterial properties, modulating materials using this strategy is comparatively slow, rendering it challenging when trying to achieve macroscale changes. Challenges with the small irradiation volume are further compounded by optical scattering through tissue, requiring longer irradiation times to achieve the same effect deeper within materials. While 2P-based material modification has been demonstrated as a powerful tool for in vitro and ex vivo experiments, it remains largely unutilized for material regulation in vivo.

6.3. Toxicity Concerns with Ruthenium Complexes In Vivo

With the prominence of the use of ruthenium complexes as new photoresponsive molecules come enhanced toxicity concerns, particularly when suggested that they be used in vivo. As these complexes were repurposed from cytotoxic photodynamic therapy designs, these concerns are valid and have led to some hesitancy regarding their further use. Early reports on the circulation and toxicity of these polypyridyl complexes suggested that freely circulating Ru molecules are rapidly flushed out via the kidneys.[100] Recent work on ruthenium polypyridyl-containing micelles demonstrated their use in vivo as well.[101–103] Upon degradation, the micelles released ruthenium photoproducts into local tissue and the bloodstream; no ruthenium buildup in any major organ (i.e., liver, spleen, kidney, lung) or tissue damage was observed.

Recent work using ruthenium complexes as hydrogel crosslinkers suggested that the toxicity of these complexes can be further mediated by ensuring that the metal cationic core remains tethered to the polymer network upon photocleavage. Ru-HA hydrogel photoproducts were minimally toxic at low-medium concentration to cell in culture, compared to the relatively high toxicity of Ru complex photoproducts.[51] This work highlights the importance of material design in minimizing tissue toxicity of these metal-based photosensitive compounds.

6.4. The Future of Low-Energy Light-Responsive Biomaterials

In seeking to develop molecules that respond to visible and near-IR light, consideration should be taken with respect to species handling during synthesis and utilization. Unwanted and premature photoactivation of low-energy light-sensitive species

can be minimized through handling in the dark or research spaces outfitted with filtered light. Though these represent issues easy to overcome, they do highlight an ongoing challenge in the area: red- and near-IR-cleavable groups should be thermally stable while photolytically unstable, all without being so photosensitive that they are impractical to work with. Though a fine line to walk, early successes in the field (as reviewed in this work) have demonstrated that this is possible.

Though efforts to red-shift activation of organic photoresponsive molecules continue to show success, newer and alternative strategies based on photoresponsive proteins or transition metal complexes have rocketed to the forefront. Optogenetics has driven innovation in genetically encoded protein-based strategies that respond to far-red and NIR light. Leveraging light-mediated protein–protein interactions will continue to open new doors in the biomaterials space.[104] Ruthenium complexes have also been demonstrated in several biomaterial applications, and may continue to be useful in future in vivo experiments as near-IR-sensitive crosslinkers. Toxicity concerns appear to be modulatory depending on compound design, though further research is warranted to determine the specific origins of their toxicity and the photocleavage process of these complexes.

Light remains a uniquely powerful stimulus for modulating dynamic biomaterial properties. Continued innovation in the synthesis of photoactive molecules that respond to low-energy light will lead to expanded opportunities for in vivo activation through complex tissue and in biologically relevant locations. Future translational opportunities for such photosensitive materials remain bright.

Conflict of Interest

The authors declare no conflict of interest.

[1] E. R. Ruskowitz, C. A. DeForest, *Nat. Rev. Mater.* **2018**, *3*, 17087.
[2] P. M. Kharkar, R. A. Scott, L. P. Olney, P. J. LeValley, E. Maverakis, K. L. Kiick, A. M. Kloxin, *Adv. Healthcare Mater.* **2017**, *6*, 1700713.
[3] D. R. Griffin, A. M. Kasko, *J. Am. Chem. Soc.* **2012**, *134*, 13103.

[4] A. M. Kloxin, A. M. Kasko, C. N. Salinas, K. S. Anseth, *Science* **2009**, *324*, 59.

[5] S. Khetan, J. S. Katz, J. A. Burdick, *Soft Matter* **2009**, *5*, 1601.

[6] C. A. DeForest, K. S. Anseth, *Nat. Chem.* **2011**, *3*, 925.

[7] R. S. Stowers, S. C. Allen, L. J. Suggs, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 1953.

[8] J. A. Burdick, W. L. Murphy, *Nat. Commun.* **2012**, *3*, 1269.

[9] N. Brandenberg, M. P. Lutolf, *Adv. Mater.* **2016**, *28*, 7450.

[10] K. A. Heintz, M. E. Bregenzer, J. L. Mantle, K. H. Lee, J. L. West, J. H. Slater, *Adv. Healthcare Mater.* **2016**, *5*, 2153.

[11] C. K. Arakawa, B. A. Badeau, Y. Zheng, C. A. DeForest, *Adv. Mater.* **2017**, *29*, 1703156.

[12] S. Khetan, M. Guvendiren, W. R. Legant, D. M. Cohen, C. S. Chen, J. A. Burdick, *Nat. Mater.* **2013**, *12*, 458.

[13] C. A. DeForest, D. A. Tirrell, *Nat. Mater.* **2015**, *14*, 523.

[14] C. Yang, M. W. Tibbitt, L. Basta, K. S. Anseth, *Nat. Mater.* **2014**, *13*, 645.

[15] K. A. Günay, T. L. Ceccato, J. S. Silver, K. L. Bannister, O. J. Bednarski, L. A. Leinwand, K. S. Anseth, *Angew. Chem.* **2019**, *131*, 10017.

[16] T. E. Brown, I. A. Marozas, K. S. Anseth, *Adv. Mater.* **2017**, *29*, 1605001.

[17] A. M. Kloxin, M. W. Tibbitt, K. S. Anseth, *Nat. Protoc.* **2010**, *5*, 1867.

[18] Y. Luo, M. S. Shoichet, *Nat. Mater.* **2004**, *3*, 249.

[19] M. S. Hahn, J. S. Miller, J. L. West, *Adv. Mater.* **2006**, *18*, 2679.

[20] C. A. DeForest, B. D. Polizzotti, K. S. Anseth, *Nat. Mater.* **2009**, *8*, 659.

[21] R. G. Wylie, S. Ahsan, Y. Aizawa, K. L. Maxwell, C. M. Morshead, M. S. Shoichet, *Nat. Mater.* **2011**, *10*, 799.

[22] K. A. Mosiewicz, L. Kolb, A. J. van der Vlies, M. M. Martino, P. S. Lienemann, J. A. Hubbell, M. Ehrbar, M. P. Lutolf, A. J. van der Vlies, M. M. Martino, *Nat. Mater.* **2013**, *12*, 1071.

[23] J. C. Grim, T. E. Brown, B. A. Aguado, D. A. Chapnick, A. L. Viert, X. Liu, K. S. Anseth, *ACS Cent. Sci.* **2018**, *4*, 909.

[24] J. A. Shadish, G. M. Benuska, C. A. DeForest, *Nat. Mater.* **2019**, *18*, 1005.

[25] M. C. Koetting, J. T. Peters, S. D. Steichen, N. A. Peppas, *Mater. Sci. Eng., R* **2015**, *93*, 1.

[26] B. A. Badeau, M. P. Comerford, C. K. Arakawa, J. A. Shadish, C. A. DeForest, *Nat. Chem.* **2018**, *10*, 251.

[27] B. A. Badeau, C. A. DeForest, *Annu. Rev. Biomed. Eng.* **2019**, *21*, 241.

[28] J. V. Frangioni, *Curr. Opin. Chem. Biol.* **2003**, *7*, 626.

[29] T. T. Lee, J. R. García, J. I. Paez, A. Singh, E. A. Phelps, S. Weis, Z. Shafiq, A. Shekaran, A. Del Campo, A. J. García, *Nat. Mater.* **2015**, *14*, 352.

[30] P. J. LeValley, A. M. Kloxin, *ACS Macro Lett.* **2019**, *8*, 7.

[31] J. S. Katz, J. A. Burdick, *Macromol. Biosci.* **2010**, *10*, 339.

[32] S. H. Yun, S. J. J. Kwok, *Nat. Biomed. Eng.* **2017**, *1*, 0008.

[33] Z. Huang, *Technol. Cancer Res. Treat.* **2005**, *4*, 283.

[34] J. P. Celli, B. Q. Spring, I. Rizvi, C. L. Evans, K. S. Samkoe, S. Verma, B. W. Pogue, T. Hasan, *Chem. Rev.* **2010**, *110*, 2795.

[35] J. F. Lovell, T. W. B. Liu, J. Chen, G. Zheng, *Chem. Rev.* **2010**, *110*, 2839.

[36] W. Fan, P. Huang, X. Chen, *Chem. Soc. Rev.* **2016**, *45*, 6488.

[37] R. R. Allison, V. S. Bagnato, C. H. Sibata, *Future Oncol.* **2010**, *6*, 929.

[38] X. Li, S. Lee, J. Yoon, *Chem. Soc. Rev.* **2018**, *47*, 1174.

[39] S. L. Jacques, *Phys. Med. Biol.* **2013**, *58*, R37.

[40] P. Taroni, A. Pifferi, A. Torricelli, D. Comelli, R. Cubeddu, *Photochem. Photobiol. Sci.* **2003**, *2*, 124.

[41] W. Zhang, A. W. Lohman, Y. Zhuravlova, X. Lu, M. D. Wiens, H. Hoi, S. Yaganoglu, M. A. Mohr, E. N. Kitova, J. S. Klassen, P. Pantazis, R. J. Thompson, R. E. Campbell, *Nat. Methods* **2017**, *14*, 391.

[42] N. J. Agard, J. A. Prescher, C. R. Bertozzi, *J. Am. Chem. Soc.* **2004**, *126*, 15046.

[43] J. A. Shadish, A. C. Strange, C. A. DeForest, *J. Am. Chem. Soc.* **2019**, *141*, 15619.

[44] L. Zayat, C. Calero, P. Albores, L. Baraldo, R. Etchenique, *J. Am. Chem. Soc.* **2003**, *125*, 882.

[45] L. Zayat, M. Salierno, R. Etchenique, *Inorg. Chem.* **2006**, *45*, 1728.

[46] J. D. Knoll, B. A. Albani, C. Turro, *Acc. Chem. Res.* **2015**, *48*, 2280.

[47] A. Li, C. Turro, J. J. Kodanko, *Chem. Commun.* **2018**, *54*, 1280.

[48] A. M. Palmer, B. Peña, R. B. Sears, O. Chen, M. El Ojaimi, R. P. Thummel, K. R. Dunbar, C. Turro, *Philos. Trans. R. Soc., A* **2013**, *371*, 20120135.

[49] W. Sun, M. Parowatkin, W. Steffen, H. J. Butt, V. Mailander, S. Wu, *Adv. Healthcare Mater.* **2016**, *5*, 467.

[50] X. Zeng, X. Zhou, S. Wu, *Macromol. Rapid Commun.* **2018**, *39*, 1800034.

[51] T. L. Rapp, C. B. Highley, B. C. Manor, J. A. Burdick, I. J. Dmochowski, *Chem. - Eur. J.* **2018**, *24*, 2328.

[52] S. Theis, A. Iturmendi, C. Gorsche, M. Orthofer, M. Lunzer, S. Baudis, A. Ovsianikov, R. Liska, U. Monkowius, I. Teasdale, *Angew. Chem., Int. Ed.* **2017**, *56*, 15857.

[53] C. Renicke, D. Schuster, S. Usherenko, L. O. Essen, C. Taxis, *Chem. Biol.* **2013**, *20*, 619.

[54] L. Fenno, O. Yizhar, K. Deisseroth, *Annu. Rev. Neurosci.* **2011**, *34*, 389.

[55] L. Liu, J. A. Shadish, C. K. Arakawa, K. Shi, J. Davis, C. A. DeForest, *Adv. Biosyst.* **2018**, *2*, 1800240.

[56] A. Stempien-Otero, D. H. Kim, J. Davis, *J. Mol. Cell. Cardiol.* **2016**, *97*, 153.

[57] T. L. Rapp, Y. Wang, M. A. Delessio, M. R. Gau, I. J. Dmochowski, *RSC Adv.* **2019**, *9*, 4942.

[58] C. Xie, W. Sun, H. Lu, A. Kretzschmann, J. Liu, M. Wagner, H.-J. Butt, X. Deng, S. Wu, *Nat. Commun.* **2018**, *9*, 3842.

[59] H. M. Rostam, S. Singh, F. Salazar, P. Magennis, A. Hook, T. Singh, N. E. Vrana, M. R. Alexander, A. M. Ghaemmaghami, *Immunobiology* **2016**, *221*, 1237.

[60] S. Chen, J. A. Jones, Y. Xu, H. Y. Low, J. M. Anderson, K. W. Leong, *Biomaterials* **2010**, *31*, 3479.

[61] M. R. MacEwan, W. G. Brodbeck, T. Matsuda, J. M. Anderson, *J. Biomed. Mater. Res., Part A* **2005**, *74A*, 285.

[62] Z. Sheikh, P. J. Brooks, O. Barzilay, N. Fine, M. Glogauer, *Materials* **2015**, *8*, 5671.

[63] Y. L. Zhao, J. Fraser Stoddart, *Langmuir* **2009**, *25*, 8442.

[64] S. Tamesue, Y. Takashima, H. Yamaguchi, S. Shinkai, A. Harada, *Angew. Chem., Int. Ed.* **2010**, *49*, 7461.

[65] A. M. Rosales, K. M. Mabry, E. M. Nehls, K. S. Anseth, *Biomacromolecules* **2015**, *16*, 798.

[66] A. M. Rosales, C. B. Rodell, M. H. Chen, M. G. Morrow, K. S. Anseth, J. A. Burdick, *Bioconjugate Chem.* **2018**, *29*, 905.

[67] D. Wang, M. Wagner, H.-J. Butt, S. Wu, *Soft Matter* **2015**, *11*, 7656.

[68] S. Samanta, A. A. Beharry, O. Sadovski, T. M. McCormick, A. Babalhavaeji, V. Tropepe, G. A. Woolley, *J. Am. Chem. Soc.* **2013**, *135*, 9777.

[69] R. Siewertsen, H. Neumann, B. Buchheim-Stehn, R. Herges, C. Näther, F. Renth, F. Temps, *J. Am. Chem. Soc.* **2009**, *131*, 15594.

[70] Y. Yang, R. P. Hughes, I. Aprahamian, *J. Am. Chem. Soc.* **2012**, *134*, 15221.

[71] A. A. Beharry, O. Sadovski, G. A. Woolley, *J. Am. Chem. Soc.* **2011**, *133*, 19684.

[72] M. Wegener, M. J. Hansen, A. J. M. Driessen, W. Szymanski, B. L. Feringa, *J. Am. Chem. Soc.* **2017**, *139*, 17979.

[73] J. I. Andorko, C. M. Jewell, *Bioeng. Transl. Med.* **2017**, *2*, 139.

[74] J. I. Andorko, K. L. Hess, K. G. Pineault, C. M. Jewell, *Acta Biomater.* **2016**, *32*, 24.

**ADVANCED
SCIENCE NEWS**

www.advancedsciencenews.com

**ADVANCED
HEALTHCARE
MATERIALS**

www.advhealthmat.de

[75] T. E. Brown, J. S. Silver, B. T. Worrell, I. A. Marozas, F. M. Yavitt, K. A. Günay, C. N. Bowman, K. S. Anseth, *J. Am. Chem. Soc.* **2018**, *140*, 11585.

[76] M. Hörner, K. Raute, B. Hummel, J. Madl, G. Creusen, O. S. Thomas, E. H. Christen, N. Hotz, R. J. Gübeli, R. Engesser, B. Rebmann, J. Lauer, B. Rolauffs, J. Timmer, W. W. A. Schamel, J. Pruszak, W. Römer, M. D. Zurbriggen, C. Friedrich, A. Walther, S. Minguet, R. Sawarkar, W. Weber, *Adv. Mater.* **2019**, *31*, 1806727.

[77] M. A. Azagarsamy, K. S. Anseth, *Angew. Chem., Int. Ed.* **2013**, *52*, 13803.

[78] M. A. Azagarsamy, D. D. McKinnon, D. L. Alge, K. S. Anseth, *ACS Macro Lett.* **2014**, *3*, 515.

[79] F. Gentile, C. Chiappini, D. Fine, R. C. Bhavane, M. S. Peluccio, M. M.-C. Cheng, X. Liu, M. Ferrari, P. Decuzzi, *J. Biomech.* **2008**, *41*, 2312.

[80] F. Buket Basmanav, G. T. Kose, V. Hasirci, *Biomaterials* **2008**, *29*, 4195.

[81] T. E. Brown, K. S. Anseth, *Chem. Soc. Rev.* **2017**, *46*, 6532.

[82] X. Xiong, A. del Campo, J. Cui, *Smart Polymers and Their Applications*, Elsevier, Amsterdam **2019**, pp. 87–153.

[83] V. X. Truong, F. Li, J. S. Forsythe, *ACS Appl. Mater. Interfaces* **2017**, *9*, 32441.

[84] R. Sridharan, B. Cavanagh, A. R. Cameron, D. J. Kelly, F. J. O'Brien, *Acta Biomater.* **2019**, *89*, 47.

[85] M. F. Berry, A. J. Engler, Y. J. Woo, T. J. Pirolli, L. T. Bish, V. Jayasankar, K. J. Morine, T. J. Gardner, D. E. Discher, H. L. Sweeney, *Am. J. Physiol.: Heart Circ. Physiol.* **2006**, *290*, H2196.

[86] J. Atance, M. J. Yost, W. Carver, *J. Cell. Physiol.* **2004**, *200*, 377.

[87] M. T. Frey, Y. L. Wang, *Soft Matter* **2009**, *5*, 1918.

[88] A. M. Kloxin, J. A. Benton, K. S. Anseth, *Biomaterials* **2010**, *31*, 1.

[89] W. Xiao, X. Zeng, H. Lin, K. Han, H. Z. Jia, X. Z. Zhang, *Chem. Commun.* **2015**, *51*, 1475.

[90] R. K. Pathak, S. Dhar, *J. Am. Chem. Soc.* **2015**, *137*, 8324.

[91] Q. Hu, W. Sun, C. Wang, Z. Gu, *Adv. Drug Delivery Rev.* **2016**, *98*, 19.

[92] S. Zhu, L. Nih, S. T. Carmichael, Y. Lu, T. Segura, *Adv. Mater.* **2015**, *27*, 3620.

[93] T. Führmann, P. N. Anandakumaran, M. S. Shoichet, *Adv. Healthcare Mater.* **2017**, *6*, 1601130.

[94] K. J. Rambhia, P. X. Ma, *J. Controlled Release* **2015**, *219*, 119.

[95] *ANSI* **2007**, *Z136*, 1.

[96] R. Vankayala, C.-C. Lin, P. Kalluru, C.-S. Chiang, K. C. Hwang, *Biomaterials* **2014**, *35*, 5527.

[97] Z. Chen, W. Sun, H.-J. Butt, S. Wu, *Chem. - Eur. J.* **2015**, *21*, 9165.

[98] Z. Chen, S. He, H.-J. Butt, S. Wu, *Adv. Mater.* **2015**, *27*, 2203.

[99] G. Jalani, R. Naccache, D. H. Rosenzweig, L. Haglund, F. Vetrone, M. Cerruti, *J. Am. Chem. Soc.* **2016**, *138*, 1078.

[100] J. H Koch, W. Rogers, F. Dwyer, E. C Gyarfas, *Aust. J. Biol. Sci.* **1957**, *10*, 342.

[101] W. Sun, S. Li, B. Häupler, J. Liu, S. Jin, W. Steffen, U. S. Schubert, H.-J. Butt, X.-J. Liang, S. Wu, *Adv. Mater.* **2017**, *29*, 1603702.

[102] W. Sun, Y. Wen, R. Thiramanas, M. Chen, J. Han, N. Gong, M. Wagner, S. Jiang, M. S. Meijer, S. Bonnet, H.-J. Butt, V. Mailänder, X.-J. Liang, S. Wu, *Adv. Funct. Mater.* **2018**, *28*, 1804227.

[103] W. Sun, R. Thiramanas, L. D. Slep, X. Zeng, V. Mailänder, S. Wu, *Chem. - Eur. J.* **2017**, *23*, 10832.

[104] Z. Yang, Y. Yang, M. Wang, T. Wang, H. Fok, B. Jiang, W. Xiao, S. Kou, Y. Guo, Y. Yan, X. Deng, W.-B. Zhang, F. Sun, *Matter* **2020**, *2*, 233.