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Photoreversible Soft Azo Dye Materials: Toward Optical Control of Bio-Interfaces

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Photoreversible optically switchable azo dye molecules in polymer-based materials can be harnessed to control a wide range of physical, chemical, and mechanical material properties in response to light, that can be exploited for optical control over the bio-interface. As a stimulus for reversibly influencing adjacent biological cells or tissue, light is an ideal triggering mechanism, since it can be highly localized (in time and space) for precise and dynamic control over a biosystem, and low-power visible light is also an inherently gentle, benign, and nondamaging stimulus in a biological environment. Azobenzene-based dyes in particular are emerging as especially attractive candidates among photoreversible molecules, and soft azobenzene-containing materials are promising due to their ease of incorporation, and efficient and robust photochemistry and photophysics. This review provides a current survey of the use of photoreversible azo soft materials in cell biology and tissue engineering bio-interface applications, to afford light control over molecular motion (orientation, flow), by inscribing surface morphological patterns or macroscopically photoactuating surfaces and structures, via three key photophysical and bioactive effects enabled by the azo groups' light-induced photo-orientation, topological optical patterning, and photomechanical actuation.

1. Introduction

1.1. Engineering the Bio-Interface

Synthetic materials that can interface with biology have been designed and applied for millennia, and the complexity and capability of these systems have increased significantly.[1] Assisted by our increasing understanding of how biological systems interact with artificial materials, researchers have now assembled a versatile toolkit of materials and processes that allows one to fine-tune interactions at the interface, tailoring specific biological responses on demand. These strategies for biocontrol can be broadly categorized as chemical (charge, ligand presence, surface groups), material changes (moisture content, stiffness), or morphological changes (molecular orientation, surface topography, or mechanical actuation). Rational design of materials for biological interface applications has a unique set of challenges due to the unique requirements of a wide range of biological systems, since different tissues present specific compositions, with their own elasticity, structural organization, and triggering mechanisms. Even within a single organ or tissue, mechanical properties can vary widely depending on the region. A recent study of viscoelasticity in live mouse brain tissue for example found a tenfold difference within the brain depending on the region and morphological structure studied.[2] However, most biological tissues are far less stiff, and far more viscoelastic than typical artificial materials employed at their interface, especially early artificial cell culture materials, which can be much stiffer than in vivo extracellular matrix by many orders of magnitude.[3]

In vivo, cells interface with a surrounding microenvironment consisting of an intricate macromolecular network of proteins and sugars swollen in an aqueous media gel. Therefore, the interaction between cells and the extracellular matrix (ECM) in the body is complex and involves a variety of cues related to topography, chemical markers, protein composition, solubility factors, and mechanical properties such as stiffness and elasticity (**Figure 1**a).[4] Yet much research over the past decades was focused on studying in vitro the effects of each of these signals on cell behavior, with a particular interest on the chemical factors, whereas only recently more advanced biomaterials with controlled physicomechanical properties have been developed, such as those with tunable and variable stiffness, alignment and orientation of key functional groups, and mechanical actuation. There is a large range of stiffness in human tissue, where bone (≈100 GPa) is nine orders of magnitude harder than brain tissue (≈0.1 kPa).[4] Therefore, biomaterial researchers assume a complex task of providing materials and processing technologies for controlling stiffness and micro- and nanostructures in

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a biologically relevant microrange. In vitro studies of the way topography affects cell behavior have shown that many of these features manifest over a relatively small length-scale, in the realm of micro and nanometers.[5] The morphology and alignment of many cell types can be influenced by parallel patterns, as shown by the elongation of an endothelial cell parallel to a micrograting pattern in Figure 1b.[5] Cellular motion can also be affected by surface topography, mimicking the way in vivo motion of certain cells is controlled by a complex set of biological markers. For example, creating a gradient of density of a surface pattern led to directional migration of fibroblasts.[6] Therefore, it is important that any approach toward fabricating materials that can guide cell behavior be capable of tuning these factors with precise patterns and shapes on the relevant size scale and within narrow windows of the values of relevant properties.

1.2. Noncontact Control via Photoswitching

Biology is inherently dynamic and it is thus a great and important challenge to design materials that can trigger, or somehow respond to, changes in biological systems with precise temporal and spatial control (Figure 1c).[7] There exists a wide variety of triggers that have been employed in designing stimuli-responsive polymeric systems, each with their own advantages and challenges.[8] Some of these stimuli, such as electrochemical or mechanical response, are less suitable for biological applications in vivo. While materials responding to changes in temperature, pH, and ionic strength can enjoy some specialized applications,[9–11] these triggers are generally unavailable in the tight physiological conditions in which they need to be applied. This leaves voltage or electric current, magnetic fields, and light as "biofriendly" input stimuli for allowing user-controlled triggering in a biological environment of temperature, fixed pH, and ionic strength. Among these three, we believe that light—the stimulus of focus for this review—is the most powerful and versatile trigger. Visible light is nondisruptive to biological systems, and additionally is a gentle and mild source of energy that can target materials with extremely high temporal and spatial resolution easily, cheaply, and with exact dosage control.[12–14] The ability to drive changes in surface properties or topography with high resolution using light as a source of power is attractive for many such applications, as it circumvents the inherent limitations to diffusion, and permits a remote (even a quite distant) wireless battery-free power supply. Light-responsive materials are usually classified by the chromophore they employ in order to achieve their photoresponsive properties, and fall into two well-defined performance classes: irreversible or reversible photoswitches.[15] In order for light to exert control over cell dynamics, the temporal and spatial coordination of many complex biological events taking place at the interface between the cells and surrounding ECM must be understood. Thus, development of such advanced biomaterials must include reacting and responding to this dynamic interplay, key to effectively manipulating live cells with high spatiotemporal accuracy. In this context, fully reversible photoswitches emerge as optimal candidates for modulating the response of biomaterials, including diarylethenes and spiropyrans, which "twist"

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The three research groups (Montreal, Tampere, and Yokohama) have collaborated since 2008, publishing more than 40 papers together on the Chemistry, Physics, and Engineering of azo-containing light-reversible soft materials.

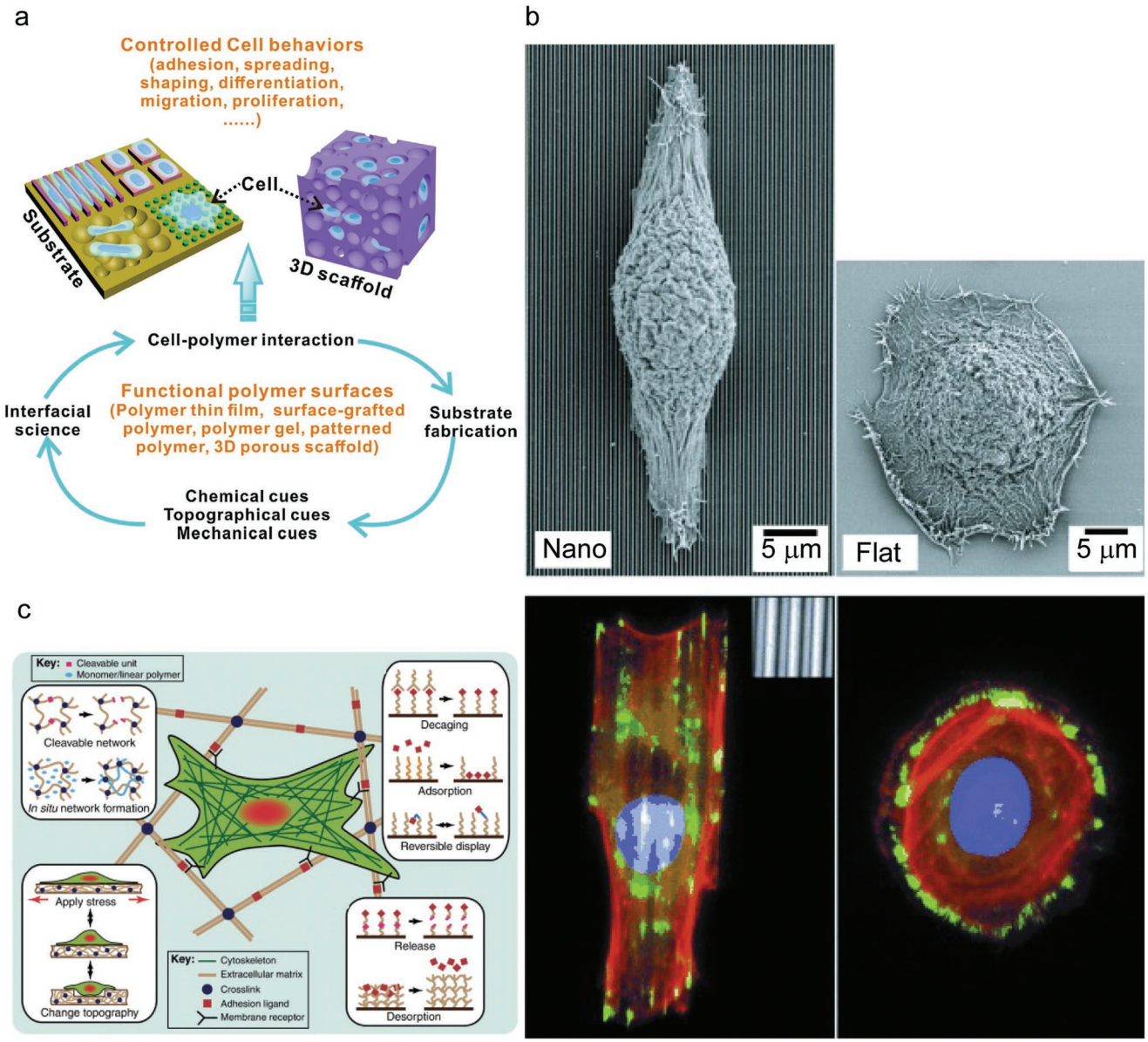

Figure 1. a) Schematic representation of different cues in 2D and 3D to control cell behavior in vitro. Reproduced with permission.[4] Copyright 2018, Elsevier. b) Epithelial cell morphologies on nanogratings and flat substrates in scanning electron microscope (SEM) (top) and fluorescence (bottom) micrographs. Reproduced with permission.[259] Copyright 2003, The Company of Biologists Ltd. c) Schematic illustration of different approaches for delivering dynamic cues during cell culture. Reproduced with permission.[7] Copyright 2012, Elsevier.

by ring-opening/closing reactions; coumarin and anthracene derivatives that dimerize reversibly with one another to "link and let go"; overcrowded imines, hydrazones, and alkenes, which can "spin" as free rotors about an axis, and those based on an azo-aromatic dye parent structure, which "snap" quickly back and forth between *trans* and *cis* geometries.[16,17]

1.3. Azo Dye Photoswitches

An ideal model photoswitch, also the most well-known and well-studied natural molecule for reversible shape change, is perhaps the rhodopsin/retinal protein/trigger system that enables vision. It is arguably the quintessential reversible photoswitch for performance and robustness, and the inspiration for many artificial biomimetic systems. In this highly efficient natural photoswitch, the small "kinked" *cis* retinal molecule is embedded non-covalently in a cage array of seven rhodopsin helices, and with the absorption of just a single photon isomerizes from this initial bent form to a linear *trans* geometry around a C=C double bond. This modest shape change is just a few angstroms, but the effect is then amplified by larger-scale disruptions to the rhodopsin helix assembly, releasing a protein "tail," which triggers a further cascade of larger geometric shape and associated chemical changes, domino-style, eventually culminating in an electrochemical signal to the brain of a vision event, the

initial energy of the input photon amplified thousands of times. Complicated biochemical pathways then revert the linear *trans* isomer back to the kinked *cis* geometric form, setting the system back up for another cascade triggered by the next incoming photon, permitting many subsequent vision cycles. The reversion mechanism back to the initial bent *cis* state is enzymatic and thus complex, so direct application of this retinal/rhodopsin photoswitch ex vivo to artificial engineering systems is prohibitive.[18,19] Azobenzene (azo) is perhaps the most promising artificial mimic however of the retinal photoswitch in terms of speed, robust reversibility, and simplicity of incorporation.[16] It has been the most successful photoswitch incorporated into polymers by far, providing a wide variety of photoresponsive materials and surfaces.[16] The fast response (picosecond photophysics), robust reversibility (of 10^5–10^6 cycles before fatigue), and absence of side reactions in the isomerization of azo groups also make them superior among their peers, so this review will focus mainly on the reversible transformation of azo dyes incorporated into various soft biocompatible host materials. The *trans* and *cis* states of azobenzene exhibit differences in absorption spectra and extinction, so azo-containing materials also change color upon absorption of light. More usefully, the geometric isomerization between the two energy states brings about differences in many secondary geometric and mechanical properties, which, while initially modest, can then be carefully harnessed and engineered, leveraging a mechanical advantage, to strongly amplify and magnify these size and shape effects to drive many useful larger macromotions.[18,20] From the biomaterial development perspective, some of the most powerful properties of azo photoswitches emerge when they are combined into polymers or other host material matrices, to fine-tune mechanical and surface biocompatibility. In particular, photo-orientation and photomechanical effects in azobenzene-based soft material systems ranging from photoinduced surface patterns in thin films of amorphous azo polymers[21] to photoactuation in liquid crystalline elastomers (LCEs),[22] and photoinduced mechanical changes in 3D matrices,[23] offer an attractive potential for biomaterial development.[24–26]

Traditionally, azobenzene chromophores can be categorized into three general classes (**Figure 2**a) characterized by the wavelength of absorption triggering the isomerization, and the thermal back relaxation of the *cis* to *trans* isomerization. Classic unsubstituted "yellow" azobenzene absorbs in the UV range and its *cis* form can be stable for days in the dark. "Orange" amino-substituted azobenzenes have an intermediate *cis* lifetime of minutes and slight redshift in the *trans* absorption band into the near-UV or deep blue, while "red" pseudostilbenes have a very fast thermal reconversion to *trans* (seconds or milliseconds) and a far redshifted absorption well into the blue-green or even low-energy yellow visible spectral regions, and are thus by far the most attractive candidates for photoreversible biomaterials.[27] This high variability in photochemical properties gives azobenzene a versatility advantage over other chromophores, as chemical substitution via a variety of possible synthetic routes affords a great deal of control over the resultant photophysical properties,[28] and allows one to tune the absorption wavelengths away from high energy damaging UV, to far deeper into the low-energy and more biologically benign visible region, yet still with 2 day *cis* half-lives or longer (Figure 2b–d).[29–35] As long as the irradiation is kept to wavelengths in the visible or near-UV, and limited to "sunlight-like" intensities of 100 mW cm^{-2} or less, in general azo molecules are stable to photobleaching or other light degradation over time. Photoresponsive materials that use azobenzenes of any spectral class hinge on capitalizing on differences between the *trans* and *cis* states. The isomerization causes a shortening of the end-to-end distance by 3.5 Å and in the parent azobenzene molecule a change in dipole of 3 Debye units—two aspects that, when well-engineered, can lead to impressive changes in materials properties.[17,36] Azobenzene-containing materials can be found as well-established key components in various applications from photomechanically responsive materials,[18] to optical and photonic devices,[37] and reversibly wettable surfaces.[38,39] This review, however, will focus on contributions that azo-containing polymers have made in applications specifically related to interfacing with biological and biomimetic systems.

Effective cell-influencing materials need to have dynamic topographical morphing capability, such as triggering a reversible presence/absence of biological ligands, tunable wettability, and/or switchable elasticity. Azobenzene-based materials can provide many of these various light-triggered dynamic properties, if properly engineered. Azobenzenes are efficient yet versatile photoswitches and can be chemically bound to a wide variety of host materials both artificial and natural, for instance, to membrane proteins to switch their regulatory action, to regulate cellular function by light.[17] They can be also attached to a hydrogel or elastomer matrix as crosslinkers in order to stretch polymer chains in the bulk of the material, thereby changing mechanical properties,[40] where the molecular-level photoswitching transfers energy to the polymer matrix, triggering phase changes and/or structural modifications. The photoisomerization process can affect a variety of material properties allowing for programming material motion at the microscale, or surface mass migration in response to light, leading to formation of micro- and nanoscale patterns that are in the relevant range for cell interaction,[41,42] for the dynamic control of bio-interfaces, and thus permitting azobenzene-based materials to be applied as "smart" cell culture supports. The aim of this review is to provide a current survey of the use of these photoreversible azo materials in cell biology and tissue engineering, generally following a "bottom-up approach": starting from the control of the molecular motion (orientation, flow) inside the material, leading to how light can be used to produce surface morphological changes, or eventually macroscopically photoactuating surfaces and structures. The three key and separate potentially bioactive effects enabled by the azo groups will first be reviewed from a photophysical perspective: that of light-induced "orientation" of the azo molecules, "topology" patterning using light to pressure-flow material into desired structures, and finally "actuation" that can be photomechanically produced.

2. Light Control Over Biosurface Properties

2.1. Photoreversible Molecular Orientation Alignment in Azo Polymers

It has now been more than 30 years since early research toward light-induced molecular alignment control in photoresponsive

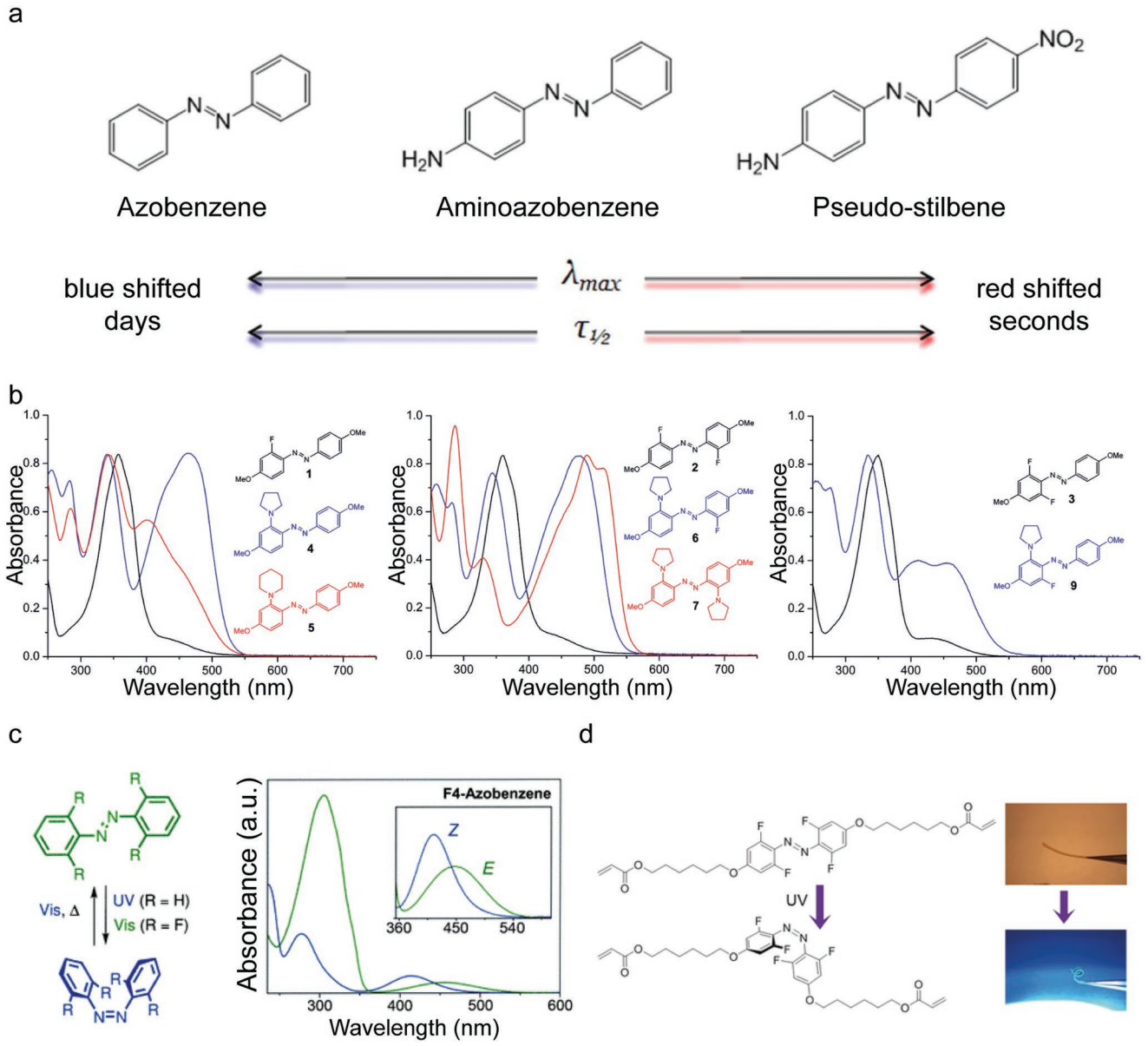

Figure 2. a) Classification of azobenzenes into three traditional spectral classes. Reproduced with permission.[16] Copyright 2013, John Wiley and Sons. b) Normalized UV–vis spectra of chemically modified azobenzenes in the ortho position. Reproduced with permission.[35] Copyright 2017, The Royal Society of Chemistry. c) Azobenzene isomerization scheme and UV–vis spectra for ortho-fluoroazobenzenes (F4, R=F). Reproduced with permission.[32] Copyright 2014, Wiley-VCH. d) Photoactuation and consequent shape change of ortho-fluorinated azobenzene polymer ribbons. Reproduced with permission.[30] Copyright 2016, Wiley-VCH.

azobenzene-containing polymer films started receiving wide attention, due to potential use in liquid crystal (LC) displays and other photonic applications. In 1984, Todorov et al. first observed that this molecular orientation (measured as birefringence) could be written/erased reversibly by linearly/circularly polarized light,[43] and in 1987, Tazuke et al. further demonstrated that it was possible to use the photoisomerization of a small amount of azo dopant in a nematic liquid crystal to induce a phase change in surrounding media.[44] In 1988, Ichimura et al. proposed a photoisomerizable azo monolayer "command surface" which could reversibly induce alignment in the bulk over many micrometers of adjacent nematic liquid crystals,[45] calling to one's mind an image of many transparent "soldier" LC molecules in the bulk receiving orders to reorient deliveries from their azo "commanders" on surface (**Figure 3**a). In 1991, Gibbons et al. extended this idea, using polarized light to photoinduce a persistent alignment of nematic liquid crystals filling an entire cell with azo dye–doped command surfaces.[46] This initial work provided early and encouraging suggestion that, in principle, a well-designed layer or command surface could exert significant control over adjacent materials and over the size and shape of biological cells, without needing

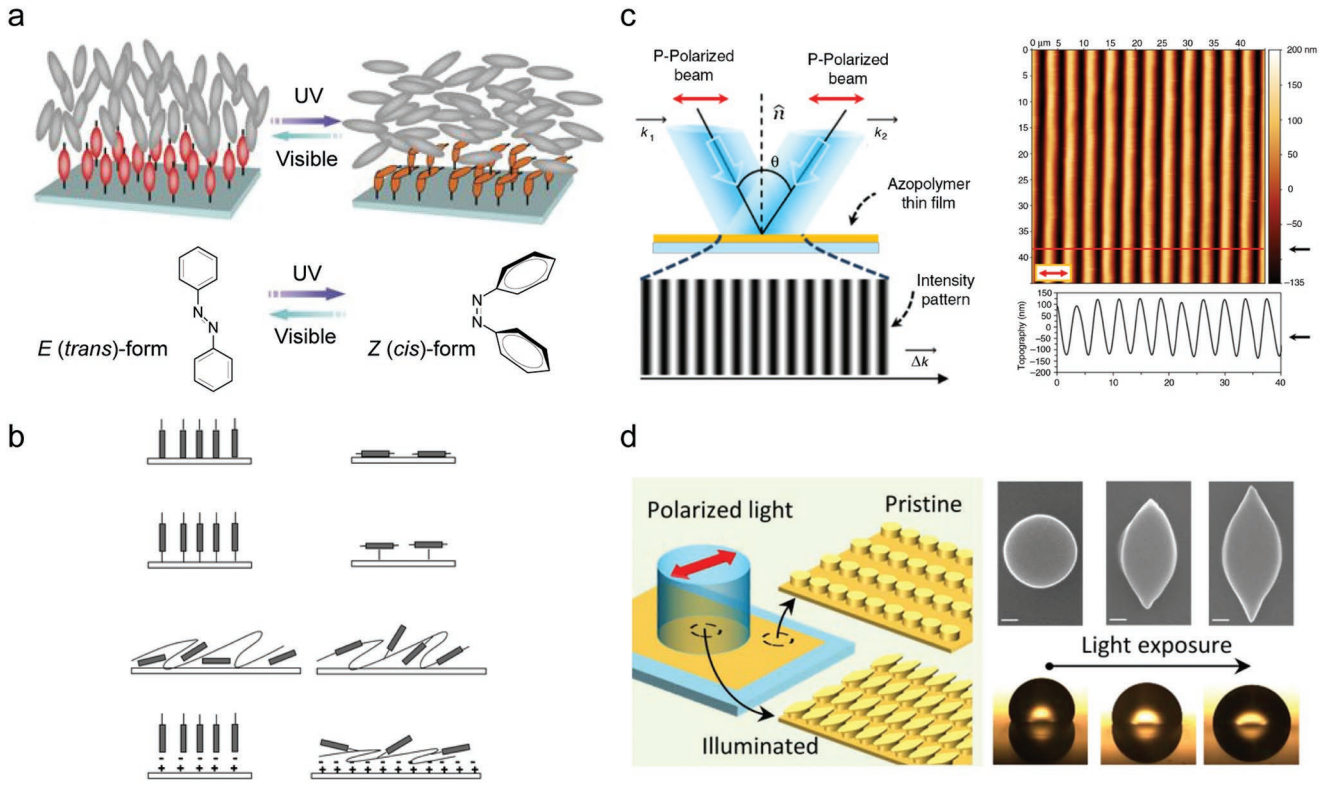

Figure 3. a) Reversible photoalignment induced by a "command surface" through azobenzene isomerization. Reproduced with permission.[52] Copyright 2014, Springer Nature. b) Schematic representation of different types of photoaligning materials. Reproduced with permission.[48] Copyright 2012, Royal Society of Chemistry. c) Schematic visualization of two-beam interference lithography process and illustrative SRGs formed onto an azo polymer film as imaged by AFM and cross-sectional profile. Reproduced with permission.[25] Copyright 2018, De Gruyter. d) Graphical representation of light-driven reshaping of an array of molded micropillars and their morphology as imaged by SEM. This principle has been used to tailor surface wettability of an azo polymer. Reprinted with permission.[86] Copyright 2017, American Chemical Society.

to penetrate the interior. Inspired by these pioneering early studies, development of new photoaligning materials and associated technologies emerged as an important topic in applied liquid crystal science (Figure 3b),[47,48] taking advantage of the reversible and remote control over the anisotropic molecular orientation, which, in turn, allows patterning and fine control over optical properties, e.g., light propagation through liquid crystals,[48–52] or other next-generation photonic applications.[53] In general, the control over photoalignment in LC materials has been generated via mechanism based on either a photoinduction of anisotropy in thin alignment layers,[54] or by doping photoresponsive molecules in small amounts into the bulk of the liquid crystal material,[55] where an azo or other dye is required to be incorporated into the device, necessary to absorb and transduce the incident light polarization into a molecular reorientation. Clearly, for control over the bio-interface, the former approach of "command surfaces" needs to be followed, as one could envisage the requirement of azo molecules to be present inside the cell or tissue as prohibitively interfering. An improved strategy was then developed by Fukuhara et al. in 2014, that employed a noninvasive azo molecular command system at the free surface as opposed to the substrate,[56] which may offer some distinct advantages for application to control over the bio-interface. Using a process based on a modified commercial inkjet printer that could deposit material onto various material surfaces, these authors demonstrated that arbitrary "designer" images of aligned mesogens could be easily and finely photopatterned in the polymer films with high spatial resolution. This general and clever strategy is widely applicable to various material systems, and importantly, requires no modification or pretreatment of the substrate surface, so in principle is readily adapted to materials already optimized as biocompatible. Following this first report, Nakai et al. reported an out-of-plane photoswitching effect, shown to be fully reversible, extending out from a "command" skin layer at the free surface.[57] The authors attribute this effect to the formation of a phase-separated azo-containing skin layer after sample annealing, "commanding" the alignment of the underlying material by the free surface side. The presence of this bilayered structure and the out-of-plane photoswitching were investigated by contact angle measurements and grazing incident small-angle X-ray scattering (GI-SAXS), whereas polarized optical microscopy showed the patterned alignment obtained upon UV irradiation with a photomask. New material design strategies for free-surface photocontrol with azobenzene command surfaces and processes[58] provide further flexibility in separate optimization of biomaterial performance, and offer exciting new materials that, in principle, could be used for next-generation photoalignment control of biological materials.

2.2. Photopatterning Azo Materials for Designer Surface Topologies

A second era of azo molecular control began in 1995, when an unprecedented and unexpected optical effect was discovered in azo polymer thin films being developed for photoalignment control. Both Rochon et al.,[59] and Kim et al.,[60] independently and simultaneously discovered a large-scale near-surface mass transport when the films were irradiated with a light interference pattern of two low-power coherent laser beams intersecting at the surface of an amorphous azo polymer, for a few minutes at room temperature (Figure 3c). A sinusoidal pattern of light interference at the sample surface led to a sinusoidal-shaped surface relief topology pattern, then referred to as a surface relief grating (SRG), as light diffraction is how the topological features were most easily monitored during inscription. However, the azo-initiated surface mass transport is not limited to just parallel line gratings, and can instead produce arbitrary complex structures, directed by the patterns of spatial intensity and polarization of the incident light.[61–63] Hence, this all-optical patterning phenomenon might more generally and accurately be called photopatterning, phototransport, or photomorphing. The surface features were found to be significantly deep/raised, up to many hundreds of nanometers, as confirmed by atomic force microscopy (AFM), suggesting that light has triggered entire polymer chains to flow across the near-surface film region over a distance of hundreds of nanometers. The topologies are stable indefinitely in an ambient or biological environment, yet can be erased by heating above T_g, or with light. The all-optical patterning process is unique to azobenzenes, as all other absorbing yet nonisomerizing dyes cannot reproduce the same curious effect and has been studied intensively with many reviews now published, detailing the various experimental results observed.[20,24,25,61,64,65] Critically, the process requires the isomerization of azobenzene chromophores, as materials with other absorbing but nonisomerizing dyes do not produce these patterns, and the feature size and patterns are limited only by the light resolution. Similar to the LC alignment layers, the wavelengths needed can be visible and the required intensity is low, so well-suited to biological applications, and the process is single step at room (or biological) temperature without the need for any further chemical or physical processing steps. Restructuring of the azo surfaces can also be provoked near-field, achieved by using the metallic tip of a SNOM (scanning near-field optical microscope), overcoming the diffraction limit.[66,67] Spontaneous self-organization of azo surfaces under one-beam irradiation has been shown to create hexagonally ordered bumps, parallel stripes, or more complex structures, mimicking similarly evolving patterns found elsewhere in nature, e.g., in the morphogenesis of animal hair (such as the stripes of tiger fur), studied first by Alan Turing in the 1950s.[68–70] This interesting self-organization has been also exploited to pattern azo materials by light-induced erasure of wrinkling structures in a stretched polydimethylsiloxane (PDMS)-azo polymer bilayer,[71] and offers exciting potential for facile inscription of complex surface topologies that mimic or influence biological cells or tissue, in principle even dynamically while in contact.

The structural patterns of the surface relief inscriptions can be harnessed as templates to organize other systems, including performing as command layers, aligning adjacent liquid crystal phases,[72–76] organizing fluorophores into various 2D micropatterns,[77,78] templating higher-order structures by arranging colloids into grooves,[79,80] and optical control over the alignment of cylindrical nanodomains in block copolymer thin films.[81] The photoinduced surface structuring enables azo polymers amenable to a variety of patterning schemes via optical lithography, such as diffraction gratings that could be transferred onto a stimuli responsive hydrogels functionalized with glucose oxidase,[82] demonstrating glucose sensors capable of continuous and quantitative measurements in solution. Soft lithography methods can also be coupled to light-induced mass migration, reshaping pre-existing structures. This technique, often referred to as "directional photofluidization lithography," employs micromolding for the fabrication of pristine 1D or 2D patterns, which can then be postmodified using light-induced movement of the azo polymer (Figure 3d).[83,84] By optimizing the conditions of irradiation, this technique allows one to fabricate ellipsoidal, circular, and even rectangular nanostructures with feature sizes as small as 30 nm, and arrays of pillars of different sizes have been superficially reshaped into anisotropic structures by changing simple illumination parameters such as intensity and polarization, providing surfaces with variable wettability, and pillars that could also be reversibly reshaped.[85–87]

These light-induced surface topologies have been employed as masks for fabricating large-area silicon nanostructures,[88] with feature sizes as small as 65 nm, as well as periodic 1D and 2D plasmonic structures.[89] Tobacco mosaic viruses were used as a mask to immobilize various compounds by placing them on an azobenzene surface, and subsequent irradiation resulted in virus immobilization on the surface by the formation of complementary grooves.[90] Finally, a focused Gaussian beam has also been shown to affect the surface modulation of an azo material in a power-dependent way. In low-power regimes, a hole with lateral lobes along the polarization direction is formed, whereas in high-power regimes (reaching hundreds of watts per square centimeter), a protrusion instead of a cavity piles up in the center.[91–93] Therefore, scanning of a focused laser allows one to "draw lines" on the surface in a reversible manner. All these techniques represent valuable strategies for obtaining tunable surface topographies which lie exactly in the biologically relevant range, on demand, and more importantly, many of them allow for reversible restructuring of the interface between the material and the adjacent biological counterpart in a biocompatible manner.

3. Polymeric Photoactuators: Moving toward Artificial Muscles

3.1. Photoactuation

In addition to photo-orientation and optical patterning of topologies, azobenzene-containing materials can also perform a 3rd separate and fundamental photoreversible effect of potential influence over the bio-interface: the exertion of mechanical stresses and forces that can result reversibly from visible

irradiation. If cleverly engineered, these molecular-level forces can be amplified into macroscopic shape-shifting "artificial muscles," or even actuating "molecular machines" powered by a transduction of light energy to mechanical motion. These azo-based "photomechanical" materials are the newest emerging class of stimuli-responsive materials and coatings for the design of artificial muscles and micromachines.[94,95] Actuators are systems where energy is converted from an input stimulus into mechanical motion, and many light-driven actuators based on photoreversible systems, able to undergo large deformation upon relatively low input stimulus, have been demonstrated.[94,95] Photomechanical actuation, where light energy is converted mechanically into changes in shape of a material, is particularly promising for various bio-interface devices, due to the possibility for precisely defined, noncontact morphing of shape and structure, triggered by gentle low-power visible light sources otherwise benign in bio applications. This effect is especially appealing for interfacing with neural cells, many of which possess an inherent mechanotaxis trigger response to minute physical stimulation. Various light-induced changes in shape have been achieved in shape-memory polymers,[96] carbon nanotube-containing composites and bilayers,[97,98] and crosslinked polymers and elastomers incorporating photochromic molecules,[21,22,99] but herein we focus on azobenzene-driven actuators, that can produce motion reversibly on a size scale and timescale of interest to living cells. Photomechanical effects in azobenzene-containing materials were first observed more than 50 years ago in azo-dyed textile fibers curling slightly while drying in the sun,[100] but it was not until 20 years later that much notice was taken of this curious effect. In the 1980s, Eisenbach prepared poly(ethyl acrylate) networks crosslinked with a similar azobenzene chromophore,[101] and these materials exhibited a photoinduced contraction of 0.2% when irradiated with UV light, and then re-expanded after visible light irradiation. In parallel studies around the same time, Matejka et al. reported that when a loading of azobenzene into the material was greater than 5%, an increased contraction of nearly 1% could be photoinduced, enabling significant azo actuation simply by increasing the azo dye loading levels.[102–104]

3.2. Photomechanical 1D Motion of Azo LC Materials

Liquid crystalline elastomers are polymeric materials in which the molecular alignment and therefore the deriving anisotropic properties have been fixed by crosslinking. LCEs exhibit a fascinating coupling between anisotropic molecular order (due to the presence of aligned LC mesogens) and elastomeric properties (brought about by the polymer network), and have emerged as strong candidate for soft-yet-strong materials for many biomaterial applications. Phase transitions can be triggered, leading to remarkable reversible shape changes due to the presence of crosslinks that are amplified by the LC molecular order. These transitions can be provoked, among others, by the photoisomerization of azobenzene. Reversible photoinduced changes in the elasticity of azo-containing semi-interpenetrating network films were reported achievable by visible and UV light irradiation.[105] In other studies, similar films of azobenzene-containing vinyl ethers with polycaprolactone achieved a "rapid" (at that time, a few seconds) anisotropic shape deformation and recovery. This photomechanical response was reported to increase with the extent of film stretching, and it was proposed to arise from an anisotropic response effected by the isomerization-induced vibration of azobenzene groups, which lowered the modulus of the amorphous regions being deformed.[106]

Azobenzene LC-based systems make excellent potential candidates for soft, elastic photomechanical actuating materials for many niche applications requiring small size, remoteness of the power source, localized actuation, and/or especially, employment in wet biological environments, where they need to be unencumbered by wires, batteries, electric current, and any internal moving parts subject to friction. Since the light polarization direction can be used to control the direction of bending in polydomain LC-based systems, fully directional photomechanical control is enabled in these materials.[107] For thin films floating on a water surface, contraction in the polarized light direction was observed for LC materials, where in contrast an expansion was reported for amorphous materials.[108] A related amplification of micromotion to macroscopic motion was achieved with the photoinduced bending of an azo dye–coated microcantilever.[109] Other important achievements in photoactuation include the macroscopic bending and 3D movement control of fibers made of azo liquid crystalline elastomers,[110–112] the fabrication of working light-driven microvalves,[113] and the achievement of fully light-driven continuous plastic motors.[114]

3.3. Higher Order Macroscopic Motion in Azo Soft Materials

In order to move from 1D surfaces and thin films into more complex and sophisticated 2D and 3D geometries, fibers can also be thermally drawn from LC mixtures of appropriate viscosity, and then crosslinked via light or other chemical reactions. The requirement for dye-doped materials undergoing photo-crosslinking is that the photoinitiator and dye have to be chosen carefully in order not to interfere with each other during polymerization. Alternatively, the use of polarized light for photopolymerization circumvents this problem.[115] As an example of chemically crosslinked fibers, Cheng et al. obtained mildly crosslinked fibers from random block copolymers containing azobenzene groups in the side chains.[116] They observed a photoinduced bending motion of these fibers away from light, ascribed to a surface volume expansion due to azobenzene isomerization. The determining factor appeared to be the location of azobenzenes inside the structure. Gelebart et al. in contrast fabricated an array of fibers by drawing them from a melted main chain LC oligomer functionalized with coreactant azobenzene moieties.[117] Here, the liquid crystalline domain aligned in the drawing direction and bent toward the light source. By illuminating this array with a complex light pattern, they were able to simulate the asymmetric movement of cilia in the lungs (**Figure 4**a). Azobenzenes have also been incorporated into dendronized polymers, synthesized by ring-opening metathesis (Figure 4b).[118] This bottom-up approach to the design of hierarchical superstructures allows for a fine control of the structure–property relationships of the final material. Uniaxially oriented films with azobenzenes in the side chain of

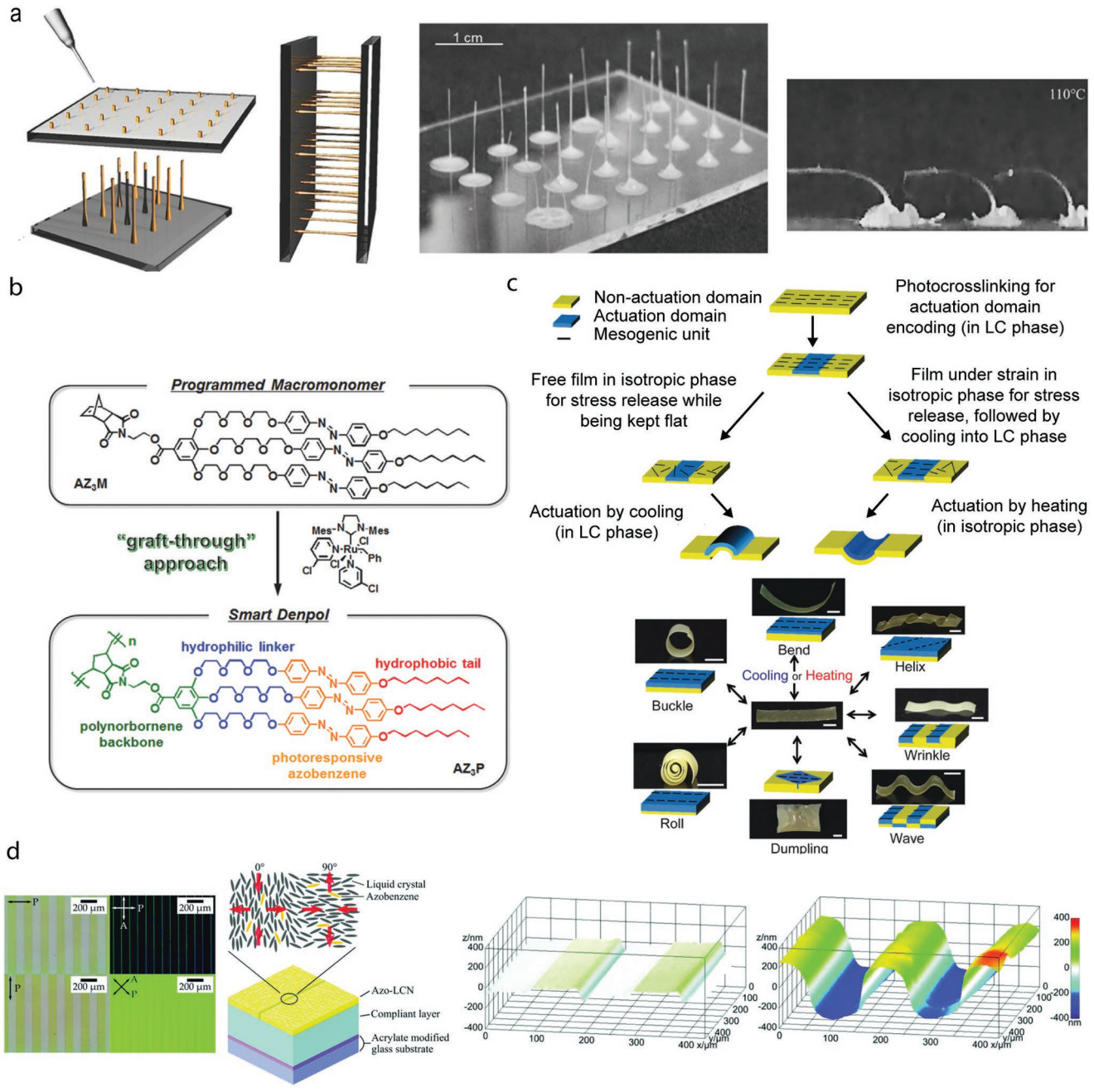

Figure 4. a) Photoactuating fiber arrays formed by thermal drawing of crosslinkable LC solution droplets. Fibers bend toward the UV light source.[117] Copyright 2017, Wiley-VCH. b) Chemical structures of azobenzene containing denpol. Reproduced with permission.[118] Copyright 2017, Wiley-VCH. c) Photo-crosslinking of domains in polymer actuator for multiple types of motion. Reproduced with permission.[119] Copyright 2017, Wiley-VCH. d) Polarized micrographs and graphical representation of the azo-LCN bilayer coating. The red arrows indicate light-induced stress. Topographical oscillation as a result of the polarization rotation. Adapted with permission.[124] Copyright 2018, Wiley-VCH.

the dendron exhibited different smectic phases, and successful photoactuation upon UV light irradiation was observed.

Soft actuators may eventually be capable of adaptive shape changes under the same stimulus, a characteristic that is at the moment still in its infancy. Yang and Zhao designed a crosslinked liquid crystalline film with eight different types of shape changes. By using spatially resolved control of photo-crosslinking, they were able to inscribe carefully designed patterns of actuation regions in the films, which resulted in the desired motion upon stimulus (Figure 4c).[119] Lahikainen et al. reported on the design of an azobenzene-containing reconfigurable liquid crystalline actuator exploiting both photochemically and photothermally driven actuations for programming and activating multiple shapes in one single sample.[120] For this purpose, a long-*cis*-lifetime azobenzene is included into the liquid crystalline mixture as crosslinker

(polymerized with a splay alignment) and a 633 nm absorbing dye (Disperse Blue 14) was instead doped into the matrix. *Trans–cis* isomerization could be used to pattern the mechanical properties of the actuator with UV light, whereas dye photothermal transduction allowed for stress release from the material. Blue-green light irradiation could bring back the system to its initial shape through *cis–trans* back isomerization. Finally, it was shown how this concept could be implemented as a light-fueled "gripper," which could grasp, hold, and then release objects.

A remarkable development in the design of higher-order photoactuators was the advance from in-plane bending and linear contraction, to out-of-plane helical or twisting motions.[121] The inspiration for increasing the complexity of photoinduced motion derives from nature: Lee et al. were the first to capture a combination of in-plane oscillation with out-of-plane twisting in azobenzene-containing liquid-crystal polymer networks.[122] An important step toward demonstrating biomimicry was achieved by Iamsaard et al. who fabricated spring-like photoactuators and demonstrated complex motions including helix winding, unwinding, and inversion,[123] illustrating that by careful preparation techniques LC polymer photoactuators may incorporate various regions that permit different dynamic behaviors, such as mixed-helicity springs where left- and right-handed helices are joined together, which upon UV irradiation wind at the right-handed side and unwind at the left-handed side, mimicking the gripping motion of plant tendrils such as wild cucumbers.

By attaching liquid crystal networks (LCNs) as coatings on a support material, different types of deformations can be induced, due to the limiting effect of the attached surface to the lateral stress.[41] Such an approach leads to surfaces whose morphology can be modified and even oscillated by means of light irradiation. This effect is most interesting for applications in self-cleaning surfaces, soft robotics, haptics, and cell culturing. A recent approach developed by Hendrikx et al. exploits a compliant intermediate layer between a glass support slide and an LCN coating with a patterned director for further enhancing the oscillation amplitude of the topography from roughly 70–100 nm to 1 µm (Figure 4d).[124] All of these initial developments in light-driven actuation paved the way for various separate motions to be combined on the same engineering platform, for higher-order composite motions, that are necessary to create functioning simple actuation devices that can perform complex prescribed tasks, such as "artificial muscles" powered only by light.

3.4. Biomimetic Microactuators and Artificial Muscles

Employing multicomponent engineering, photoinduced shape changes, and actuation can achieve bioinspired proof-of-principle micromachines capable of generating continuous mechanical work under irradiation. A first and leading example of such a "machine" was reported in 2008 by Yamada et al., who translated the photoinduced deformations of a "ring" of crosslinked liquid-crystalline polymer film into a rolling rotational motion,[115] that could then be mounted onto a stationary pulley system as a "belt," driven continuously in a counter-clockwise direction of rotational motion when light was introduced. Other examples of basic robotic motions by the same research collaboration followed, such as an "inchworm" locomotion from a sheet of azo-LCN adhered to a flexible polyethylene (PE) substrate, with an asymmetric sliding friction providing a "gripping heel" and a "sliding foot" working in concert.[125] A robotic arm-like actuation was also demonstrated by fabricating flexible azo polymer "hinge" joints of a PE film laminated with azo-LCNs to provide optical control (expansion or contraction) at specific individually addressable positions of the arm. The light-responsive azo-LCN regions then functioned as hinge joints on the arms, acting as remotely addressable bending "elbows" and "wrists." Lv et al. also reported a clever strategy with light-driven tubular microactuators fabricated from photoresponsive liquid crystalline polymers to manipulate fluid slugs,[126] by photoresponsive asymmetric deformation of the actuator to induce capillary forces for liquid propulsion. In another work from the same group, Huang et al. fabricated several shapes of microactuators such as straight, helical, serpentine, and "Y"-shaped, creating light-driven microswimming "robots" with a gripper that demonstrated crude swimming, grabbing, carrying, and transport.[127] These hybrid materials represent complex driving and control of various microrobotic motions and structures achieved remotely by light.

Iamsaard et al. developed photoactuators where the helical deformations were predetermined by including azo dyes as chiral dopants, and by controlling the relative orientation of the liquid crystals aligned within each spring.[123] A right-handed or left-handed liquid crystalline film could be induced to twist, curl, or do both upon light irradiation. Complex extensile and contractile coiling and twisting helical motions were demonstrated, with mechanical energy extracted from the system by a pair of embedded magnets. Photo-deforming molecular systems were also demonstrated on a larger macroscopic scale,[30] employing fluorinated azobenzenes to slow the room temperature *cis* back to *trans* shape reconversion, retaining their photo-deformed shape in excess of 8 days, to overcome thermal relaxation on a timescale useful for most devices envisaged. Wie et al. also demonstrated impressive photomotility using thin strips of liquid crystalline networks containing azo dyes,[128] where on broadband UV irradiation their polymer films added a variable and controlled directionality and photomotility speed, via the liquid crystalline orientation. Recently, Ferrantini et al. developed a biocompatible azo-acrylate LCE with photoresponsive contraction that could be tuned to mimic the contraction frequency and behavior of human cardiac muscles.[129]

Direction control that is more complex can be gained by photomechanical fibers that function as artificial muscles, where the bending direction can be controlled simply by repositioning the source of illumination.[130,131] Interestingly, these homeotropically aligned, crosslinked LC polymer films were observed to exhibit a completely different bending behavior, where upon UV light exposure they bent away from the light source, due to isotropic expansion of the surface upon *trans–cis* isomerization.[132] As another way to control the direction of the photoinduced bending beyond the initial chromophore alignment, Tabiryan et al. showed that the direction of polarization of the incident light also affects the bending direction, which they attributed to a light-induced reorientation of the azobenzene groups.[133]

Nocentini et al. demonstrated a method for fabricating LCE fibers by pulling them from LC monomer droplets with or without stretching and twisting of the material, which resulted in different rotations upon exposure with UV light.[134] Van Oosten et al. showed that control could also be gained over the bending direction by introducing internal composition gradients within the LC polymer network.[135] By adjusting the irradiation stimulus power, elastic fibers are able to rotate by specific angles, completing even full revolutions around their axis. As a final example, Priimagi et al. showed that the direction of bending can also be controlled by the nature of the bonding between the crosslinked polymer network and the azobenzene groups.[136]

3.5. Bioinspired Devices and Light-Powered Soft Robotics

Demonstrations of more sophisticated photomechanical actuators continue to be reported. Zeng et al. demonstrated a light-powered walking LCE "artificial muscle" powered microrobot,[137] where the actuating unit comprises four conical legs on the LCE body, and could in effect "walk" via sequential expansion/contraction cycles of the body with a micropositioned 532 nm laser. Palagi et al. also demonstrated a light-driven LCE microrobot that performed sophisticated biomimetic motions,[138] driven by complex travelling waves of carefully coded structured incident light fields. Rogoz et al. prepared a caterpillar-like soft machine on a natural size scale,[139] where the robot performed various tasks such as squeezing through a narrow hole, walking up a slope, and pushing around large objects. Zeng et al. were also inspired by caterpillar motion and developed an inching robot in miniature made from a monolithic LCE film, designed with alternating splay-aligned segments such that on and off cycles from a visible light source lead to cycles of deformation resulting in a walking/inchworm motion.[140]

Smart optical components that are capable of self-adjusting their action to the surrounding light conditions would be extremely interesting in biotechnological applications. Usually, optical elements like diaphragms are able to mechanically regulate the amount of transmitted light, but these systems require an external power input to actuate, whereas in nature the iris of an animal's eyes is capable of self-regulation. In a recent approach by Zeng et al., LCEs are used to bring actuators to a new performance level,[141] by assembling LC moieties in a radial molecular orientation through a photoalignment layer and exploiting the initial bending stress (opposite to the direction of light-induced bending[142]) to obtain an opening/closing iris-like device (**Figure 5**a). This device is then capable of spontaneously closing in response to changing light conditions, thereby self-regulating the light transmission through the device. A "predatory" biomimetic microrobot was proposed by Wani et al.[143] who constructed a device they dubbed an "artificial flytrap" via a light-driven gripping motion which mimicked the capture motion of a real Venus Flytrap plant. This "intelligent" LCE microactuator was positioned onto the tip of an optical fiber, which served as both the source of power and as a contactless probe to sense the local environment, providing an important control and feedback loop to enable closing the gripper when an object crossed the field of view, providing a higher level of autonomy for such a "smart" microrobot. An autonomous light-responsive gripping device able to distinguish between particles of different color has been also produced at the microscale by Martella et al. (Figure 5b).[144]

Another use of azobenzene-based LCEs in a biomimetic device has been recently proposed by Kizilkan et al. for the photocontrol of adhesive forces.[145] They used a multilayered structure where a porous azobenzene-containing LCE is combined with a microstructured PDMS surface to mimic a dynamic gecko foot pad surface. Upon UV light illumination, adhesion was reduced up to 2.7 times and then it was quickly recovered upon switching off the light stimulus (Figure 5c). The system has been successfully implemented to work as a pick-up and drop-down system for transporting 2D and 3D objects. Further examples of LC-based devices can be found in many comprehensive reviews on the topic.[146–149]

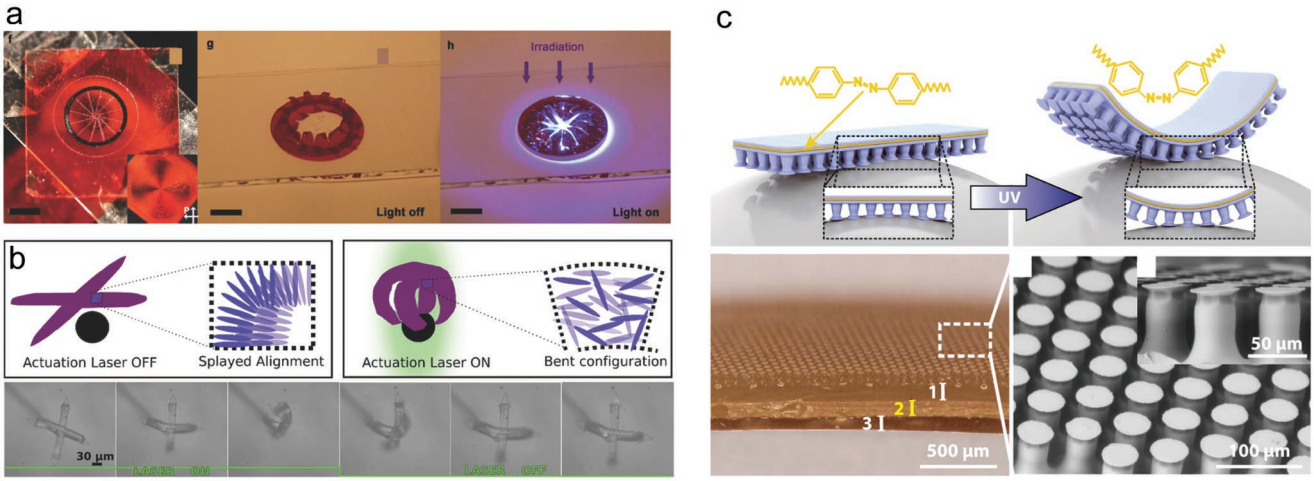

Figure 5. a) Polymerized LCE film cut in 12 radial slices. The LCE iris is open-state when the light is off, whereas it closes upon light illumination. Scale bars are 5 mm. Adapted with permission.[141] Copyright 2017, Wiley-VCH. b) Microhand design and molecular alignment scheme. Microhand closure and opening upon light switch. Reproduced with permission.[144] Copyright 2017, Wiley-VCH. c) Mechanism of reversible adhesion by azobenzene photoswitching. Microscopy images of the multilayered structure and SEM image of pillars. Reproduced with permission.[145] Copyright 2017, AAAS.

In these examples of various light-driven actuation, the input energy source was either visible or UV light, giving rise to a locally addressable photoinduced expansion/contraction of the photoresponsive polymer structures. However, UV light can be harmful to many living organisms, so it is of great interest to develop "redshifted" light-driven actuators responsive at longer and more benign wavelengths, deep into the visible and far from the UV, for any realistic biorelated applications. In general, there is also an added advantage in avoiding UV light to lessen photo-degradation of these soft and often-fragile organic materials, and to improve the penetration depth into aqueous-based biological environments. Some of the first longer-wavelength visible-light-driven photomobile materials, incorporating deep red photoresponsive azotolane moieties, were developed by Yin et al.,[150] fabricating long visible-light-driven microrobots that were shown capable of lifting and moving objects weighing ten times that of the robotic arm itself.[151,152] This complex system consisted of several integrated azo-LCN layers on PE supports connected by joints that mimicked the fingers, hand, and wrist. The robotic arm was shown to complete complex movements by individually addressing the various photoactive sections, so that an object could be first picked up and then later dropped by targeting the "fingers," while when focusing light instead at different "elbow" locations, the entire arm could be moved in concert. Following this report, Lee et al. then demonstrated a clever photofueled catapult motion that was capable of launching objects using low-intensity blue-visible light irradiation.[153] Wu et al. also designed a similar composite material, but one truly powered by long-wavelength light, where up-converting nanophosphors successfully enabled a photoinduced deformation using near-infrared (near-IR; 980 nm) irradiation.[154] Lastly, this same research group developed a similar red-light soft actuator driven and controlled by a low-power excited triplet–triplet annihilation mechanism based on up-conversion luminescence (TTA-UCL),[155] where red-light-controllable actuation offers advantages in potential biological interface applications, as the long wavelengths used eliminate undesirable thermal effects, and permit deeper penetration into live tissues. Sensitivity to visible light can also be brought about by a combination of different absorbing units, achieved by Cheng et al. with a nanocomposite LCE,[156] where by adding graphene oxide to a polymer-dispersed liquid crystalline material containing azobenzenes, they broadened the responsivity of the system via both photochemical and photothermal processes.

Additive manufacturing techniques, such as two-photon lithography and 3D printing, are becoming more and more relevant in biotechnology, giving the possibility to produce complex 3D structures on different length-scales and becoming amenable to many different materials.[157–160] It has been shown that LC materials can be utilized with these techniques with the characteristic molecular order remaining preserved during the photo-crosslinking process,[161] or even induced and patterned due to shear forces in the 3D printing process.[162–164] Van Oosten et al. achieved the construction of bioinspired artificial cilia for mixing applications and microfluidic pumping (Figure 5d).[165] Using commercial inkjet printers, droplets of reactive LC azo monomers were deposited onto a surface of polyvinyl alcohol (PVA) and a thin layer of polyamide was rubbed to induce LC alignment. After self-assembly, the LC monomers were crosslinked, and then a second azobenzene monomer-based ink layer is added, to create cilia that were capable of responding to different wavelengths of light. Dissolving the PVA support releases the cilia, which were then demonstrated as capable of bending upward depending on intensity, when irradiated from above with UV light. The bicomponent cilia were shown capable of various bending movements enabled by their separately addressable sections, and the addressing of these two components sequentially with different irradiation wavelengths could thus achieve a nonsymmetric reciprocal motion, permitting the cilia to effectively pump fluids.[166]

4. Interfacing Azo to Bio: An Eye toward Influencing Living Systems

4.1. Photocontrol of Material Properties for Biological Applications

While azo-based materials for biological applications share a common concern of potential toxicity with all new artificial materials proposed, in general azo dye incorporation does not exacerbate this common problem, and many azo materials have now been demonstrated ex vivo as nontoxic with good cell felicity. It is also helpful to note that there remains a large class of molecular azo dyes that are still approved for human consumption as food colorings, from which one can draw for lowest-toxicity incorporation. Many of the existing methods for cellular control via topographic or mechanical cues are static, consisting of nano- and microimprinted grooves, pillars, and pits or other prefabricated surfaces.[5] However, the interaction between cells and the ECM in the body is not static. Fibroblasts migrating in the body to repair wounds are partially guided by the changing density of the ECM as it is undergoing damage and repair.[6] Reversibly switchable materials are a step toward true biomimetic cell guidance. There are several approaches to making a surface which can undergo switchable mechanical or topographic changes, including temperature-responsive or pH-responsive polymer brushes grafted to a surface which can control cell adhesion and detachment,[4] or liquid crystal alignment to influence self-propelling bacteria.[167] It is difficult to locally target pH and temperature, so photoswitchable materials are clearly superior for these applications of control over cell behavior.[4] Photocontrol of cellular interactions using azobenzenes tethered to surfaces can be traced back to pioneering work by Auernheimer et al. who demonstrated that azobenzene-functionalized arginine-glycine-aspartic acid (RGD) peptides grafted onto a silicon surface could achieve photoreversible control of cell adhesion.[168] Another approach is to create appropriately tailored substrates using polyelectrolyte multilayers, where light allowed for exposure of RGD ligands which enhanced the growth of cells via adhesion pathways mediated by integrin.[169]

The hydrophilicity and the surface energy of synthetic cell surfaces have previously been shown to have an effect on cell adhesion, attachment, and proliferation.[4] Pan et al. created a method for making surfaces with tunable wetting control (**Figure 6**a), where spin-coated surfaces with a functionalized azo derivative (fluoro-AZO) resulted in a material that had

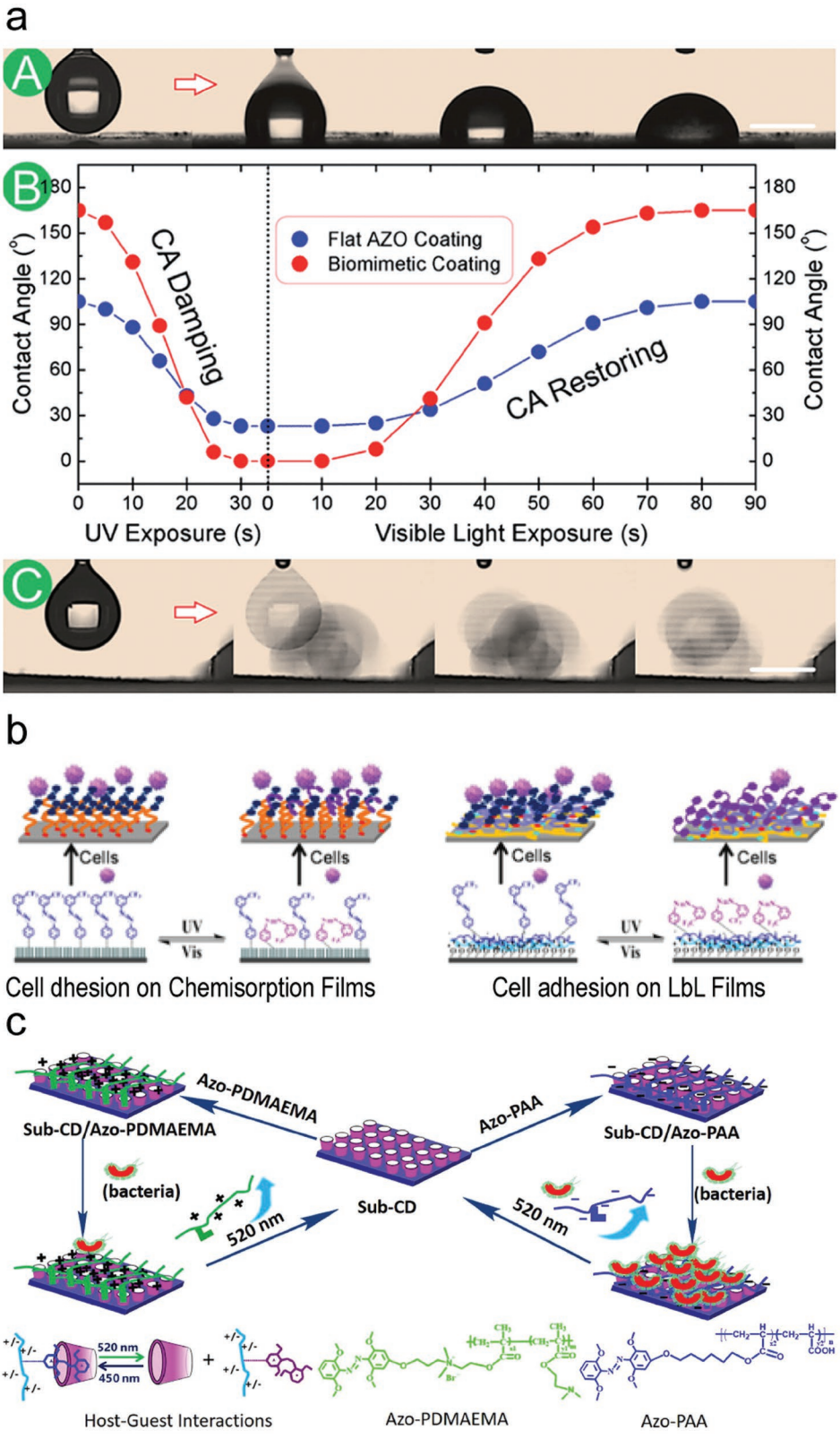

Figure 6. a) Switchable wetting behaviors of surfaces via light behavior. Reproduced with permission.[170] Copyright 2014, Royal Society of Chemistry. b) Schematic representation of reversible switching of cell adhesion on chemisorbed LbL surfaces. Reproduced with permission.[175] Copyright 2016, Wiley-VCH. c) Synthetic routes for preparation of antibacterial and bioadhesive surfaces based on host–guest interactions. Reproduced with permission.[176] Copyright 2018, Elsevier.

photoswitchable wettability, where the contact angles switched between 23° to 105° upon irradiation with light. Further modification, to create what they described as a biomimetic surface, allowed for a dramatic increase in the change in wettability from super-hydrophobic to extremely hydrophilic, switching the contact angle between 0° (complete wetting) to 165° with light. They grafted fluoro-AZO to iron-oxide-coated silica nanoparticles to create photoresponsive hydrophobic nanoparticles, which were then sprayed onto silicon wafer substrates. Light irradiation provoked a photoisomerization of the azobenzene derivatives, leading to a structural change in the surface coating and the contact angle dropping sharply.[170] While changing the hydrophobicity of the nanoparticle coating is one method of changing surface wettability, another approach is through the azobenzene functionalization of surfaces via chemisorption and layer-by-layer (LbL) techniques. This has also successfully produced light-responsive surfaces for the control of cell adhesion by changing surface wettability.[38]

The mechanical stiffness of a surface can have a strong effect on the behavior of cells. For example, the cell differentiation behavior of PC-12 rat adrenal gland cells was shown to change depending on whether the cells were grown on a soft polymer 40 nm nanopillar surface versus a hard Si nanopillar surface. Aside from stiffness, other factors were kept consistent between the surfaces, including pillar size, pattern, and surface chemistry.[171] These surfaces were made using a nanostructured template method, and postfabrication modification of the surface stiffness was complex and tedious. In contrast, photosoftening in azo-containing polymer films would allow for facile photocontrol of the stiffness of cell culture surfaces. An early study showed that it was possible to induce a large decrease in the Young's modulus of azo-doped crosslinked LC polymer films upon irradiation with UV light.[172] A nanoindentation study of this photosoftening effect showed that it was different from thermal softening.[173] Photosoftening can be observed both when the azo dye is covalently bound to the polymer as well as when it is hydrogen-bonded to the polymer in a supramolecular complex.[174] Depending on the material, photosoftening can be induced with either UV or visible light.[172,174] Two azobenzene materials, 7-[(3-trifluoromethyl-4-phenylazo)phenoxy]pentanoic acid (CF3Azo) and poly[2-[(3-trifluoromethyl-4-phenylazo)phenoxy]ethyl acrylate-*co*-acrylic acid] (PTAPE), were respectively assembled on a surface by chemisorption and by electrostatic LbL assembly (Figure 6b). Even if the packing of azobenzene units via chemisorption was more dense than via LbL, the photoinduced changes obtained by alternating UV and visible light were larger for LbL films.[175] The same experiment was performed on a microstructured surface with micropillars with CF3Azo and PTAPE and it yielded a 15-fold larger effect on the LbL-deposited surface than that of the chemisorbed film. Most notably, MCF-7 cells and T24 cells largely adhered onto microstructured LbL films, whereas in UV pretreated samples cell adhesion significantly decreased, demonstrating the efficacy of this facile approach for the fabrication of photoresponsive cell adhesion substrates.

The use of visible-light-responsive polymers is also reported for the fabrication of reusable platforms for biotechnological applications, such as for a tetra-ortho-methoxy-substituted azobenzene, responsive to green light, chemically linked to polyanionic and polycationic polymers and then reversibly attached to cyclodextrine-terminated substrates by host–guest interactions (Figure 6c).[176] Azo-functionalized polycations, presenting quaternary ammonium groups, showed antimicrobial properties, while azo-functionalized polyanions also showed good bioadhesive properties. These materials could be alternately immobilized onto the cyclodextrine-terminated surface via visible-light-triggered assembly.

4.2. Cellular Control with Azo Polymer Surface Patterns

Surface relief patterns have been used in cell biology for influencing cell behavior with photoinscribed surface patterns. The cell lines mainly used are neuronal cells and fibroblasts, for their well-known ability to follow topographical cues through an outside-in signaling with the extracellular environment. The first reported use of azo polymers for instructing cell behavior was carried out in 2004 by Baac et al.,[177] on a glass slide spin-coated with a commercially available azobenzene copolymer (poly[(methylmethacrylate)-*co*-(Disperse Red 1 acrylate)]) and patterns were imprinted with interference lithography with a 488 nm laser. Subsequently, primary human astrocytes were seeded and cultured, where cells were able to attach and grow on the patterned material, clearly orienting and elongating along the pattern axis direction.

Barillé et al. used azobenzene photomobility under irradiation to pattern a biocompatible azo polymer substrate using light. PC-12 cells were shown as able to sense this nanotopography, induced to guide down the channels compared to a non-irradiated poly-l-lysine (PLL) control.[178] Hurduc et al. used a series of custom azobenzene-functionalized siloxanes with cultured human hepatoma derived cells (HepaRG), and other work by the same group adjusted the degree of substitution and the substituents on the polymer side chain influenced polymer T_g, tuned near body temperature of 37 °C.[179–181] Therefore, in physiological conditions the photoinscribed topographies were unstable and could revert to their initial condition in a "shape-memory" fashion.[182,183] Rianna et al. also used surface relief patterns on a glassy azo polymer for culturing NIH-3T3 murine fibroblasts, attempting to exploit the erasure of azo polymer photopatterning using light.[184] 1D- and 2D-gratings with different periods were inscribed on the azo material (**Figure 7**a) and an incoherent light source (a 15 mW mercury lamp) filtered in the blue region provided erasure, forming ordered microbumps at the surface, forcing cells to align along this new topographical cue. Azo polymer brushes could also be patterned at the microscale using interference lithography, with an increased stability due to covalent tethering to the substrate.[185,186] These studies showed the surfaces to be amenable to controlled culture of human umbilical vein endothelial cells (HUVECs), which aligned on the photoinscribed topography axis.[187] In all these examples, the use of surface topography patterns for cell culturing has been optimized for controlling cell attachment, and alignment appears well demonstrated, yet there remains far less work reported on azobenzene-based photoresponsive materials for controlling more complex cell behavior or function, exploiting the strong potential for the unique features of

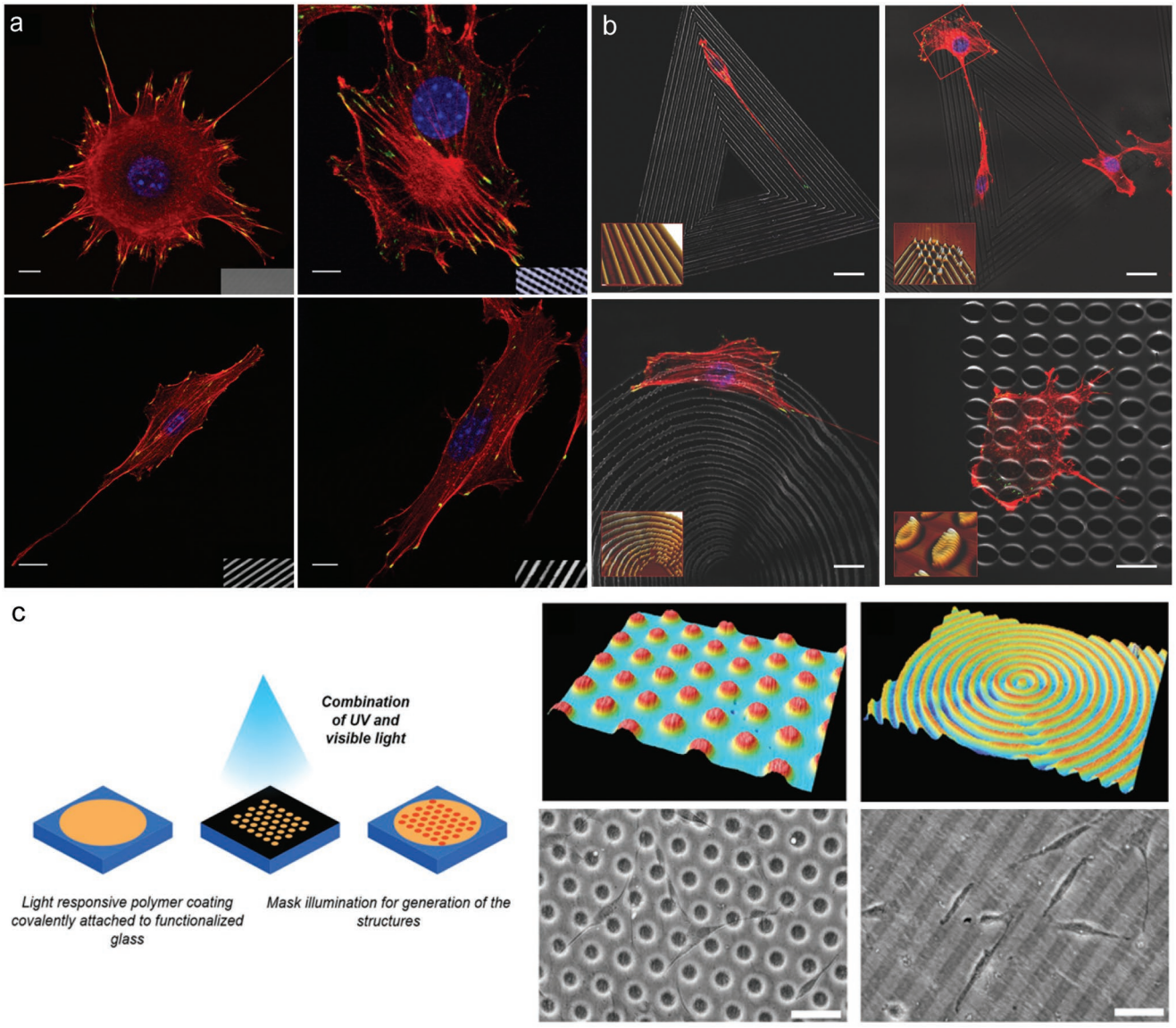

Figure 7. a) Confocal images of fibroblasts on flat and photopatterned azobenzene substrates. Reproduced with permission.[184] Copyright 2015, American Chemical Society. b) Cell orientation on azo patterns produced by laser scanning technique. In the inset, AFM micrographs of the substrates. Reproduced with permission.[191] Copyright 2016, Wiley-VCH. c) Topography imprinting process in LCN coatings. 3D representation and phase contrast images of fibroblasts on the patterns. Reproduced with permission.[26] Copyright 2017, Wiley-VCH.

these materials, such as the dynamic in situ inscription capabilities, or the reversibility of patterning,[184,188] arguably the most exciting potential future applications.

4.3. Azobenzene Coatings as Dynamic Cell Growth Surfaces

Azo polymers can act as powerful substrate coatings for dynamic photocontrolled cell culture, as real-time photocontrol of topography during cell culture relies on smart materials that can be precisely controlled spatially and temporally, dynamically, ex vivo in physiological conditions. This can be possible by coupling azobenzene-based materials responsive in the visible range, in a temperature- and pH-controlled environment. This requirement can be realized by using a common laser scanning microscope equipped with a thermochamber, where a focused laser beam can be scanned over the surface of the azo polymers, leading to the inscription of desired surface topographies (Figure 7b).[91,189–191] Embossed patterns can be reversibly erased by incoherent light illumination after cell medium removal for about 30 s, and this technique has also been used for directing angiogenesis via an in vitro spheroid assay.[192] Rossano et al. then demonstrated the successful use of imprinted circular topographies via focused laser scanning in a cell-populated area.[193] Here, the inscription of concentric rings with different spacing was exploited to affect NIH-3T3 cell morphology. In fact, many recent reports demonstrated that the induction of complex cell curvatures

through topographies (e.g., zigzag, sinusoidal, or circular patterns) is able to alter cell adhesion, migration, and even stem cell commitment.[6,194] After the pattern inscription with a focused 514 nm laser beam, cell morphology was monitored for 24 h and evaluated in terms of local alignment to the underlying pattern. Ridges were measured to be 300 nm high and the periodicity was 2 μm for all trials, spaced apart either 5, 10, or 15 μm. The onset of cell alignment was 1 h for 15 μm distant rings and 2 h for 10 μm distant rings, but on the latter, cells maintained the induced alignment longer. Interestingly, cell elastic modulus after 24 h (as measured by AFM) was significantly lower on these patterns than on flat surfaces due to the formation of a diffused cytoskeleton and small focal adhesions. Importantly, photoresponsive azo polymers remain compatible with established imaging techniques usually employed for monitoring cell behavior.[195] In these studies, fibroblasts were mostly the chosen cell type; however, these materials and techniques for locally inscribing topographies hold great potential for influencing multicellular systems and locally altering intercellular communication. For instance, it would be interesting to investigate the response of systems such as epithelium, endothelium, and neuronal networks to photoswitchable topographical changes. Azobenzene-conjugated polymers have been also used as smart coatings for inducing biofilm disruption.[196] As described in the previous section, liquid crystalline elastomers have been broadly implemented as actuators, soft microrobots, and sensors.[22,95,197,198] Phase transition temperatures in LCEs are typically much higher than physiological temperature; so their implementation as cell culturing substrates has been so far modest and restricted to nondynamic applications. However, advances in their application as biomaterials in the near future are foreseeable both for their anisotropy, as well as for their photoactuation capabilities.[199–202]

Employing LC-based materials may provide some inherent advantages in biocompatibility, as many biological materials exhibit liquid crystalline order, for example, in the exoskeleton of certain beetles, which results in iridescence.[3] Even cells in biological tissue have been shown to order in nematic domains and it is possible that certain functions require liquid crystalline order.[203] A major design paradigm in tissue engineering is organizing cells into a proper alignment and morphology; so due to their order and flexibility, LCEs can offer great potential applications in biological applications. Toward this goal, Lockwood et al. developed nontoxic thermotropic fluorine-substituted liquid crystal which they coated with Matrigel, where they were able to successfully culture human embryonic stem cells on this surface.[204] As further steps toward making artificial muscle tissue, Bera et al. used acrylate polymers to make biocompatible nematic LCE 3D scaffolds.[205] Gao et al. developed 3D LCE foams with tubular porosity and the ability to recover from compression and demonstrated biocompatibility of these scaffolds by culturing myoblast cells on the surface,[202] and Prévôt et al. in the same group developed porous lactone- and lactide-based LCE foams that supported the growth of SH-SY5Y cells.[206] Martella et al. were able to culture muscle cells with some degree of alignment on the LCE.[207] This contractile cell line has been studied on a mechanically anisotropic substrate, and recently Martella et al. fabricated a liquid crystalline polymer where they changed the molecular arrangement to create an aligned scaffold for control of myoblast alignment,[208] but moving toward photoswitchable azobenzene-containing LCE would be particularly intriguing.

The sole example so far of a soft LCE with photoreversible topography was demonstrated by Koçer et al. (Figure 7c).[26] An LCN coating was designed with a chiral nematic phase aligned by shear forces and then photo-crosslinked. Subsequently, azobenzene isomerization was patterned with a mask and resulted in local swelling and softening, with the consequent formation of topographical cues for cell culture (either fixed or reversible, depending on the LC mixture composition). Different cell migration tracks and speeds were observed in the irradiated and unirradiated regions, due to the contact guidance of both photoinduced changes in topographies and nanoscale roughness. Multistable LCN coatings have also been recently shown to be promising substrates for biological studies.[209] Here, fluorinated azobenzenes have been incorporated as crosslinking units in a photopolymerizable LC mixture and shaped into different topographies. Blue and green light have been used to switch between different topographies in presence of NIH-3T3 fibroblasts.

Reversibly photoactuating hydrogels have also been suggested as promising candidates for dynamic 3D culture.[210–212] For example, a poly(ethylene-glycol) (PEG)-based hydrogel was polymerized with an azobenzene-containing crosslinker, where the elastic modulus of the hydrogel could be controlled by azobenzene photoisomerization. Changes in the mechanical properties of the hydrogel were studied by rheological measurements under light irradiation, and a reversible reduction in the storage modulus G′ was observed in response to UV irradiation, increasing with the azobenzene content. Moreover, valvular interstitial cells were observed to grow and differentiate into myofibroblasts (**Figure 8**a).[40] Gelatin hydrogel has also been engineered for two-photon lithography and crosslinked with azobenzene moieties in a modular platform for locally stimulating attached NIH-3T3 fibroblasts (Figure 8b).[212] Polyacrylamide (PA) hydrogels are used extensively as cell culturing materials, where mechanical properties can be tuned by the crosslinker percentage in the mixture composition. Similarly, it would be even more beneficial to be able to tune mechanical properties reversibly and dynamically in response to an external stimulus such as light. Toward this aim, a bisacrylamide derivative of azobenzene has been successfully introduced into the formulation of a PA hydrogel, yielding a dual-wavelength responsive hydrogel useful for dynamic cell culture.[213] This material could be softened from 8.3 down to 2.0 kPa by UV irradiation for 30 min after waiting 3 h, and the Young's modulus could be recovered thermally or upon blue light irradiation. Bone-marrow-derived mesenchymal stem cells were seeded on previously UV-treated hydrogels in order to avoid direct UV light irradiation and then were irradiated with blue light, where their morphology was then found consistently changing and adapting to the mechanical variations in the hydrogel (Figure 8c).

4.4. Azo Photoswitches Incorporated into Natural Polymers

A major difficulty in the development of implantable medical devices is the body's response to the materials used to fabricate

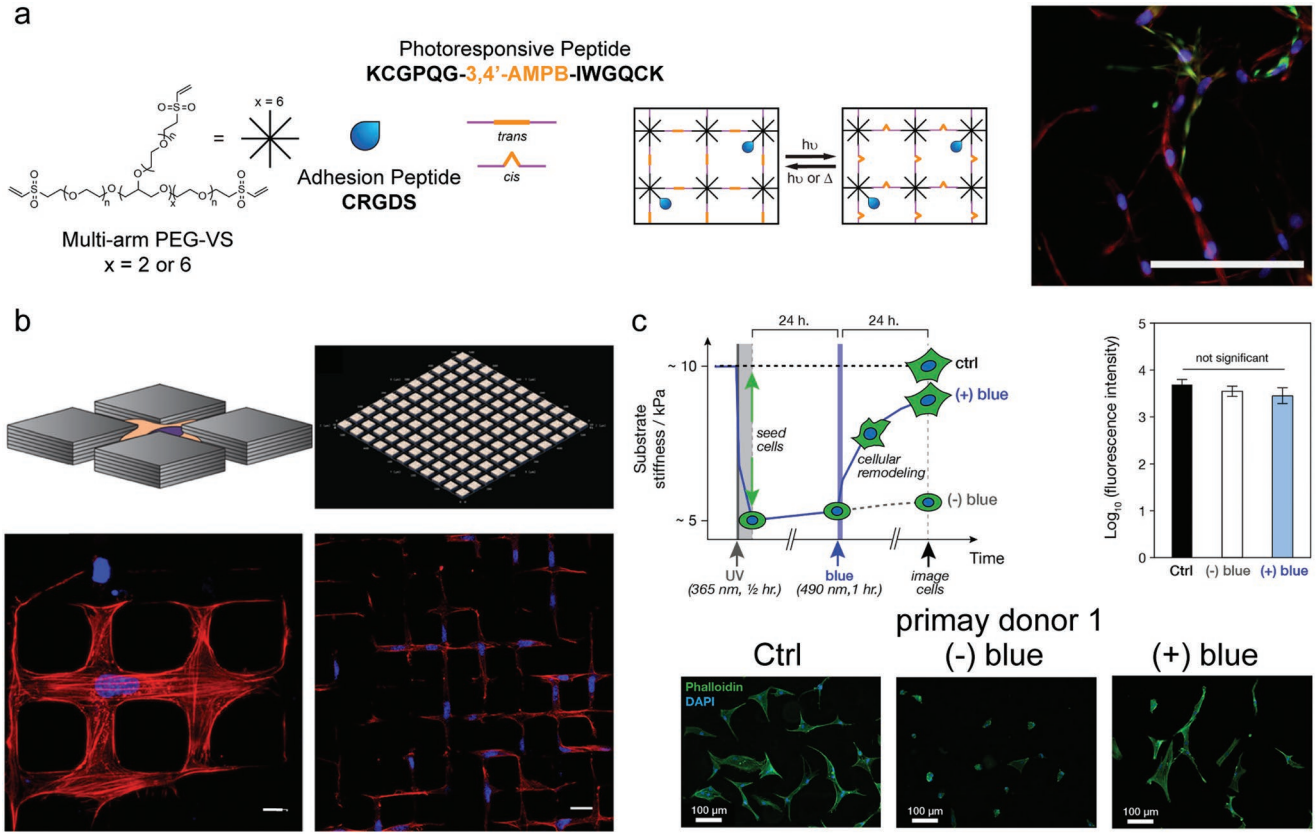

Figure 8. a) Photoresponsive hydrogels composed by multiarm PEG vinylsulfones (PEG-VS) and thiol-containing peptides. VIC cells immunostained on the hydrogel after 5 days of culture. Adapted with permission.[40] Copyright 2015, American Chemical Society. b) Photoresponsive gelatin structure arrays. Fixed cells cultured on the gelatin platform after 24 h. Reproduced with permission.[212] Copyright 2017, American Chemical Society. c) Fibronectin-coated photoresponsive gels cultured with mesenchymal stem cells. Cell morphology is represented as a function of photoinduced stiffening/softening. Plot of cell viability. Fluorescence microscopy images of immunostained mesenchymal stem cells (MSCs) on photoresponsive hydrogels. Reproduced with permission.[213] Copyright 2018, American Chemical Society.

them. Natural polymers, such as polysaccharides and proteins, are emerging as promising alternative host materials. Polysaccharides are a large class of natural polymers which form supramolecular structures and serve as energy storage in many organisms and plants, such as cellulose, hemicelluloses, chitin, and various starches. These all have an "O-linked" pyranose or furanose ring polymer backbone and various individual functional groups that can be chemically postmodified.[214] Azobenzene moieties can either be doped into, or covalently bound to various polysaccharides in order to add photostructuring and photoswitching capabilities.[214] An early example demonstrating successful azo functionalization of polysaccharides is the photoresponsive azo-cellulose compounds synthesized by Arai and Udagawa 30 years ago.[215]

Celluloses and hemicelluloses are polysaccharides that are the major components of plant cell walls. They are widely abundant in nature and possess a host of interesting features, including amorphous and crystalline regions, the ability to form supramolecular structures, to order into cholesteric phases,[216] and to form fibers and films.[217] Cellulose derivatives can be fabricated into materials with many desirable functions; for example, hydroxypropyl cellulose is a biocompatible material that is used widely in various food industry and medical applications, and can be used to prepare anisotropic films with supramolecular liquid crystalline ordering.[218] Cellulose can be used for soft lithography of photonic structures,[219] and cellulose films, fibers, and mats with desired characteristics can be prepared with control over molecular ordering with amorphous, chiral ordered, and nanocrystalline regions.[220] Geng et al. used the humidity-induced actuation of cellulose LC films to design a humidity-driven cellulose LC motor.[218] Azobenzene compounds have been incorporated into the cellulose network to create a photoresponsive material, where Qin et al. functionalized hydroxypropyl cellulose with azobenzene groups to produce an azo-cellulose material with light-switchable thermotropic cholesteric liquid crystalline properties. Upon irradiation with UV and visible light, the azo moiety undergoes reversible *trans* to *cis* isomerization.[221] Matsumori et al. developed highly ordered liquid crystalline films composed of an azobenzene dye doped in a triacetyl cellulose polymer matrix, to produce a material that is dually responsive, as the cellulose is humidity-responsive and the azobenzene is photoresponsive. By photoaligning the film using linearly polarized light and then exposing it to humidity to induce nematic to anisotropic columnar ordering, they were able to create films with a high order parameter of 0.81.[222]

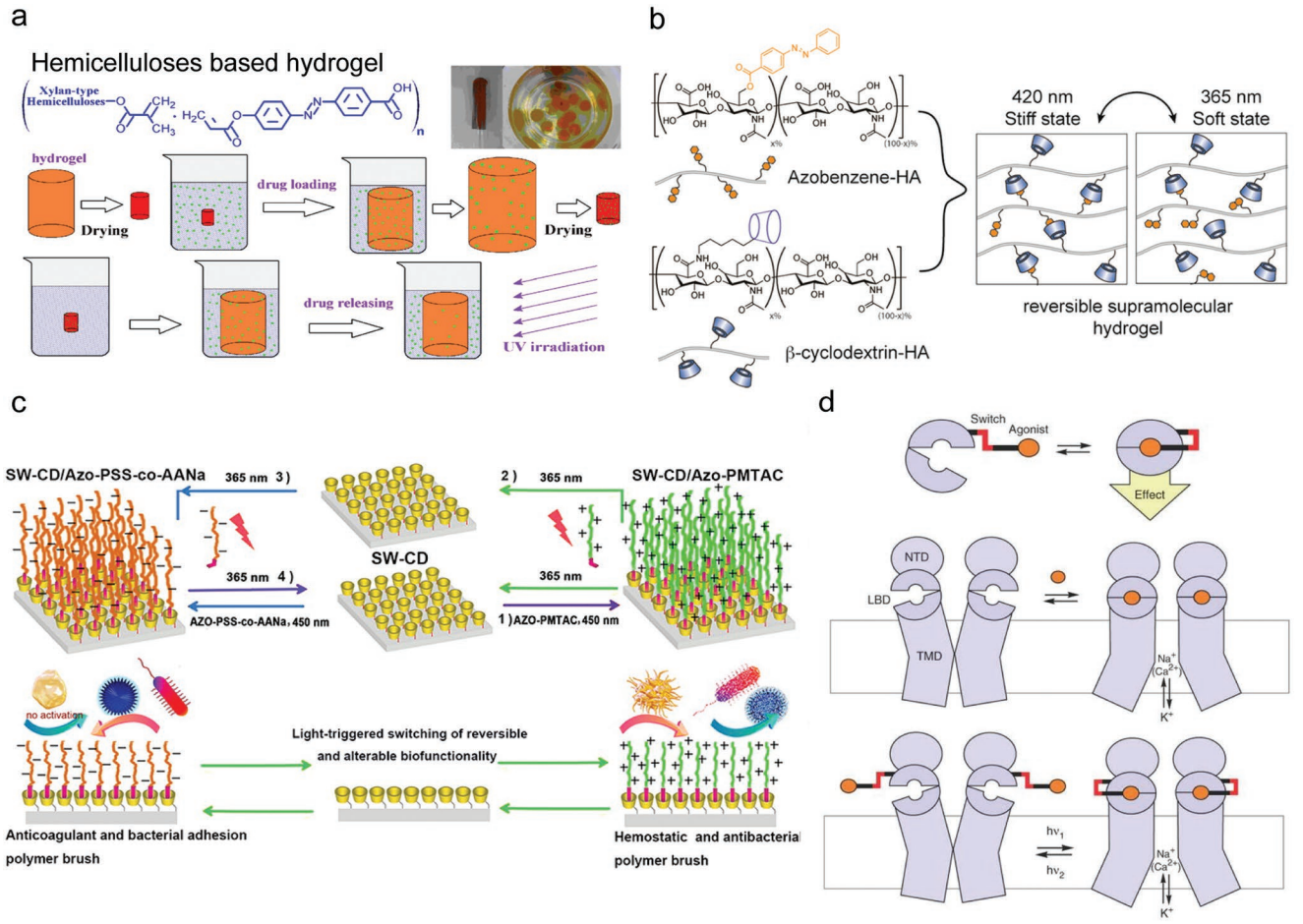

Figure 9. a) Photoresponsive hydrogels of hemicellulose methacrylate with 4-[(4-acryloyloxyphenyl)azo]benzoic acid (AOPAB) showing multiresponsive behavior to pH, water/ethanol solutions, and light. Reprinted with permission.[224] Copyright 2014, American Chemical Society. b) Reversibly tunable HA hydrogel with light, using supramolecular interactions between azobenzene and β-cyclodextrin. Reproduced with permission.[230] Copyright 2018, American Chemical Society. c) Schematic depiction of a photoswitchable bioactive surface. Reproduced with permission.[238] Copyright 2014, American Chemical Society. d) Design of an allosteric photoswitch. Reproduced with permission.[247] Copyright 2005, Springer Nature.

A major application area of azo-functionalized polysaccharides is photoresponsive hydrogels: soft, flexible, hydrophilic polymers that swell in water, long used for tissue engineering scaffolds, as they are hydrophilic and have 3D structure, and the mechanical properties and chemical structure can be adjusted to mimic the properties of natural ECM and tissue.[223] Dynamic adjustment of the properties of hydrogels using light would allow for additional biological applications. Cao et al. developed an azo-hemicellulose hydrogel based on the copolymerization of methacrylate with 4-[(4-acryloyloxyphenyl)azo]benzoic acid, where photoisomerization of the azobenzene moiety from *trans* to *cis* changed the hydrophilicity of the hydrogel (**Figure 9**a).[224] Another useful property of such hydrogels that can be changed is the sol–gel transition, via azo-modified celluloses and hemicelluloses, where *trans* to *cis* photoisomerization of the azobenzene moiety results in disruption of π–π stacking which then leads to a change in phase.[225] In an early example, Arai and Kawabata changed the cloud point of an aqueous azobenzene-modified methylcellulose system by *trans* to *cis* photoisomerization.[226] Zheng et al. further developed an azo-hydroxypropyl-methylcellulose system where the sol–gel transition temperature could be changed upon irradiation with UV light. The transition temperature of the system increased after irradiation, and this effect could be modulated with the addition of α-cyclodextrin, resulting in a decrease in the transition temperature after irradiation.[227]

In addition to cellulose and hemicellulose hydrogels, other photoresponsive polysaccharide hydrogels have been developed, such as those by Su et al., who prepared a dual-responsive starch-based azobenzene-functionalized hydrogel. The monomers were made by esterification of the starch and PVA, and the hydrogel was prepared through radical crosslinking of the starch-based monomer, the PVA-based monomer, and an unsaturated azobenzene-containing monomer, and the resulting hydrogel was shown to be both pH and light responsive.[228] Hyaluronic acid (HA) is sometimes found in ECM, so HA-based polymer hydrogels have been developed and proposed as 3D cell culture media.[229] Rosales et al., for example, have developed a photocontrollable HA hydrogel by crosslinking HA with azobenzene and β-cyclodextrin (Figure 9b), a cyclic polysaccharide composed of 7 D-glucose units, where there is a difference in binding affinity of *trans* versus *cis* azobenzene

β-cyclodextrin. Irradiation with light reversibly changed the stiffness of the hydrogel due to this difference in binding affinity.[230] Harada and co-workers also used α-cyclodextrin (composed of 6 D-glucose units) linked to curdlan and azobenzene linked to polyacrylic acid to make a photoresponsive hydrogel where the sol–gel transition could be controlled with light.[231]

In addition to the aforementioned photoactive hydrogels, a difference in binding affinity between cyclodextrins and *trans* versus *cis* azobenzene has been exploited for many interesting biomimetic applications.[232] Examples of applications of these azobenzene/cyclodextrin host/guest systems include acting as a cation carrier,[233] shielding and exposing a catalytic site,[234] controlling self-aggregation in aqueous media,[235] and as externally controllable hydrophobic drug-delivery systems.[236,237] Cyclodextrin/azobenzene systems have also been used for photocontrol of cell behavior. Deng et al. developed a biofunctional polymer brush with the photoswitch ability of the β-cyclodextrin/azobenzene host/guest system (Figure 9c). The behavior of the monolayer brush surface could be photocontrolled, resulting in the ability to affect the cell adhesion and survival of *Escherichia coli* and *Staphylococcus aureus* bacteria cells on the surface.[238] Bian et al. used a similar β-cyclodextrin/azobenzene and cell capture aptamer-functionalized material for photocontrolled capture and release of cancer cells.[239] Further examples of biological applications of cyclodextrin/azobenzene-functionalized materials can be found in a review by Zhan et al.[240] As many early examples of these systems are UV-responsive,[241] recent work has endeavored to redshift the absorption toward visible and near-IR–responsive systems for greater biocompatibility.[232] Further information and examples of azobenzene/cyclodextrin host/guest systems can be found in reviews by Harada et al. and Wang et al.[232,241]

Another important class of functionalized natural polymers involves azobenzene-functionalized amino acids, whether as polypeptides or as proteins. Early work in the area involved photoinduced order–disorder transitions in azo-modified polypeptides, which may undergo transitions to disordered solutions from ordered chiral helices,[242–244] or even undergo reversible conversions between β-sheets and α-helices.[245] These azo-functionalized amino acids were incorporated into proteins within a living cell for optocontrol of cellular functions. Bose et. al. synthesized an azo-functionalized phenylalanine (AzoPhe) as well as a tRNA synthase pair that allowed them to incorporate AzoPhe into proteins in *E. coli*. They were able to change the binding between DNA and a transcriptional activator through photoisomerization of AzoPhe.[246] Azo-functionalized amino acids have also been used to photocontrol cell signaling, for example, by Volgraf et al., who designed a photocontrollable ionotropic glutamate receptor by linking an agonist and an azobenzene to the receptor (Figure 9d).[247] In the naturally occurring glutamate receptor, the ion channel opens and closes depending on whether the agonist is bound. In the modified receptor, when azobenzene is in the *trans* state, the linked agonist is not able to bind to the receptor site, and when the azobenzene is photoswitched to the *cis* state, the linked agonist binds to the receptor and the ion channel opens.[247] Further work was built on this early photoswitchable receptor with varying levels of complexity. Berry et al. made an azobenzene-functionalized version of a G protein-coupled receptor, which they used to partially restore vision in blind mice.[248] Further discussion of azo-functionalized protein switches can be found in other reviews.[249]

In addition to acting as photoswitches for cell signaling, protein hydrogels and fibrous protein scaffolds have also shown promise for tissue engineering and 3D cell culture. One promising material is silk fibroin, a naturally occurring protein that is one component of cocoons spun by *Bombyx mori* silkworms. It is flexible but strong and can be processed into various materials, including sponges, films, gels, and fibers. In addition, silk is biocompatible, induces a low immune response, and biodegrades within the body. Silk sutures have been FDA-approved for use in surgery,[250] and silk materials have been developed as effective extracellular matrix analogs for cell growth and direction.[251] Silk can be chemically modified in a variety of ways to produce materials with additional functionalities,[252] including with azobenzene functional groups, via the tyrosine residues on silk fibroin that can be chemically modified to form azobenzene derivatives on the silk through diazonium coupling (**Figure 10**a).[253] 5.3% of the amino acids composing silk fibroin are tyrosine,[254] which is distributed through the silk fibroin protein sequence,[253] and this azobenzene postmodified silk fibroin is then referred to as "azosilk" or "optosilk." This diazonium coupling method, developed by Murphy and Kaplan, can be performed in mild conditions and results in a relatively high conversion of tyrosine residues to azobenzene residues (≈40%).[254] It is now the typical method used for synthesizing azosilk,[255–257] and the modification is usually completed on silk fibroin dissolved in an aqueous solution before it is cast into its final structure, though Raynal et al. have developed an approach for modifying silk fibroin that has already been cast on a surface.[258]

Murphy et al. changed the functional groups on the azobenzene derivatives (X in Figure 10a), to tune the hydrophilicity of the resulting azosilk, and they showed that hydrophilic azosilks resulted in more proliferation of human mesenchymal stem cells, while more hydrophobic azosilks resulted in the formation of confluent monolayers of human mesenchymal stem cells (hMSCs) (Figure 10c).[253] Diazonium-modified silk has also been used to make biodegradable sponges for use as tissue fillers due to its hydrophilic swelling properties and shape-memory capabilities.[255]

Coupling azobenzene with silk creates a material with great potential for optically addressing biophysical properties. Silk fibroin itself has been explored as a material for optical components because in addition to being biocompatible, it has a high refractive index and optical transparency,[256] for example, Palermo et al. have created reversibly switchable optical components using azosilk, such as a diffraction grating using azosilk layered on polydimethylsiloxane. The resulting structure's diffractive properties could be tuned using light at a wavelength (405 nm) that induced azobenzene *trans–cis* photoisomerization.[256] A more relevant application to the bio-interface of the optically addressable properties of azosilk is in photopatterning. Landry et al. developed a method for creating permanent topographical patterns in hydrated azosilk films using two-photon laser scanning lithography.[257] Azobenzene photoisomerization is generally performed in the UV–vis region, but this two-photon approach allows for

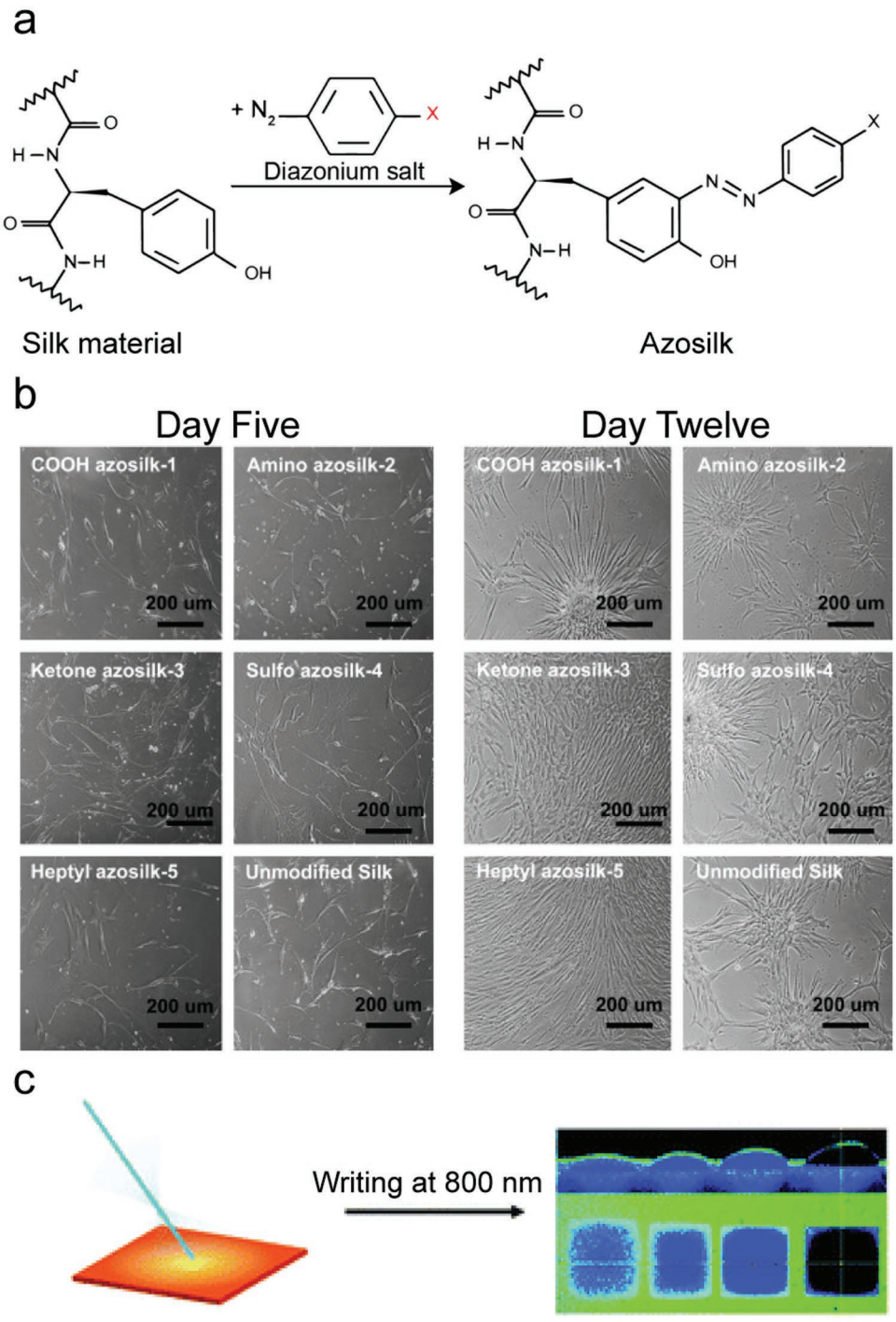

Figure 10. a) Diazonium coupling reaction scheme in silk. Reproduced with permission.[257] Copyright 2017, American Chemical Society. b) Phase contrast micrographs of human mesenchymal stem cells cultured on the azosilk derivatives (COOH, Amino, Ketone, Sulfo, Heptyl) and unmodified azosilk after 5 as well as 12 days. When reaching 70% of confluency (day 12), hMSCs exhibited a morphology typical of undifferentiated hMSCs on Ketone and Heptyl azosilk. Reproduced with permission.[253] Copyright 2008, Elsevier. c) Confocal fluorescence image of hydrated azosilk with photostimulated regions. Reproduced with permission.[257] Copyright 2017, American Chemical Society.

use of significantly redshifted light, which offers a reduced impact on cells compared with light in the UV region. Patterns were written to the azosilk film using a femtosecond 800 nm IR laser (Figure 10b),[257] to produce precise and complex irradiation patterns that can be easily visualized due to the resulting increase in observed fluorescence emission. In these regions, the *trans–cis* isomerization in the folded beta-sheets of the silk created sufficient geometric and mechanical

disruption to unfold the sheets, then filling with surrounding water by osmotic pressure, so that the azosilk formed a soft, raised "blister" bubble coincident with the light patterns, where both the morphology and the modulus of the silk were significantly changed. Depending on the depth of writing, a change in height of up to 13 μm was observed, and the modulus dropped from ≈12 to 0.6 kPa.[257] This change in topography and modulus, combined with biocompatible materials and patterning method, could allow for applications toward an optically tunable cell culture medium with the capacity for cell guidance, via dynamic irradiation, in situ.

5. Conclusions and Perspectives

It is hoped that this review demonstrated the great potential of photoreversible optically switchable azo dye molecules in polymer materials to control a wide variety of physical, chemical, and mechanical material properties in response to light, that can be exploited for optical control over the bio-interface. As a stimulus for reversible influence over properties of adjacent biological cells or tissue, and as a triggering mechanism, light is ideal since it is highly selective, and can be localized (in time and space) for precise and dynamic control over a biosystem, and low-power visible light is also an inherently gentle, benign, and low-damaging stimulus in a biological environment. Thus, for efficient and facile motion and actuation, photoresponsive materials offer great promise in general. Azobenzene in particular is arguably the leader among potential photoreversible molecules, and soft azobenzene-containing materials hold great potential for achieving success in realizing application of these bio-interface systems discussed in this review, due to their ease of incorporation, and robust, versatile, and efficient photochemistry and photophysics.

Azo polymers have been shown here to be promising candidates for a variety of cell control applications, via photoalignment, optically induced flow patterning of surface topographies, or photomechanical actuation. The optical inscription of topographical features in particular is becoming well advanced, and now represents a tangible step toward the reversible real-time triggering and control over complex cell signaling events. Azobenzene-based soft actuators and hydrogels in particular can reproduce well the features of a dynamic 3D extracellular environment, and provide controlled input of mechanical stimuli to living cells. With further development of such next-generation light-responsive biomaterials, exploiting the light-triggering activity of azobenzene molecules, one can readily foresee the opportunity to gain deeper insight into the complex processes involved in dynamic cell–material interactions and in cell signaling, offering exciting new possibilities for advances in basic biology as well as in biomaterial sciences. These materials are already enjoying significant impact in various research areas, and the materials now being developed have grown in their complexity and sophistication. As many fundamental foci of this research are reaching maturity, intensity now needs to turn toward deliverable applications in various biomedical fields using the materials and techniques described. The emerging research areas reviewed here are focused on the interface to and reversible control over biological systems. In future, the energy harvested and transduced by the azo dye molecules may also gain interest as mimics of real light-harvesting biological polymers, such as rhodopsin/retinal systems that enable vision, or chlorophyll that enables photosynthesis—the ultimate optobiological photosystem.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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