Light-Responsive Biomaterials: Development and Applications

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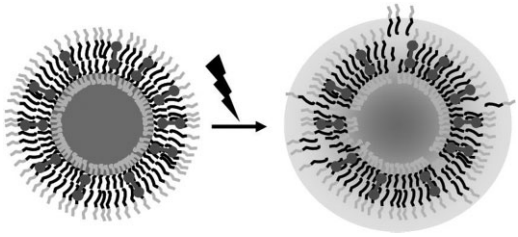
Novel biomaterials are beneficial to the growing fields of drug delivery, cell biology, microdevices, and tissue engineering. With recent advances in chemistry and materials science, light is becoming an attractive option as a method to control biomaterial behavior and properties. In this Feature Article, we explore some of the early and recent advances in the design of light-responsive biomaterials. Particular attention is paid to macromolecular assemblies for drug delivery, multi-component surface patterning for advanced cell assays, and polymer networks that undergo chemical or shape changes upon light exposure. We conclude with some remarks about future directions of the field.



Introduction

The past couple of decades have seen a rapid evolution in the development of novel materials for applications in drug delivery, tissue engineering, microdevices, and cellular biology.[1] Early efforts in these fields focused on materials that were supportive of the desired applications, though not necessarily direct participants. Following these initial efforts, the focus turned to the design of functional materials whose properties were more important in achieving successful outcomes, such as degradation, functional group presentation, or mechanical robustness. As the development of these materials has advanced, there has been further interest in control over material properties in time and space. One approach to achieve this precise control is through the employment of exogenous agents that can act on the material, such as enzymes, acoustic energy, magnetic fields, or light.[2]

Light is a particularly attractive source of energy for use in controlling biomaterial behavior. Its intensity and wavelength can easily be controlled through the use of filters, and photomasks or lasers allow for fabrication of complex features and exposure areas with resolution as small as approximately 1 μm. The advent of confocal microscopy has enabled a further increase in resolution, in addition to three-dimensional control.[3,4] The majority of light-responsive chemical moieties are responsive in the UV spectral range, which is generally not limiting in an in vitro environment, and may be sufficient for many applications. However, systems that would be desirable for translation to an in vivo setting are limited by the narrow spectral range in the near-infrared (NIR) region in which light has sufficient penetration through tissue for utility.[5] However, recent advances in two-photon microscopy and lasers and the development of NIR-active molecules suggest that this drawback can be overcome and has allowed for the development of a range of useful materials.

Light-responsive polymeric materials can be designed through the incorporation of specific chemical moieties into the polymer network or through composite systems in which a light-responsive group is suspended within the network, though not covalently attached. In this article we explore some of the recent advances in the control of biomaterial behavior using light. Specifically, we focus on the incorporation of light-responsive moieties into

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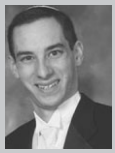
macromolecular assemblies for drug delivery, the development of bio-friendly methods for light-controlled patterning of two-dimensional cellular substrates and three-dimensional gels, and the harnessing of light to induce shape changes in biomaterials.

Macromolecular Assemblies

Macromolecular assemblies are formed by the self-assembly of polymers in aqueous solutions, driven by non-covalent interactions between the macromolecules.[6,7] The classical example of a macromolecular assembly found in nature is the phospholipid vesicle (Figure 1a), where a membrane is formed through hydrophobic interactions between the hydrocarbon tails of phospholipids and is stabilized in solution by the polar head groups. The ratio of hydrophobic to hydrophilic segments within a macromolecule determines its self-assembly behavior; molecules that are largely hydrophobic tend to aggregate as membranes (e.g., vesicles), while those that are more hydrophilic form micelles (Figure 1b,c).[8] Micelles and vesicles have shown significant promise for use as drug delivery vehicles in vivo, and have been synthesized from a wide variety of molecules ranging from lipids to di-and tri-block copolymers.[9,10] Controlling the release of encapsulated agents by an external stimulus is of great interest for the drug delivery field and has been extensively reviewed for many different stimuli.[11–13] The synthesis of micelles and vesicles that release their content upon light exposure could allow for improved site-specific delivery of cargo to diseased tissues such as tumors, through selective irradiation of the target site. Many drugs and drug candidates are currently limited by a narrow therapeutic index[14] and site-specific delivery could enable larger dose release at a target site while reducing systemic doses that lead to debilitating side-effects.

Light-induced release of encapsulated contents within macromolecular assemblies has been explored through both chemical and physical changes to the assembly. An early study explored phospholipid vesicles (liposomes) that were sensitive to photo-oxidation induced by a sensitizer.[15] Phosphocholine was modified to include a vinyl ether linkage between the polar head group and the palmitoyl tail. Liposomes were formulated to encapsulate glucose (for release) and zinc phthalocyanine, a photosensitizer. Exposure of these liposomes to >640 nm light enhanced glucose release over unexposed controls. However, the vesicles were quite leaky, releasing nearly 30% of their content within 1 h in the dark (compared to roughly double that under light exposure), likely rendering these vesicles unsuitable for clinical use.

A more recent example incorporated a photo-isomerizable azo group into the tails of a phosphocholine-based lipid.[16] These liposomes were formulated to accommodate the active loading of drugs (aciridine orange and doxorubicin), allowing for high drug encapsulation. When in the *trans* conformation, the lipid bilayer was well packed and relatively stable with little release observed. However, UV-induced isomerization from *trans* to the *cis* conformation of the azo group disrupted the packing of the membrane and induced release of the two encapsulated model drugs (Figure 1d). This same chemistry was exploited for gene delivery using the small cationic surfactant azobenzene trimethylammonium bromide.[17] In this case, isomerization from *trans* to *cis* induced a decrease in the hydrophobicity of the molecule, causing vesicle rupture. These vesicles can complex with DNA and were used as a



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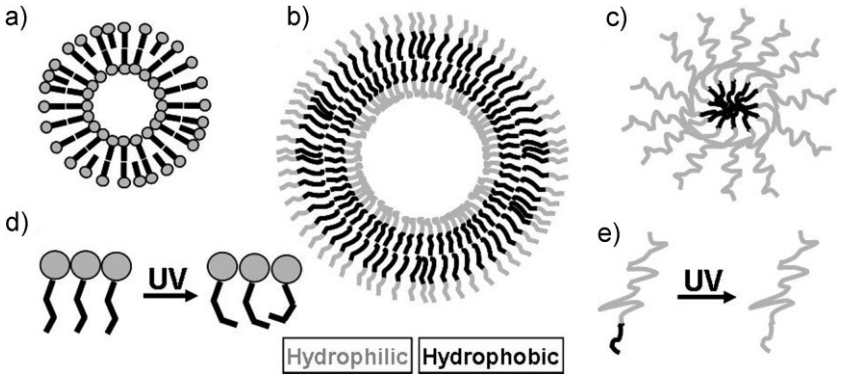

Figure 1. Self-assembled macromolecular structures. (a) Lipid molecules (hydrophilic head and hydrophobic tail) self-assemble into vesicle structures. Diblock copolymers of hydrophilic and hydrophobic blocks assemble into vesicles with a larger hydrophobic content [polymersomes, (b)] and micelles with a greater hydrophilic content (c). These structures can be designed to respond to light. For example, (d) UV-light induced isomerization of azo-containing lipids interferes with their packing ability, leading to the leakage of any loaded contents and (e) irreversible cleavage of a hydrophobic protecting group changes the hydrophobic/hydrophilic balance of the macromolecule, leading to micelle breakdown. In all cartoons, black is hydrophobic; gray is hydrophilic.

transfection vehicle for an enhanced green fluorescent protein (eGFP) plasmid to fibroblasts. While the vesicles themselves were successful transfection agents, UV light roughly doubled the efficiency and greatly enhanced endosomal escape of the DNA, an important issue in gene therapy approaches.

Photo-isomerizable groups, including the azobenzene group discussed above,[18] azopyridine,[19] stilbenes,[20] and spiropyrans[21,22] have also been used to control the assembly and disassembly of micelles and polymeric vesicles. In one particularly interesting study, Mabrouk and coworkers formed polymersomes with an asymmetric membrane, the inner leaflet consisting of poly(butadiene) and the outer being a liquid crystalline diazobenzene-based methacrylate.[23] UV light exposure induced the liquid crystal to transform from a nematic to isotropic state, inducing a small, unstable pore penetrating the membrane. The pores induced by this change in state caused a curling instability of the outer leaflet of the polymersome, leading to outward spiraling of the leaflet and, ultimately, bursting of the vesicle.

Several aromatic light-sensitive protecting groups have been exploited to allow irreversible chemical changes to drug delivery assemblies such as liposomes, micelles, and vesicles. Chandra and coworkers designed lipids where a hydrophilic amino acid (aspartate, glutamate or lysine) was coupled to a saturated hydrocarbon through a nitrobenzyl linker.[24] UV light cleaves the molecules, separating the amino acid from the hydrophobic tail. Incorporation of these lipids at only 5% into distearoylphosphatidylcholine (DSPC) liposomes was sufficient to release encapsulated contents upon exposure to UV light. Interestingly, the required wavelength for cleavage varied by the charge on the head group; the glutamate- and aspartate-based lipids cleaved at 365 nm and the basic lysine cleaved at 254 nm, presumably due to the amine group on the lysine interacting with the photocleavable group.

The nitrobenzyl group has also been incorporated into micelle-forming block copolymers.[25,26] In these systems, the group protects a side-chain carboxylic acid in a hydrophobic state; the hydrophobicity within the protected block drives micelle formation. Cleavage of the protecting group with UV light irreversibly alters the hydrophobicity of the molecule (now a free carboxylic acid) and drives the rearrangement of the micelle and release of the contents (Figure 1e). Several other photo-sensitive protecting groups have also been utilized in micelle and vesicle systems. Jinqiang Jiang and coworkers designed micelles from poly(ethylene glycol)-*block*-poly(pyrene methacrylate).[27] The bulky hydrophobic pyrene drives micellization, but could be cleaved from the methacrylate with exposure to 365 nm light, yielding a fully water-soluble poly(ethylene glycol)-*block*-poly(methacrylic acid) polymer. Yugui Jiang and coworkers designed reversible vesicles using a poly(ethylene glycol) (PEG)-terminated malachite green derivative.[28] This malachite green derivative has a terminal cyano group that was cleaved to yield a positive charge when exposed to UV light. This positive charge was sufficient to disassemble the vesicles. Removal of the light source led to reattachment of the cyano group and vesicle reassembly.

Two groups have recently reported micelle systems that are responsive to two-photon illumination.[29,30] As discussed earlier, two-photon exposure has several advantages over simple UV irradiation, including improved spatial resolution and significantly deeper tissue penetration in vivo. Goodwin and coworkers capped a PEG molecule with a 2-diazo-1,2-naphthoquinone moiety.[30] Upon exposure to a 350 nm or 795 nm laser in the presence of water, the naphthoquinone underwent a Wolff rearrangement, yielding an indene carboxylate which is significantly more hydrophilic. The rearrangement drove disassembly of the micelles and release of their contents. Babin and coworkers designed a PEG-poly(methacrylic acid) polymer, where the acid groups were protected by a coumarin derivative.[29] Similar to the naphthoquinone, the coumarin is also sensitive to one- (≈350 nm) or two-photon (≈795 nm) light, in this case, cleaving from the methacrylate, yielding the free acid. As with other systems, this drastic change in

hydrophilicity drove the micelles to disassemble in solution and release their contents. The 2-nitrobenzyl group's susceptibility to two-photon cleavage has been explored,[31] but to the best of our knowledge, assembly disruption using this protecting group with NIR light has yet to be reported for a self-assembled system.

In addition to chemical changes affecting release from macromolecular assemblies, several light-sensitive particles have been encapsulated within vesicles that cause a physical change upon light exposure, leading to vesicle disruption. Paasonen and coworkers encapsulated gold nanoparticles within liposomes.[32] The nanoparticles, 1–3 nm in diameter, were loaded into the aqueous core, hydrophobic membrane or tethered to the membrane, depending on the surface chemistry of the nanoparticles. Exposure to UV light (250 nm) induced heating of the gold nanoparticles, consequently leading to permeabilization of the liposome membrane and release of contents. One major drawback to this system, however, is the extremely low wavelength required to heat the particle and cause release. Two other similar systems are significantly more likely to be translatable to the clinic, as they absorb in the NIR regime. Wu and coworkers encapsulated hollow gold nanoshells, rather than nanoparticles, into and onto liposomes.[33] These gold nanoshells rapidly heat upon exposure to 800 nm light, creating small bubbles of vapor that cavitate the membrane. As a result, the membranes are not destroyed but do transiently (for the duration of laser illumination) allow for release of encapsulated contents. Troutman and coworkers developed a method to coat liposomes with small ($<$10 nm) clusters of gold.[34] The clusters, in close proximity to each other, produced plasmon resonances that exist at wavelengths of approximately 1 000 nm. Upon NIR excitation, the plasmon bands produced in the clusters were converted to thermal energy which allowed for liposome destabilization and release of encapsulated contents.

One recently-reported composite system is of particular note,[35] as it combines NIR-induced release with polymersomes, which are significantly more stable than liposomes,[36] and avoids the use of potentially-toxic metals. Robbins and coworkers designed a three component system for inducing vesicle release. Polymersomes were fabricated from polybutadiene-*block*-poly(ethylene glycol); a protein (ferritin, myoglobin or albumin) was loaded into the aqueous core, while a NIR porphyrin dye was loaded into the membrane.[37] Upon excitation of the porphyrin, the vesicles were observed to bud and collapse, releasing encapsulated molecules, such as biotin. Fluorescence recovery after photobleaching studies suggested that some of the protein was intercalating with the membrane, perhaps aiding in the light-sensitivity.

While the technology is clearly being advanced for light-responsive macromolecular assembly delivery systems, there have yet to be any reports translating these systems to an in vivo setting. Non-responsive systems have already been shown to have utility for in vivo imaging and disease treatment,[37–39] and such studies exploring drug delivery and disease treatment using light-sensitive materials should be forthcoming in the near future.

Two-Dimensional Protein Patterning

In the interest of understanding in vivo processes, there is a growing need for fundamental biological studies to better understand cell behavior in vitro. Though many cellular processes are governed by μm-scale patterns of proteins,[40] including immunological and neural synapse activation,[41,42] integrin clustering,[43,44] motility,[45] and differentiation,[46] relatively few cellular studies actually take place on surfaces that present ligands in a spatially-defined manner. Further advances in patterning technologies will help to facilitate more studies that better mimic the in vivo cellular environment and will help to better understand cells in native tissues and during disease, as well as our ability to control cells in therapy development.

Photochemical (using light to effect a change to molecules attached to a surface) and photolithographic (using light to change the solubility of a molecule protecting a surface) technologies offer many benefits over other patterning methods. A single mask can be used to quickly generate many complex surfaces with high fidelity and resolution. However, because of the need for harsh solvents, intense UV light, and dehydration, many classical photo-based techniques are not compatible with biological systems to generate surface patterns of ligands.[47–49] Only recently have several groups reported the development of "bio-friendly" photolithographic and photochemical patterning methods for patterning multiple ligands on a surface.

Early work in the development of light-patterned biological surfaces centered on the use of caged biotins, which, through streptavidin bridges, could be used to spatially pattern any biotinylated molecule (i.e. proteins, antibodies, DNA, etc.). Sundberg and coworkers tethered a nitrobenzyl-caged biotin to surfaces that, upon UV exposure, cleaved the nitrobenzyl group and presented free biotin.[50] By employing successive exposures to different regions of the slide, different fluorescent biotinylated antibodies could be coupled. In this case, they avoided exposure of protein-bound regions by masking already-exposed regions. However, as a result, this severely limits the possible resolution to several hundred of μm, areas too large for patterns at the cellular level. A similar approach was employed by Blawas and coworkers who, rather than covalently attaching a biotin to a glass substrate, coupled caged biotin to bovine serum albumin which could then

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adsorb onto glass.[51] UV light released the cage, exposing free biotin that could bind streptavidin and subsequently other biotinylated molecules. As with the first case, though, the spatial resolution of this system for patterns of multiple proteins is too low for patterning at the cellular level. Holden and Cremer addressed the issue of spatial resolution of patterning by changing the method used to make biotin available to proteins.[52] Rather than using an uncaging procedure that requires UV light and could be damaging to proteins, they attached different fluorophores to different biological molecules, an immunoglobulin G (IgG) and a biotin. Exposure of the fluorophores to their excitation wavelength (in the visible spectrum) generated radicals that could react with the surface, immobilizing attached molecules. Because the excitation wavelengths are non-damaging to biological molecules, patterns, including overlapping patterns, of different molecules could easily be generated at high resolution.

The above examples are all instances of photochemical patterning, rather than the use of photolithography. Only recently have photoresist systems been developed that can be used to pattern multiple biological ligands in a spatially-defined manner. Doh and Irvine developed a carboxylic acid-generating photoresist that could be used to pattern multiple proteins in complementary regions of a surface (Figure 2).[53] Their photoresist, a random terpolymer of 2-nitrobenzyl methacrylate, methyl methacrylate, and hydroxyl-terminated PEG methacrylate, exhibited pH dependent water solubility following UV exposure. Furthermore, the exposed resist is also a polyelectrolyte, allowing a small (10 nm) layer of resist to remain behind on a cationic surface. Biotinylation of the resist prior to casting, therefore, created a biotinylated surface even after removal of the exposed photoresist. Consequently, a first pattern could be etched, and the remainder of the resist exposed. Before removal of the second portion of the resist, a first protein could be bound to the surface of the resist under mild acidity, where the resist is not water soluble but proteins are still highly stable, through a streptavidin-biotin bridge. Following this initial binding, the resist still present after the first exposure could be removed by increasing the pH, also removing any protein that bound in that region. A new layer of resist is then exposed to which a second protein could be bound by the same chemistry. Interestingly, it was found that another formulation of this same terpolymer could act as a negative-tone photoresist, where an originally water-soluble photoresist is transiently stabilized through intermolecular hydrogen bonds following UV exposure.[54] In a method similar to the one above, two proteins could again be patterned in spatially defined regions without exposing either protein to UV light or dehydration. In a beautiful example of the applicability of this technology, the authors demonstrated how surface patterns of anti-CD3 and ICAM-1 could – with proper spatial organization – stimulate the formation of immunological synapses with CD4 + T cells, leading to T cell activation (Figure 2b).[55]

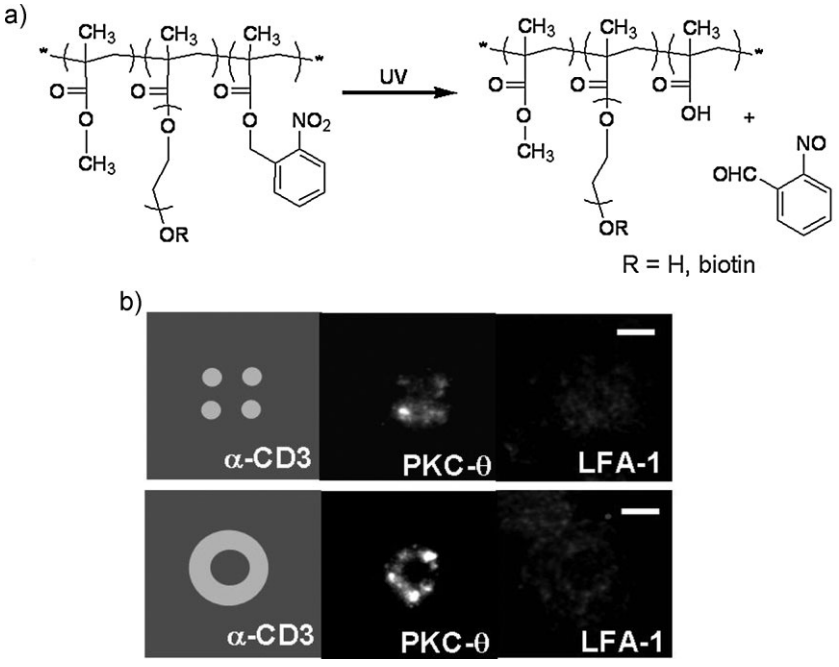

Figure 2. Light-controlled multi-component protein patterning. (a) Bio-friendly photoresist terpolymer that is sensitive to light. Selective light exposure of the photoresist alters the film solubility, allowing the spatial patterning of proteins selectively with intermittent light exposure. (b) The patterns can be used to investigate T cell response to patterns of ligands (anti-CD3 surrounded by ICAM-1). T cell surface receptor PKC-θ and intracellular signaling molecule LFA-1 localize well with the patterned ligand. Scale bar: 5 μm. Figure adapted from ref.[55] with permission. Copyright 2006 National Academy of Sciences, USA.

Several other recent advances are worth noting, though they have yet to be expanded to multicomponent patterning. Alvarez and coworkers employed two-photon techniques to pattern carboxylic acids on a quartz surface that could then be functionalized with biotin or other biological molecules.[56] While the use of two photon microscopy slows the process for producing patterns rapidly, there are two advantages compared to simple lithographic techniques. First, the authors demonstrated the ability to generate different densities of ligand on the surface based on the intensity or duration of exposure. Second, confocal techniques

allow for resolutions that are finer than the limitation that is usually imparted by the wavelength of the light, meaning smaller features can be patterned on a surface. Similarly, towards the transfer to a third dimension, Hahn and coworkers used confocal microscopy to pattern arginine-glycine-aspartic acid (RGD) ligands on PEG hydrogels.[57] The ability to pattern on the surface of gels is promising as it will enable the generation of surfaces that can be spatially tailored for chemical functionality and be designed to have specific physical properties, which are known to affect cell behavior.[58,59] Their specific system has a few limitations in that it relies on incomplete polymerization of the gel in order to couple the ligand. Edahiro and coworkers expanded patterning from proteins to actual cells.[60] Combining a spirobenzopyran, which reversibly forms a zwitterionic structure upon non-toxic UV irradiation, with *N*-isopropylacrylamide, a thermo-sensitive polymer, cells could be selectively removed from a surface by a combination of UV exposure and low-temperature washing. Perhaps due to the nature of cell growth, though the patterns produced were coarse and not well-defined. However, the field as a whole is certainly progressing to achieve higher levels of complexity to further enhance our ability to understand and control cellular behavior.

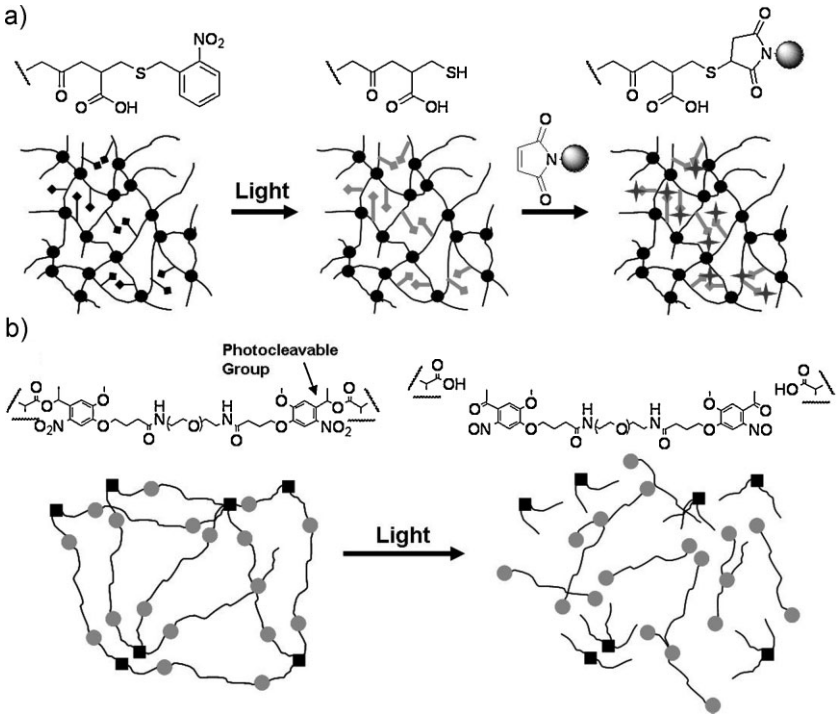

Figure 3. Patterning of chemistry and structure in hydrogels with light. (a) Photochemical patterning of a hydrogel, where UV light activates protected moieties covalently attached to a gel. These activated moieties can then, in a second step, bind to biologically active molecules. (b) Photo-degradation of a hydrogel. A hydrogel containing crosslinks that contain UV-cleavable sites can be broken down upon UV exposure. With both of these techniques, patterning is possible by spatially controlling light exposure.

Three-Dimensional Patterning of Hydrogels

While two-dimensional surfaces have been shown to be quite useful for fundamental cell studies, any attempt at engineering full tissues requires three-dimensional scaffolds that support cellular growth and behavior. Recently, several groups have reported methods for scaling cell patterning into the third dimension. A significant amount of this work has focused on further crosslinking or interpenetrating networks of hydrogels as a method to spatially alter local mechanics or inhibit the spreading of encapsulated cells. While this is beyond the scope of our focus, we reference several notable reports for the interested reader.[61–63] The rest of the research in this field can be approximately broken down into two categories – ligand presentation and scaffold degradation. The field of ligand presentation has been pioneered by Shoichet and her lab (Figure 3a).[64–66] In their work, they utilized the 2-nitrobenzyl- or coumarin-derived protecting group as a thiol-protected cysteine, which they coupled to agarose gels. Irradiation of specific regions of the gel site-specifically cleaved the nitrobenzyl group, yielding free sulfhydryls that could react with thiol-reactive compounds. In areas where the gels were patterned with the cell-responsive glycine-arginine-glycine-aspartic acid-serine (GRGDS) ligand, neurites seeded on the surface of the gel were seen to grow into the patterned regions.[64] The original work reported was limited by control of the pattern in only two dimensions, as there is little control over the pattern throughout the depth, and only patterns such as columns could be fabricated. To improve on this, Wosnick and Shoichet expanded this work to pattern the gels using confocal microscopy, enabling complete freedom in pattern choice in all dimensions, with reported feature sizes as small as ≈5 μm.[66] For this work, however, they focus only on the pattern fabrication itself and do not report any studies with cells.

Only recently was the first photo-degradable hydrogel system reported for tissue engineering.[67] In groundbreaking work, Kloxin and coworkers modified PEG diacrylate to include a 2-nitrobenzyl linker between the

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PEG and acrylate end groups. This diacrylate acts as a crosslinker in a radically-polymerized gel system. Upon UV exposure, using either a UV lamp or confocal microscope, portions of the gel could be eroded away by rearrangement of the nitrobenzyl groups and cleavage of the crosslink (Figure 3b). Erosion of channels within gels containing fibronectin created regions that were permissive to cellular movement. As a second example of the utility of this chemistry in gel systems, an RGDS peptide was coupled to the same nitrobenzyl-acrylate moiety. Copolymerizing the photo-cleavable peptide into PEG gels created gels in which the presence of RGDS ligand could be tailored in a spatial and temporal manner. It was found that human mesenchymal stem cells undergo chondrogenesis when transiently exposed to RGDS for a 10 d period followed by 11 d of no ligand, whereas no chondrogenesis was observed if the ligand was or was not consistently present for the entire culture period. In addition to the interesting findings observed in this paper, this work further opens the door for the design of new biomaterials for tissue engineering that can be spatially and temporally tailored for specific and varying mechanical properties, crosslink densities, and ligand concentrations through combinations of photo-cleavable groups and corresponding non-cleavable molecules. This example is just the first step towards added complexity in designing three-dimensional systems with added cellular control.

Shape-Memory and Shape-Changing Biomaterials

In addition to the importance of spatial control of cell and ligand populations in the development of advanced tissue engineering scaffolds, control of the spatial dimensions and shape of a biomaterial could provide improved versatility for biomedical implants. Shape-memory polymers are materials that can be set in temporary shape until a stimulus allows it to return to a more permanent and desired conformation. Shape-changing polymers are materials that undergo a temporary change in shape upon suitable stimulation but may revert back to their original shape when the stimulus is removed.[68] In this section, we explore several recent advances in shape-memory and shape-changing polymers controlled by exposure to light. Similar to our discussion of macromolecular assemblies above, shape transitions initiated by light can be induced through either chemical changes to a polymer network or through composite systems in which an absorbing species causes a physical change to the network.

Photoinduced transitions in gels were first reported nearly twenty years ago for poly(*N*-isopropyl acrylamide) (NIPAm) gels.[69] In the first of these systems, a UV-ionizable moiety, bis[4-(dimethylamino)phenyl](4-vinylphenyl)-methyl leucocyanide, was introduced into the polymer gel backbone. Upon irradiation, cyanide is released, inducing an osmotic force and driving water into the gel, leading to increased swelling. As the ionization is reversible, upon removal of the light stimulus, the gel reverted back to its original swelling state. There are, however, several drawbacks to this system. It was difficult to control the rate of swelling and deswelling, as ion recombination is governed by diffusion of the cyanide ions. Further, for biomedical applications, cyanide ions would not be ideal, because of their toxicity. Some of these issues were addressed in another paper that appeared shortly after this original publication.[70] In this study, the ionizable moiety was replaced by a copper-containing chromophore which absorbs visible light. The chromophore, upon irradiation, locally heated the gel, leading to a variable ionization state of the NIPAm. The new ionization state in turn led to localized deswelling of the gel. As with the prior system, upon removal of the light stimulus, the gel reverted back to its original state.

A major limitation of both systems described above is that light can only be used to control swelling of a material. However, often times, there may be an interest in changing the entire shape, rather than only the dimensions. This issue was addressed by Lendlein and coworkers in a seminal paper.[71] In their work, they grafted cinnamic acid (CA) into the backbone of hydroxyethyl acrylate hydrogels. When exposed to ultraviolet radiation of $\lambda > 260$ nm, the CA molecules underwent a [2 + 2] cycloaddition, adding new crosslinks into the material. These crosslinks are reversible, though, as they were rapidly cleaved with light, $\lambda < 260$ nm. To use this as a shape-memory material, prior to secondary (UV) crosslinking, the gel was deformed into a temporary (i.e. elongated) shape. Once exposed to $\lambda > 260$ nm light, the gel was permanently fixed in this new shape until stimulated with light, $\lambda < 260$ nm, breaking the CA crosslinks, allowing the gel to revert to its original shape. As a second demonstration of the utility of this system, the authors also used the cinnamic moiety (cinnamylidene acetic acid, CAA) to build a crosslinked interpenetrating network around a preformed gel to hold it in a temporary (corkscrew) shape until exposed to $\lambda < 260$ nm light, breaking down the secondary network. Between these two demonstrations, it is clear that the CA molecule could potentially be used to control shape memory of nearly any gel system, either through covalent incorporation of it into the polymer backbone of the gel or through an interpenetrating polymer network, though there are obvious limitations to biological systems resulting from the required wavelengths for the chemical group's activity.

Recently, several groups have explored the use of composite systems in order to harness light for gels that exhibit thermo-responsive behavior. In these systems, as with macromolecular assemblies, a light-absorbing

material is dispersed within the polymer matrix, providing a route to convert light into thermal energy, leading to a heat-induced phase change for the polymer. Two independent groups have loaded gold particles into NIPAm microgels. Budhlall and coworkers loaded gold nanoparticles into NIPAm microparticles and observed their deswelling behavior in response to light irradiation.[72] Rather than dispersing the gold throughout the microgels, these particles had a core/shell structure, with the gold comprising the core. However, light exposure still led to significant deswelling of the gels, as the polymer was heated above its lower critical solution temperature (LCST). West and coworkers loaded different formulations of gold nanoshells into NIPAm microgels swollen with various proteins.[73,74] Upon irradiation in the NIR regime, the nanoshells heated the NIPAm above its LCST causing the gels to collapse and release their contents in a time-dependent manner, where the rate of release was inversely correlated to the molecular weight of the encapsulated agent. While this system offers much promise for localized delivery of therapeutics using NIR light, more advanced formulations will likely be required to accommodate for the fairly significant release (ca. 20% of amount released with irradiation) that was observed in the absence of light.

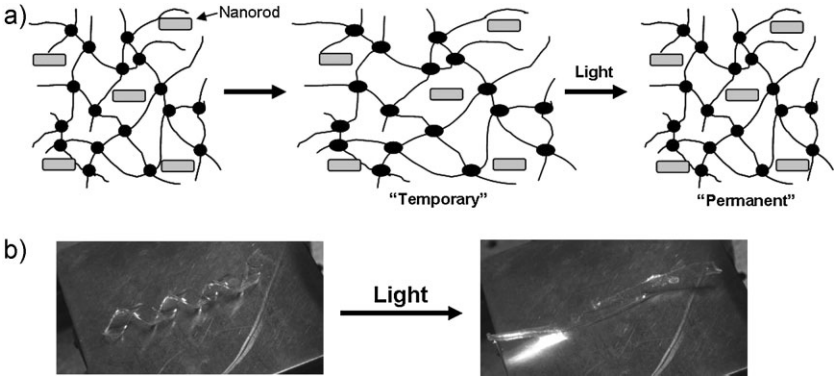

Figure 4. Light-induced transitions in shape-memory polymers. (a) Schematic of polymer network, where a material is heated above its T_g and set in a temporary shape and then cooled. UV light can then be introduced to heat the polymer via entrapped nanorods to again raise the temperature above the T_g to allow the material to return to its permanent shape. (b) Example of a coiled sample that returns to its rectangular shape with NIR light exposure.

While the examples cited above have again focused on simple swelling/deswelling of gels upon irradiation, we recently reported a light-responsive composite shape memory system (Figure 4).[75] Similar to the nanoshells employed by West and coworkers,[73,74] we used gold nanorods as a NIR absorbing material. Gold nanorods are an exciting material for use as NIR absorbers, as they can be easily synthesized with a tunable absorbance and have the ability to be surface functionalized through gold surface-thiol reactions.[76–79] We loaded gold nanorods at a low (<1 vol.-%) concentration into *tert*-butyl acrylate polymer slabs, crosslinked with a poly(*β*-amino ester) polymer.[80] This system could easily be tuned for a specific glass-transition temperature by altering the monomer/crosslinker ratio, and *tert*-butyl acrylate had previously been characterized for shape-memory applications.[81,82] The formulation was shown to be non-toxic both in vitro and in vivo. Exposure to NIR light (770 nm) led to rapid temperature changes of over 30 °C within the slabs. As a demonstration of shape-memory ability, a heated polymer slab was shaped into a coil and cooled back to room temperature. Upon irradiation with NIR light, the sample quickly heated and returned to its original rectangular shape (Figure 4b). As with Lendlein's system described above, this method is versatile and could be easily translatable to other polymeric materials with thermal-responsive behavior.

Several other composites have been developed to induce swelling within polymeric materials. Fujigaya and coworkers employed carbon nanotubes as a NIR light absorbing material in NIPAm gels.[83] Similar to the gold nanoparticles, the carbon nanotubes could easily absorb NIR light and dissipate the energy as localized heat in the gel, leading to rapid deswelling of the material. The swelling/deswelling was repeatable, showing fidelity over 1 200 cycles of light/dark. Tatsuma and coworkers loaded titanium dioxide nanoparticles into acrylic acid gels.[84] Titanium dioxide catalyzed the reduction of silver ions to silver nanoparticles upon UV irradiation. The process is reversible with visible light. The composite gels were soaked in silver nitrate to load silver ions, which coordinated the acid side chains on the polymer, and shrank the gel. Upon exposure to UV light, the silver ions were reduced to silver nanoparticles, liberating the acid side chains, allowing the gel to swell. Visible light reoxidized the silver, leading to collapse of the gel. The use of photomasks enabled the creation of complex swelling morphologies. One could imagine this material being especially useful for applications that require antibacterial properties, as the silver could serve both to control the material swelling properties as well as impart antibacterial qualities.[85]

Conclusions and Future Directions

There have been many promising advances in the development of light-responsive biomaterials, but there is still much that needs to be done to make these advances translatable to the clinic. The advent of two-photon systems

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and NIR-responsive materials has opened the field to many new possibilities that were previously unattainable due to poor in vivo light penetration. At present, though, most confocal systems are likely too complicated to translate easily for routine in vivo use, but the materials that have been generated are quite useful for studies in vitro. As more materials are developed in the field of molecular imaging for in vivo use, we imagine that the applicable chemistries will be translated for use also in materials for drug delivery and tissue engineering. As the demand develops further through more applicable advances in the materials field, we are confident that the technology and systems to control these materials will be developed as well. Indeed, while more research is necessary to develop clinically useful materials, the future holds much promise for employing light to better control drug delivery and regenerative medicine.

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