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Engineering the Cell Microenvironment Using Novel Photoresponsive Hydrogels

Yuqing Dong,[†,‡,⊥] Guorui Jin,[‡,¶,⊥] Yuan Hong,[‡,¶] Hongyuan Zhu,[‡,¶] Tian Jian Lu,[‡,§] Feng Xu,[‡] Dan Bai,[*,‡,¶,∥] and Min Lin[*,‡,¶]

[†]State Key Laboratory for Mechanical Behavior of Materials, School of Materials Science and Engineering, Xi'an Jiaotong University, Xi'an, Shaanxi 710049, People's Republic of China

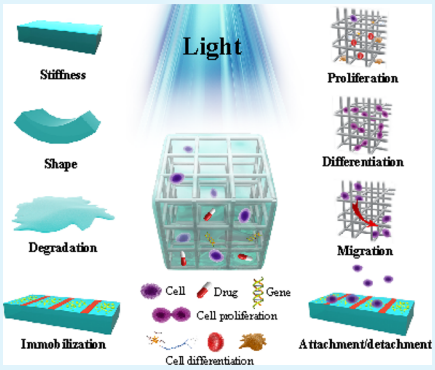
[‡]Bioinspired Engineering & Biomechanics Center (BEBC), Xi'an Jiaotong University, Xi'an, Shaanxi 710049, P. R. China

[¶]The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, Shaanxi 710049, China

[§]MOE Key Laboratory for Multifunctional Materials and Structures, Xi'an Jiaotong University, Xi'an, Shaanxi 710049, P. R. China

[∥]Department of Biochemistry and Molecular Biology, School of Medicine, Xi'an Jiaotong University, Xi'an, Shaanxi 710061, P. R. China

ABSTRACT: In vivo, cells are located in a dynamic, three-dimensional (3D) cell microenvironment, and various biomaterials have been used to engineer 3D cell microenvironments in vitro to study the effects of the cell microenvironment on the regulation of cell fate. However, conventional hydrogels can only mimic the static cell microenvironment without any synchronous regulations. Therefore, novel hydrogels that are capable of responding to specific stimuli (e.g., light, temperature, pH, and magnetic and electrical stimulations) have emerged as versatile platforms to precisely mimic the dynamic native 3D cell microenvironment. Among these novel hydrogels, photoresponsive hydrogels (PRHs) that are capable of changing their physical and chemical properties after exposure to light irradiation enable the dynamic, native cell microenvironment to be mimicked and show great promise in deciphering the unknown mechanisms of the 3D cell microenvironment in regulating the cell fate. Several reviews have already summarized the advances of PRHs and have focused on specific photosensitive chemical groups and photoresponsive elements or on the reaction categories and mechanism of PRHs. However, a holistic view of novel PRHs, which highlights the multiple physical and chemical properties that can be tuned by remote light activation, as well as their applications in engineering a dynamic cell microenvironment for the regulation of cell behaviors in vitro is still missing and is the focus of this review.



KEYWORDS: *3D cell microenvironment, extracellular matrix, photoresponsive hydrogels, physical and chemical properties, cell regulation*

1. INTRODUCTION

The three-dimensional (3D) cell microenvironment, which is composed of the extracellular matrix (ECM), homotypic or heterotypic cells, cell−cell/cell−ECM interactions, and various biochemical (e.g., soluble factors) or biophysical cues (e.g., stiffness/stress), plays an important role in dynamically regulating cell behaviors such as migration,[1] proliferation,[2] differentiation,[3] and apoptosis.[4] During tissue development, homeostasis/repair, and disease progression, cells interact dynamically with their microenvironment through biochemical and biophysical interactions.[5] For instance, cells continually remodel the surrounding ECM and its properties (e.g., stiffness and porosity), which in turn directs the cell behavior.[6−8] Although the cell microenvironment regulates the cell fate, the underlying mechanisms remain elusive because of the complexity of the cell microenvironment. One strategy to address this is through engineering the cell microenvironment in vitro to reveal how the cells sense and respond to various microenvironmental cues, which requires interdisciplinary efforts by coupling basic cell biology, biomechanics, materials science, and advanced micro/nanofabrication techniques.

Biomaterials that mimic the native ECM have emerged as a versatile tool in engineering the cell microenvironment in vitro to regulate the cell behavior.[9] Nevertheless, many studies have demonstrated that conventional two-dimensional (2D) biomaterials (e.g., fibrous sheets and biofilms) cannot maintain the phenotypes of cells derived from complex multicellular tissues because of the dramatic differences in the physical and chemical properties of a 2D culture in vitro and the native cell microenvironment.[10] To overcome this obstacle, three-dimen-

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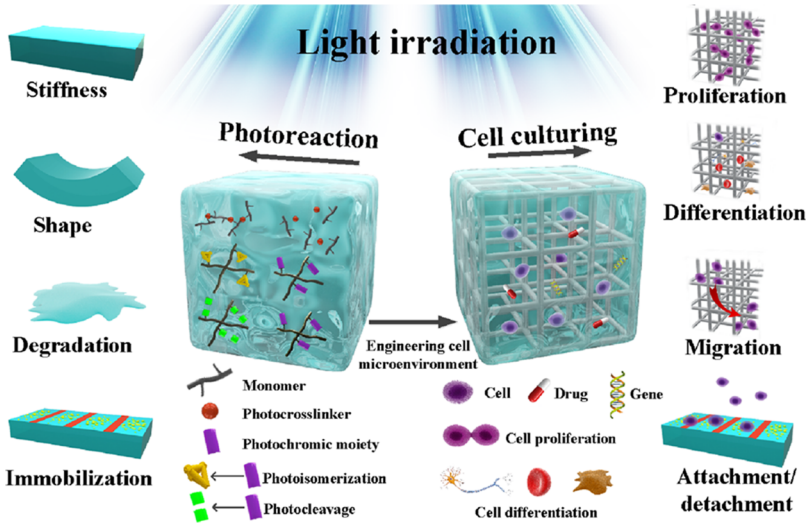

Figure 1. Schematic representation of the photoresponsive behaviors of PRHs and their applications in engineering the cell microenvironment for the regulation of cell behaviors in vitro under light irradiation. Photoresponsive reactions (e.g., photo-cross-linking, photoisomerization, and photocleavage) and photoresponsive behaviors (e.g., stiffness change, shape change, degradation, and surface immobilization) are exhibited in the left. Applications of PRHs in engineering the cell microenvironment, controlling cell proliferation, differentiation, migration, and adhesion are presented in the right.

sional (3D) cell culture using biomimetic hydrogels has emerged as an alternative strategy to recapitulate the native cell microenvironment in vitro.[11] The high water content and vast extended polymeric 3D network make hydrogels a versatile tool for creating customized 3D microenvironments with highly tunable biochemical and biophysical properties. Therefore, hydrogels synthesized from native and synthetic materials with highly tunable biochemical and biophysical properties in both the spatial and temporal scales have been explored extensively for mimicking the in vivo 3D cell microenvironment.[12−15] However, conventional hydrogels with modifications to the bulk material can only mimic the static aspects of the cell microenvironment.[16] Novel hydrogels that are capable of responding to external stimuli (e.g., light, temperature, pH, and magnetic and electrical stimulations) have emerged as versatile platforms for in vitro 3D cell culture and for the effective regulation of cell behaviors because of their ability to mimic the dynamics of the native cellular microenvironment.[17−21]

Stimuli-responsive hydrogels, such as those based on poly(*N*-isopropylacrylamides) and polyesters, can respond to external stimuli (e.g., temperature, pH, or a redox environment) to adjust their polymer chains, leading to altered physical and chemical properties.[22,23] As a result, these systems are oligomer-specific and sensitive to changes to the gel components, which lead to variability in the gelation efficacy and hydrogel properties.[24] By contrast, various materials can be applied to fabricate photoresponsive hydrogels (PRHs) because most synthetic or natural polymers can be modified with photochromes and then covalently cross-linked upon photoirradiation.[25] In addition, light irradiation allows for the remote alteration of the dimensional and structural composition of the PRHs with highly precise and localized actuation.[26−28] Therefore, by simply changing the light parameters (e.g., light intensity, wavelength, exposure time, and angle of incidence) and choosing photochromic moieties, PRHs are capable of mimicking the dynamic cell microenvironment because of the controllability of their physical properties, including stiffness, strength, and degradation, and their chemical properties, including hydrophilicity and functional molecular immobilization.

Recent advances in PRHs have been summarized in several reviews, either with a focus on specific photosensitive chemical groups and photoresponsive elements or on the reaction categories and mechanism of PRHs.[29,30] A holistic view of novel PRHs, which focuses on their multiple physical and chemical properties that can be changed by remote light activation, as well as their applications in engineering dynamic cell microenvironments for the regulation of cell behaviors in vitro is missing and is the focus of this review (Figure 1). Here, we start with a brief summary of the different ways of modulating the physical and chemical properties of PRHs using light irradiation. Then, we emphasize the versatile capability of PRHs in engineering the cell microenvironment for guiding the cell behavior.

2. REGULATION OF HYDROGEL PROPERTIES USING LIGHTS

PRHs typically consist of a polymeric network and a photoreactive moiety (usually photochromes) that can transduce light-induced modifications to the polymeric network.[31] Factors such as photochromic molecular structure and concentration, irradiation time, and light intensity are important in regulating the properties of PRHs. During the photosensitive process in PRHs, the photochromic molecules capture the optical signal and then convert it into a chemical signal via photoreactions (e.g., isomerization, rearrangement, cleavage, dimerization, and energy conversion), which can further regulate the physical and chemical properties of PRHs. Thus, the desired physical and chemical properties of PRHs can be regulated by carefully choosing the photochromic moieties and irradiation conditions. In this section, we will review light irradiation-induced changes in the physical properties (e.g., stiffness, shape, and size as well as degradation) and chemical properties (e.g., hydrophilicity and functional molecular immobilization) of PRHs.

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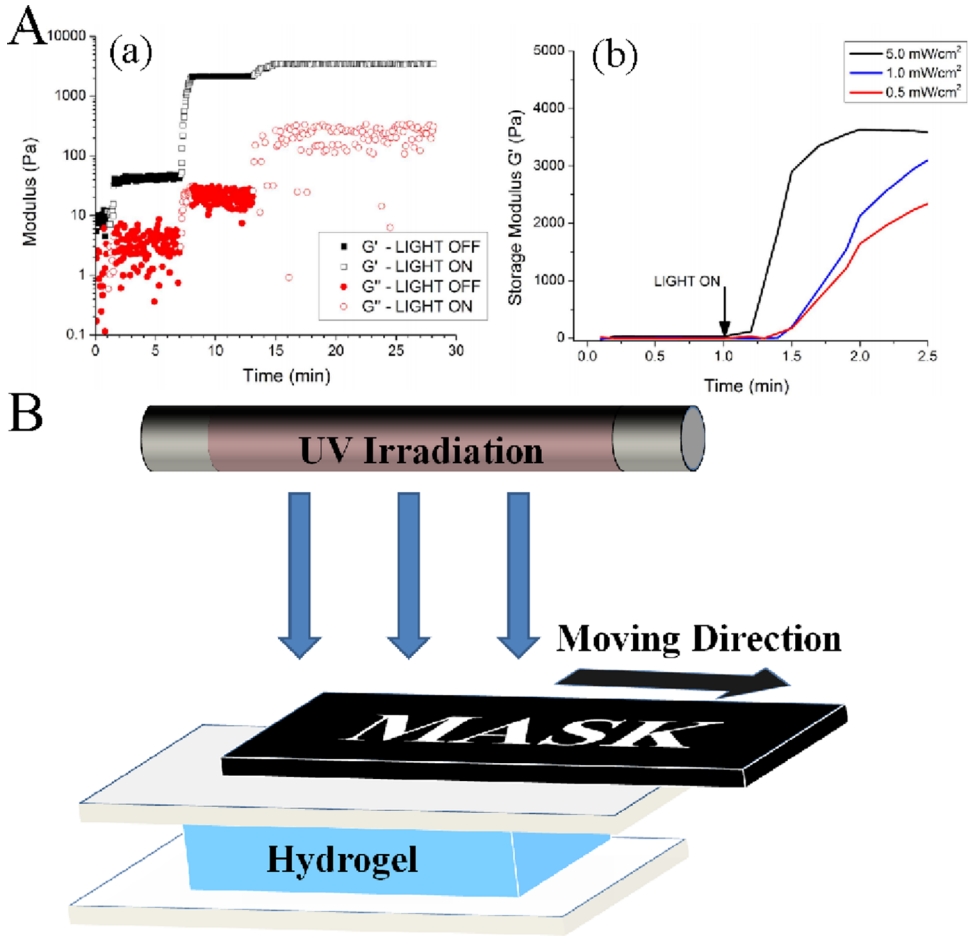

Figure 2. Photoregulation of the hydrogel stiffness. (A) (a) Hydrogel solutions were irradiated at 1.5, 7, and 13 min by UV light and obtained approximately 45 Pa (G′, storage modulus), 2.2, and 3.5 kPa, respectively. The samples were not exposed to UV irradiation in the intervening periods. (b) Hydrogel solutions exposed to the higher irradiation intensity of 5 mW/cm^2 cross-linked within 15 s and much faster than those exposed to 1 and 0.5 mW/cm^2 irradiation intensities. (B) Hydrogel stiffness gradient was obtained from the UV irradiation (365 nm) gradient, which was induced by moving the mask (in the direction of the arrow). Reprinted with permission from ref 47.

2.1. Photoregulation of the Physical Properties of Hydrogels. Other modification technologies have been used to adjust the physical properties of a hydrogel (e.g., thermal and conductive properties) using multiresponsive systems, including photothermo, photoelectric, and photo-pH-responsive systems.[32−34] Here, we mainly focus on the stiffness, degradation, shape, and size changes in PRHs, as induced by light irradiation.

2.1.1. Stiffness. Hydrogel stiffness is important for guiding cell fates.[35,36] To synthesize hydrogels with a specific stiffness, various approaches, such as the use of chemical initiator (e.g., Ca^{2+}) to adjust the degree of cross-linking and the structural design (e.g., digital plasmonic patterning) of hydrogels and photo-cross-linking, have been undertaken. Of these approaches, photo-cross-linking that realizes light-controlled hydrogel gelation and stiffening by photoinitiators has attracted great interest because of the spatiotemporal controllability of light, which renders photo-cross-linking a more direct means of manipulating the stiffness of the hydrogel than afforded by conventional chemical strategies. There are two basic types of photo-cross-linking, namely chain growth reactions and step growth reactions.[37−41] Chain growth reactions are conventional photo-cross-linking strategies that involve one cycling step, propagation of the radical species to carbon−carbon double bonds, and the generation of a new carbon-centered radical at the end of the polymer chain.[42] Macromers [e.g., poly(ethylene glycol) (PEG), polyvinyl alcohol, gelatin, and hyaluronic acid] modified by methacrylate can easily accomplish photo-cross-linking by this mechanism, which has many applications.[43] In contrast to chain growth, step growth reactions have a totally different reaction mechanism, which involves two alternate steps. For example, thiol−ene photopolymerization includes the propagation of the thiyl radical to the −ene group to form a carbon-centered radical; chain transfer of the carbon-centered radical by subtraction of a hydrogen from the thiol group forms a new thiyl radical.[44] Compared with the chain growth reaction, the step growth reaction is faster, free of oxygen inhibition, and more cytocompatible.[43−46] Furthermore, a comprehensive comparison of the mechanical properties of hydrogels formed by step growth and chain growth reactions shows that, compared with chain growth, step growth produces hydrogels with higher ductility, tensile toughness, and shear strain because of the more homogeneous networks of PRHs formed.[46] These new explorations on photo-cross-linkable hydrogels enrich the content of PRHs and provide possible applications for PRHs in the cell microenvironment.

In photo-cross-linking reactions, PRH macromer concentration, light intensity, and exposure time play major roles in determining the PRH stiffness. Generally, the stiffness of PRHs is in direct proportion to the macromer concentration, light

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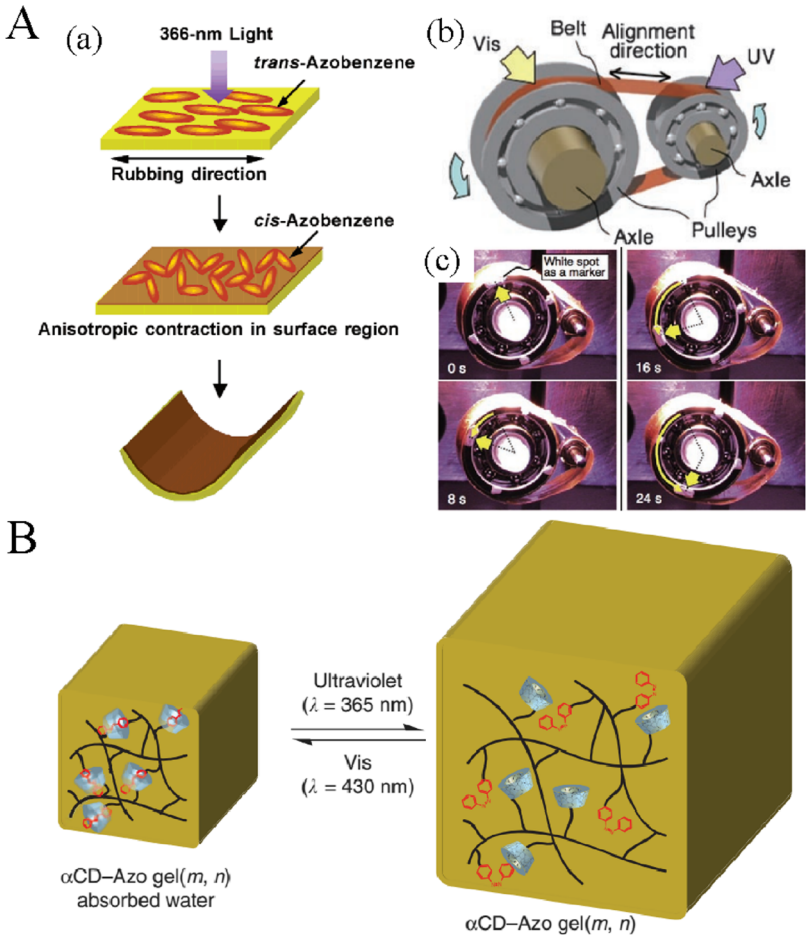

Figure 3. Photoregulation of the hydrogel shape and size. (A) (a) Mechanism of the common liquid-crystal hydrogel film bending when exposed to UV light. (b) Schematic illustration of a light-driven plastic motor system. (c) The running procedure of the motor system actuated by specific light. (B) Schematic illustration of the expansion−contraction of α-CD−AZO gel irradiated by UV and visible light. Reprinted with permission from refs 58 and 60.

intensity, and exposure time. For example, Kiick et al. demonstrated the feasibility of light irradiation (with different duration times and irradiation intensities) in adjusting the stiffness of PRHs to observe cell migration behavior (Figure 2A).[47] The stiffness of the hydrogel significantly increased from 45 Pa to 2.2 kPa and finally to 3.5 kPa with increasing exposure time to 365 nm ultraviolet (UV) light excitation. Switching off the UV light stopped the increase in hydrogel stiffness, demonstrating that the hydrogel cross-linking process was precisely guided by light. Additionally, with the increased light intensity, the photo-cross-linking reaction of the hydrogels could be completed in a shorter amount of time. Apart from directly changing the light exposure time and intensity to control the hydrogel stiffness, Sunyer et al. constantly pulled an opaque mask, located on top of a polyacrylamide (PAA) hydrogel, to create an irradiation time gradient that resulted in the formation of a stiffness gradient in the PAA hydrogel using bisacrylamide as the photo-cross-linker (Figure 2B).[48] Despite the great feasibility and controllability of photo-cross-linking in the fabrication of PRHs with the desired stiffness, the limited penetration depth of the excitation light is one of the major drawbacks restricting the thickness of the fabricated PRHs. Moreover, the area of the hydrogels that can be regulated is restricted by the light source (such as the irradiation area). Additionally, the increase in the PRH stiffness usually leads to a decrease in the PRH permeability and the deteriorating viability of the encapsulated cells. To decouple the dependency of the permeability of the hydrogel on the stiffness of the hydrogel, Cha et al. chemically cross-linked methacrylic alginate with PEG dimethacrylate at a proper concentration and substitution degree.[49] Approaches such as the fabrication of thermal/pH-responsive hydrogels to regulate hydrogel stiffness have also been tried.[50] However, thermal-responsive hydrogels for in vivo use are usually associated with limited changes in the stiffness because of the stability of the body temperature. Likewise, obtaining pH-responsive hydrogels with alterable stiffness lacks dynamic control because of the limited pH variation in the in vivo microenvironment.[23] Nevertheless, the photoregulation of hydrogel stiffness is flexible, feasible, efficient, accurate, and temporally and spatially controllable. Apart from the in situ modification of the stiffness of PRHs, controlling the shape and size of PRHs is also desired.

2.1.2. Shape and Size. Shape memory is a remarkable feature of PRHs used in the engineering of the cell microenvironment. The light-driven PRH actuator can provide a dynamic shear stress to the cells attached to this PRH. Moreover, PRHs capable of automatic shape-changing under light activation can eliminate the risk of contaminating cells compared with manual manipulation.[51] Thus, these PRHs are suitable for dynamically mimicking the microenvironment of endothelial cells in a blood vessel, which always bear shear stress. Normally, PRHs that contain the typical photochromic

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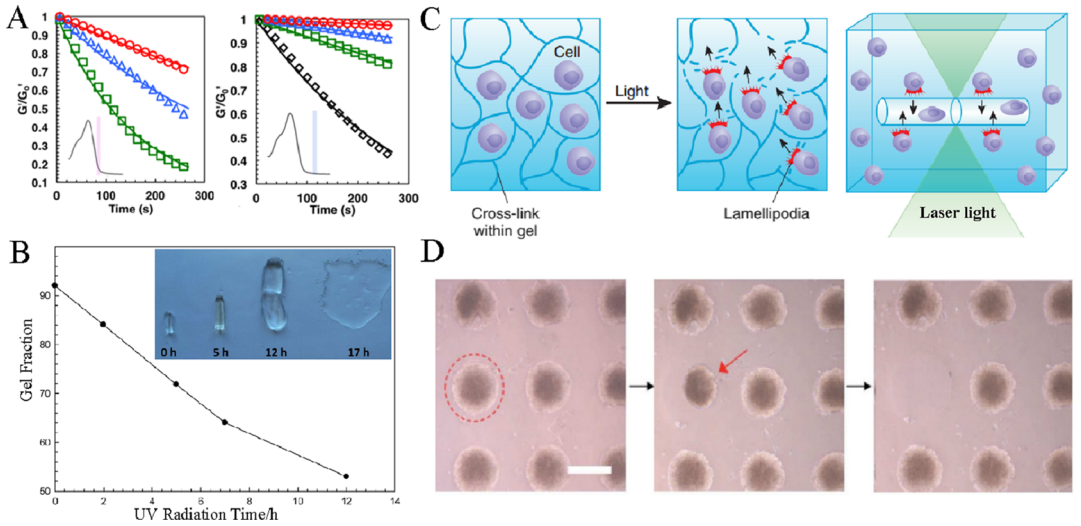

Figure 4. Modification of hydrogel chemical properties on photodegradation. (A) Coumarin-based photodegradable hydrogel degradation kinetics: in situ rheology of the coumarin gel degrading in response to 365 nm light (left) and 405 nm light (right) with different intensities (left: 10.4 mW/cm^2, green; 4.0 mW/cm^2, blue; and 1.4 mW/cm^2, red; right: 40 mW/cm^2, black; 10.4 mW/cm^2, green; and 4.0 mW/cm^2, blue). (B) Gel fractions vs different irradiation time intervals of photodecomposable hydrogels. The inset shows the equilibrium swollen-state photograph of photodegradable hydrogel samples before and after irradiation. (C) Local gel degradation enables spreading of entrapped cells (arrows indicate the direction of spreading). (D) Images demonstrating a sequence of steps leading to release of one stem cell colony. Left image—focusing on the colony to be released, middle image—gel around the colony is affected by brief exposure to UV light through a microscope objective, and right image—the site from which the colony was retrieved. Reprinted with permission from refs.[66,69]

moieties, such as azobenzene (AZO) or spiropyran (SP), possess shape memory characteristics. UV irradiation induces the bending behavior of the PRH film, whereas visible light irradiation recovers such bending through the isomeric reaction of AZO or SP upon UV and visible light irradiation (Figure 3A(a)).[52−57] By using the photoinduced bending and unbending behaviors in PRHs, Ikeda et al. designed a motor device that converts light energy directly into a continuous rotation using AZO-containing PRHs, as shown in Figure 3A(b,c).[58] Further, Lee et al. used the shape memory PRHs to create light-driven hydrogel actuators that exhibit rapid and tunable motions such as fingerlike flexing and crawling that is controlled by the light position, intensity, and direction.[59]

The photoinduced shape changes of PRHs are usually accompanied by size changes. Takashima et al. studied the light-regulated shape and size changes of hydrogels, which directly affect hydrogel permeability. They designed a photo-responsive supramolecular hydrogel that integrated the host (α-cyclodextrin) and guest (AZO derivative) interactions, which showed well-controlled expansion or shrinkage when irradiated by UV (365 nm) or visible (430 nm) light, respectively.[60] After soaking the gel in water, UV irradiation induced isomerization from the trans to cis form, and the complex between α-cyclodextrin and AZO units changed from a conjunction state into a decomposed state, leading to the expansion of PRH. By contrast, visible light irradiation caused the shrinkage of the PRH by inducing isomerization from the cis to trans form between α-cyclodextrin and the AZO units (Figure 3B). The size change behavior was verified by the change in the PRH water content, indicating the change in the PRH permeability. The change in the PRH shape and size, actuated by two lights (visible and UV), exhibited high controllability and feasibility in the whole reversible reaction process.

Apart from the AZO derivative, Florea and colleagues synthesized PRH by incorporating the *N*-isopropylacrylamide-*co*-acrylic SP-*co*-acrylic acid polymer.[61] The synthesized PRH has the ability to contract upon exposure to white light and reswell in the dark after a relatively short time period (seconds to minutes). Upon irradiation with white light, the less hydrophilic SP was formed because of the isomerization of merocyanine (MC−H$^+$), which resulted in a decrease in the hydrogel volume because of the contraction of the hydrogel. In the dark, the acrylic acid monomer dissociates in water, resulting in the protonation of SP to the hydrophilic protonated merocyanine (MC−H$^+$) and leading to the swelling of the hydrogel. Compared with the AZO-derived hydrogel, this method is easy to operate but lacks the necessary controllability for obtaining the desired PRH size.

The in situ PRH shape change regulated by a beam of light holds great promise for biomedical applications such as controlled cell encapsulation and release as well as remotely, spatially, and temporally controlled mechanical loading (tensile or compressive forces) to the attached/encapsulated cells. Through dynamic mechanical loading, the shape memorable PRHs can be a powerful tool in regulating the attached/encapsulated cell fate. However, regulation of the PRH shape change always requires short-wavelength light, which has a limited tissue penetration capacity. Thus, for in vivo use, longer wavelengths that are able to penetrate more deeply into the tissue are in higher demand. To achieve this, the hydrogels could be embedded with upconversion nanoparticles (UCNPs) that convert the excitation light from near-infrared (NIR) to UV, facilitating the response and reaction of the photochromic moieties in the deep tissue.[62] Photoresponse in situ size change PRHs are versatile tools for the encapsulation and release of cells or drugs (discussed in section 3).

2.1.3. Degradation. Light-regulated hydrogel degradation can be realized via incorporation of photoresponsive moieties within the polymer network of PRHs. The incorporated photoresponsive moieties undergo photocleavage, leading to the degradation of PRHs. The PRH degradation rate is affected by the quantum yield (Φ), the molar absorptivity (I_0), the

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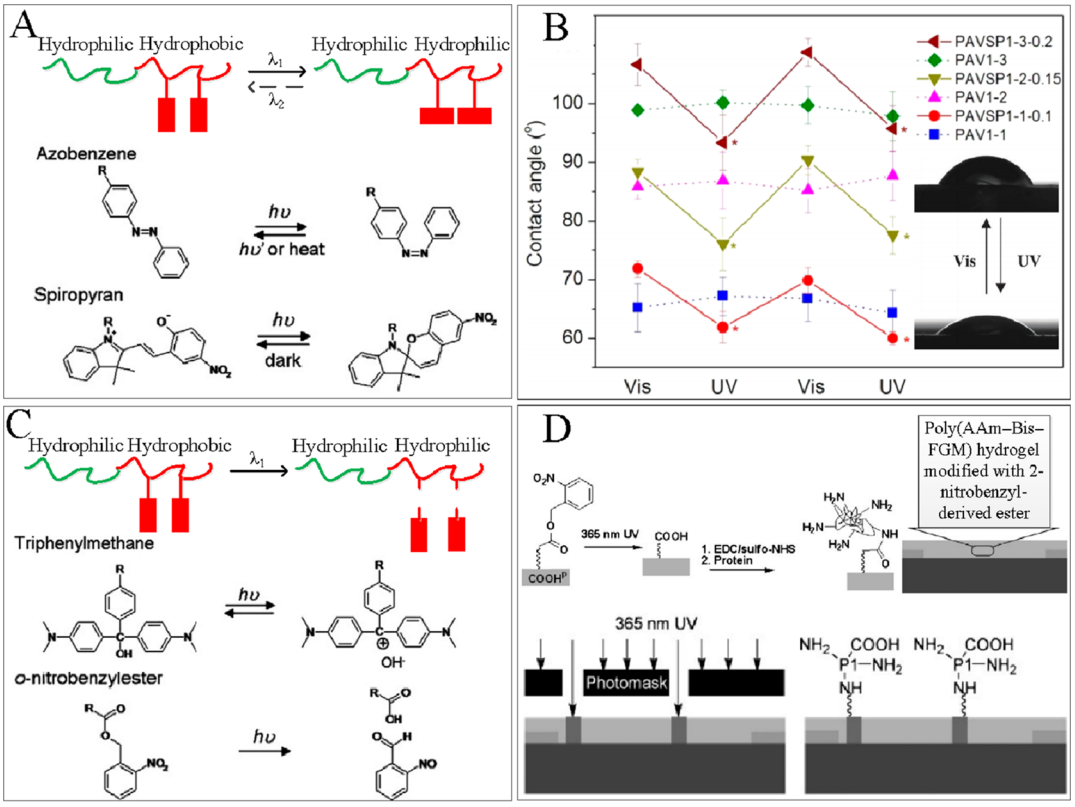

Figure 5. Photoregulation of hydrogel hydrophilicity. (A) Schematic illustration of photoisomerization of photochromic moieties-incorporated hydrogels. (B) Changes in the water CA on PAV and PAVSP hydrogels after two cycles of visible−UV light irradiation. Data are presented as mean ± standard deviation. The inset exhibits the hydrogel surface water CA. (C) Schematic illustration of the photocleavage of photochromic moieties-incorporated hydrogels. (D) Hydrogel surface hydrophilic change and specific immobilization of proteins for patterning under UV irradiation. Reprinted with permission from refs.[84,86,87]

incident irradiation (ε), and the wavelength of light (λ), as defined by the first-order degradation model proposed by Anseth and colleagues.[63,64] Anseth et al. fabricated a PRH using coumarin as the photocleavable cross-linker and studied the effect of light intensity on the degradation of the synthesized PRH (Figure 4A).[65,66] Coumarin-cross-linked PRH was exposed to both 365 and 405 nm light at varying intensities. PRHs exposed0 to light (365 or 405 nm) with a higher intensity degraded faster, as reflected by the reduction in the shear elastic modulus because of the faster photocleavage of coumarin in the hydrogel polymer network.[66] Additionally, the prolonged light irradiation time also promoted the degradation of PRHs.[67] To study the influence of light irradiation time on hydrogel degradation, Selen et al. prepared a photoresponsive PAA hydrogel in the presence of the photodecomposable cross-linker, diacrylated Irgacure-2959. After light irradiation, the remaining hydrogels were quantified by the gravimetric method. A faster PRH degradation rate could be realized by increasing the irradiation time (Figure 4B). In addition, approximately 50% of the hydrogel degraded after 12 h of irradiation, and then the remaining PRH began to dissolve. These results demonstrate the feasibility of light-regulated PRH degradation in a programmable way, which enables PRH to be a powerful tool in regulating the cell migration under light irradiation. Jay and Saltzman incorporated *o*-nitrobenzyl esters as the photoresponsive moiety in a polymer network of PRH to regulate the migration of human mesenchymal stem cells (hMSCs) encapsulated within it. The embedded laser beam within the hydrogel bulk (as shown in Figure 4C) induced photodegradation of the surrounding PRH polymer network. The hMSCs migrated toward the degraded locations around the laser beam (Figure 4C).[68] You et al. used a photodegradable heparin-based hydrogel to cultivate and retrieve mouse embryonic stem cells (mESCs).[69] Upon exposure to 365 nm UV light for 3 min, the irradiated surface of the heparin-based PRH began to degrade, and the mESC colonies detached from the surface after 1 h (Figure 4D). The ability to retrieve the stem cell colonies without disturbing the neighboring, nonirradiated area opens up the possibility of characterizing the in situ heterogeneity of the stem cell phenotype while conserving the cells/reagents. However, exposure to UV light may cause unwanted damage to the cells.

Apart from the degradation of PRH induced by photocleavage, another degradation process, regulated by the structural changes of the photochromic moieties incorporated in the PRH, has also been achieved. A PRH with host−guest interactions was synthesized via the polymerization of β-cyclodextrin-grafted alginate (β-CD−Alg) with diazobenzene-modified PEG (Az_2−PEG) to study the degradation behavior of PRHs.[70] The trans-to-cis photoisomerization process induced by UV light (365 nm) irradiation led to the dissociation of *cis*-AZO from β-CD and substantial structural degradation of the hydrogel and then enabled the rapid release of the trapped molecules. A similarly designed PRH was used for the light-triggered release of RNA by the light irradiation-induced degradation of the PRH.[71]

The hydrogel degradation process is always accompanied by scaffold dissociation and molecular release, which has great

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potential for drug delivery and other biomedical applications.[72] However, natural or synthetic hydrogels that degrade through hydrolysis and enzymolysis are limited by the lack of accurate controllability. By contrast, PRHs, with their light irradiation-induced spatially and temporally controlled degradation, have already been used for controlled release of therapeutic proteins.[73,74] However, UV light is important to control the PRH degradation, and thus the irradiation time is confined to second/minute levels to avoid the potential cell damage induced by long-time UV exposure. To solve this problem, photodegradable hydrogels responding to NIR light have also been designed and used for drug release.[75−77] These new photodegradable hydrogels undergo a quick mass loss after a few minutes of NIR irradiation, thanks to its deep tissue penetration property, which greatly broadens the biomedical applications of PRHs.

2.2. Modification of the Chemical Properties of Hydrogels Using Photostimulation. The chemical properties of the ECM, such as surface hydrophilicity and specific molecule immobilization, play important roles in regulating cell behaviors such as attachment, migration, and proliferation.[78,79] The ability of the hydrogel surface to switch between hydrophilic and hydrophobic is important in cell biology.[80,81] In contrast to hydrophobic surfaces, biomimetic scaffolds, such as hydrogels with hydrophilic surfaces, promote their interactions with cells and lead to better cell adhesion and proliferation. The dynamic alteration of the PRH surface hydrophilicity by light can affect remote modifications to cell adhesion and detachment for in vitro cell expansion. Moreover, the light-induced increase in the hydrophobicity of the PRH surface enables the adjustment of other cell behaviors, such as cell proliferation and migration, via adjustment of the number of adhesion sites between the cell and the PRH. These dynamic alterations to the PRH chemical properties provide a powerful tool for studying cell behaviors in situ and effecting remote modifications to the chemical properties of the hydrogel. In this section, we summarize various approaches and their mechanisms for remotely adjusting the chemical properties of PRHs with light.

2.2.1. Hydrophilicity. PRHs that respond to light irradiation by changing their hydrophilicity have been fabricated by conjugation of the photochromic moieties to the hydrogel polymer chains.[82,83] Usually, on the basis of the mechanism of the photoreaction, these photochromic moieties can be classified into photoisomerization and photocleavage groups. The photoisomerization group, such as AZO, SP, dithienylethene, stilbene, and diazonaphthoquinone (DNQ), undergoes isomerization when exposed to light. The photocleavage group, such as pyrene, *o*-nitrobenzyl, and coumarins, undergoes light irradiation-induced cleavage.

The schematic illustration of photoinduced hydrophilicity changes in PRHs via photoisomerization is shown in Figure 5A. The incorporated photochromic moieties in the polymer network of PRHs, such as AZO and SP, change their molecular structures under light irradiation, which leads to changes in the molecular networks of PRHs and finally results in a change in hydrophilicity. The photoisomerization reactions can be controlled using UV or visible light, and most of the photoisomerization reactions are reversible. These photoisomerization reactions, such as the trans to cis isomerization for stilbene, the Wolff rearrangement for DNQ, the ring-open and ring-closed isomerization for dithienylethene, and the isomerization of SP to merocyanine, are all capable of changing the hydrophilicity of PRHs under light irradiation.

SP, one of the most used photochromic moieties, has shown great promise in adjusting the hydrophilicity of PRHs. Wang et al. fabricated photoresponsive P(AAm-*co*-VDT-*co*-SPAA) hydrogels via polymerization of hydrophilic hydrogen-bonding monomers (acrylamide, AAm), hydrophobic hydrogen-bonding monomers (2-vinyl-4,6-diamino-1,3,5-triazine, VDT), and SP-containing monomers (SPAA) in the presence of the cross-linker PEG diacrylate.[84] After 15 min of UV irradiation, the hydrophilicity of the prepared PAVSP hydrogel was significantly increased, as indicated by the contact angles (CAs) of the surface water (Figure 5B). The phenomenon originates from the isomerization of the SP group from a closed-ring, hydrophobic SP form to an open-ring, hydrophilic merocyanine form on the hydrogel surface under UV irradiation. After UV irradiation, a 13.33° decrease in the CAs of PAVSP hydrogels was observed compared with that of PAV hydrogels without SP, indicating that the isomerization of SP incorporated into PAVSP hydrogels contributes to the improved hydrophilicity.[85] In addition, the subsequent visible light irradiation recovers the decreased CA. Light-regulated, reversible changes in hydrogel hydrophilicity were reproduced in an SP-incorporated PEG hydrogel (Figure 5B inset).[86]

Another approach in adjusting the hydrophilicity of PRHs by light is through a photocleavage reaction, as illustrated in Figure 5C. The photocleavable moieties that are conjugated to the polymer network of PRH usually have an aryl methyl ester group. Upon light irradiation, photochromic moieties, such as pyrene, *o*-nitrobenzyl, and coumarin, undergo cleavage that exposes the hydrophilic carboxylic acids from the aryl methyl ester group, resulting in an increased hydrophilicity. Batt and colleagues prepared a functional moiety 2-nitrobenzyl (NB) methacrylate-modified PRH and used UV light to regulate the hydrophilicity of the PRH for protein immobilization.[87] UV irradiation induces the formation of carboxylic acids on the hydrogel surface by the cleavage of NB, leading to the increased regional hydrophilicity of PRH (Figure 5D). Then, the locations with increased hydrophilicity of PRH were used to immobilize proteins to create multiplexed protein patterns. Similarly, Doh and colleagues also fabricated PRH using *o*-nitrobenzyl methacrylate (*o*-NBMA) as the photoresponsive group for protein patterning.[88]

Photoisomerization reactions controlled by light are reversible and result in changes to the PRH side chains decorated with photoresponsive groups and further induces hydrophilic/hydrophobic changes to the PRH surface. Photoisomerization of AZO/SP-containing PRH can be used to control hydrogel film bending and the hydrogel bulk size as well as to regulate cell release, adhesion, and migration on the PRH surface.[89] Progress has been made in the photocontrolled release of macromolecules conjugated to hydrogel surfaces undergoing photoisomerization, and hydrogels are being used in biosensors, substance diffusion and transport, and tissue engineering and controlled drug-release systems.[90]

Another approach, gas plasma treatment, has also been used to increase the hydrophilicity of biomaterials, usually by exposing the carboxyl groups on the surface of the biomaterials.[91,92] However, this approach only realizes the superficial (2D) hydrophilicity changes of biomaterials because of the limited penetration of the plasma. By contrast, light irradiation can temporally and spatially regulate the hydrophilicity of PRHs in three dimension. In addition, the effects of

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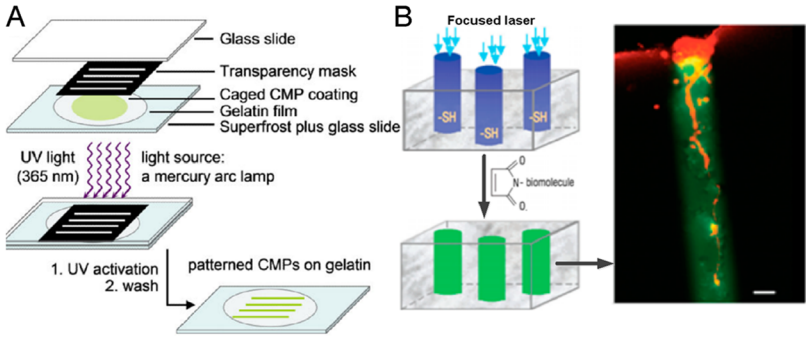

Figure 6. Photoimmobilization of bioactive molecules on PRHs. (A) Experimental setup and process of photopatterning gelatin films with caged CMPs. (B) Schematic of photocleavage within a 3D hydrogel (left) and image (right) about the immobilized green fluorescently labeled fibronectin-mimetic peptide with dorsal root ganglion cells (red) attached on the peptide surface. Reprinted with permission from refs 103 and 104.

plasma treatment on biomaterials will decline with preserving time, and further practical applications of the biomaterials will be affected.[93] In a previous report, combining ammonia plasma treatment reserved and enhanced the effects of the plasma treatment, but the ammonia gas used in the method was poisonous, which could pollute the environment and erode the equipment.[94] Surface modification of the membranes by chemical coupling of a hydrophilic polymer onto the hydrogel surface lacks the dynamic regulation of hydrogel hydrophilicity.[95] Although the use of temperature-sensitive hydrogels that induce surface structure changes enable the dynamic regulation of the hydrophilicity of the hydrogel, it lacks the accurate modification of the sites in the required area.[96] In addition, by incorporating specific photochromes, such as AZO and SP, into PRH polymer chains, the PRH hydrophilicity is reversibly regulated, highlighting the robust ability of PRHs to have their chemical properties dynamically altered. For tissue engineering applications, the interactions between cells and scaffolds are of critical importance.[97] In addition to modifying the hydrophilicity of PRHs, immobilization of bioactive molecules onto PRHs via light irradiation is another powerful approach for controlling the behaviors of cells and their interactions between scaffolds.[98] Therefore, the photoimmobilization of bioactive molecules on PRHs for dynamically controlled cell−substrate interactions is discussed below.

2.2.2. Photoimmobilization of Bioactive Molecules. Although nonresponsive hydrogels made from natural and synthetic materials can provide a 2D/3D cell culture microenvironment,[11,99] they usually lack the dynamic interactions that occur between cells and hydrogels. To solve this issue, immobilization of bioactive molecules, such as peptides and growth factors, onto PRHs using light irradiation has emerged as a promising method for dynamically and spatiotemporally controlling cell−ECM interactions.[100−102] For instance, Yu et al. immobilized a caged collagen mimetic peptide (caged CMP) onto a gelatin hydrogel.[103] They first incorporated photoresponsive NB groups into the CMP backbone, which inhibited the triple helical hybridization between CMPs and the gelatin polymer chains. After UV irradiation, photocleavage of NB resulted in the reduction of the steric hindrance and then immediately triggered the immobilization of the peptide onto the hydrogel. With the help of a photomask, photopatterning of spatially controlled cell adhesion remotely within the hydrogel could be achieved (Figure 6A). These photoimmobilization and photopatterning methods hold great promises for immobilizing various drugs and bioactive molecules onto scaffolds for dynamic control of multiple cellular interactions with ECM. Likewise, Luo and Shoichet also conjugated the *o*-NB-based *S*-2-nitrobenzyl-cysteine (*S*-NBC) group to the agarose hydrogel and then realized photoimmobilization of cell-adhesive peptides onto the agarose hydrogel.[104] Photoirradiation of *S*-NBC exposed free sulfhydryl groups, which then reacted with maleimido-terminated biomolecules for immobilization. With photoimmobilization, they successfully created molecular channels in agarose hydrogel matrices, resulting in alternating volumes of cell-adhesive (peptide) channels separated by nonadhesive (agarose) volumes (Figure 6B). Compared with traditional chemical (e.g., Diels−Alder cycloaddition, carbodiimide chemistry) and physical (thin film deposition from glow discharge plasma) modification techniques,[105−107] photoimmobilization has spatiotemporal controllability, dynamic adjustment of the chemical properties of PRHs, and a broad choice of materials because both bioactive molecules and polymer chains of hydrogels can be modified with photochromes. Therefore, photoimmobilization allows PRHs as a versatile tool in studying the dynamic behaviors of cultured cells and their interactions with PRHs.

Various approaches in the synthesis of PRHs have been summarized above, and the underlying mechanisms of light in regulating the physical and chemical properties of PRHs have also been discussed in detail. Compared with conventional hydrogels that can only mimic the static aspects of the cell microenvironment, PRHs that can respond to light stimuli have emerged as versatile platforms for better mimicking the dynamics of the native cell microenvironment found in vivo. Additionally, light irradiation can precisely alert the dimensional and structural compositions of the PRHs as well as their physical and chemical properties, which is highly desirable in controlling the cell behaviors. However, it should be noticed that it is difficult to achieve specific site immobilization of proteins on PRHs because the designed site that reacts between the PRH surface and the specific protein groups might change the protein structure, which further affects the functionality of proteins. In addition, most of the photochromes are activated by UV light, which could cause unwanted damage to the cells. Moreover, the excitation wavelength of most of the photochromes is usually below 600 nm, leading to the limited penetration depth and the competition for light between photochromes and endogenous biological chromophores.[108] Potential solutions include development of biocompatible photochromes that respond to NIR light or embedding PRHs with UCNPs that can convert NIR to UV and visible light. PRHs with tunable properties allow the study of the effect of dynamically changed biochemical and biomechanical signals

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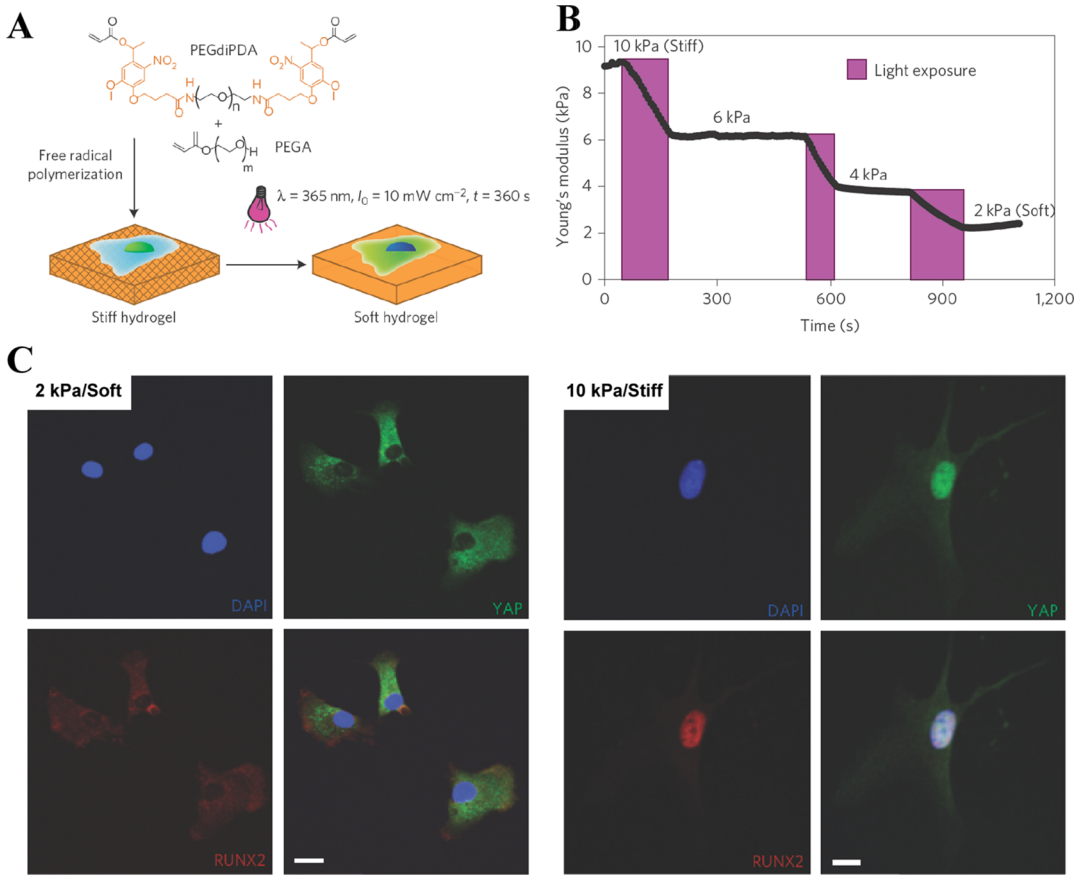

Figure 7. Stem cell behavior regulated by PRHs. (A) Fabrication of photodegradable hydrogels from the free-radical polymerization of PEG diphotodegradable acrylate cross-linker, with a monoacrylated PEG. The moduli of these hydrogels can be tuned by UV irradiation (λ = 365 nm) for 360 s. (B) Light irradiation can be used to fabricate the culture substrate with a range of stiffness (2, 4, 6, and 10 kPa). (C) Immunofluorescence staining results revealed the expression of IYAP and RUNX2 in hMSCs on both soft hydrogels (2 kPa) and stiff hydrogels (10 kPa). DAPI, blue; YAP, green; RUNX2, red. The scale bar is 20 μm. Reprinted with permission from ref 124.

on cell behaviors, which are more close to the cell microenvironment in vivo. Examples of applying PRHs for dynamic tailoring of the microenvironment of a cell have been discussed in the following section.

3. ENGINEERING THE CELL MICROENVIRONMENT USING PRHS

3.1. Mechanical Microenvironment. The mechanical cell microenvironment plays a vital role in regulating the cell behavior in terms of adhesion, spreading, migration, proliferation, and differentiation.[19,109,110] In vivo, the cells sense various mechanical cues from the surrounding ECM and body fluid through the transmission of force from transmembrane complexes (e.g., focal adhesion) to the cytoskeleton and exert traction force against the matrix through actomyosin dynamics.[111,112] The stiffness of the ECM resists the deformation caused by traction cells and regulates the cellular mechano-transduction process. Therefore, there is a pressing need for tools to investigate the effect of the ECM stiffness on cell function. Various biomaterials have been used to mimic the mechanical cell microenvironment.[10,113−115] Of these biomaterials, hydrogels offer the advantages of a high water content, biocompatibility and biodegradability, and tunable chemical and mechanical properties.[11,116] A variety of strategies based on hydrogels have, therefore, been developed to study the response of cells to matrix stiffness.[117,118] For instance, specific lineages of hMSCs (e.g., adipocytes, neurocytes, myocytes, and osteocytes) have been derived from protein-functionalized PAA hydrogels with different stiffnesses ranging from 0.1 to 40 kPa.[109] hMSCs adhered, spread, and exhibited a filopodia-rich morphology on a soft hydrogel (0.1−1 kPa) that mimicked the stiffness of the brain. After 1 week in culture, the hMSCs differentiated into primary neurons, as determined by the expression of specific proteins, including R-III tubulin and P-NFH. When cultured on a stiffer hydrogel that mimicked the stiffness of the muscle (8−17 kPa) and the collagen of osteoids (40 kPa), hMSCs specifically differentiated into myoblasts and osteoblasts, respectively. Matrix stiffness can induce the cross-talk of various intracellular signaling pathways through integrin complexes. For instance, the Smad pathway can be activated by matrix stiffness and induces the neural specification of hMSCs, whereas focal adhesion kinase induces osteogenesis. Matrix stiffness enhances the adhesion, spreading, and proliferation of other cell types, such as myoblasts and cardiac fibroblasts.[119,120]

Although many efforts have been made to demonstrate the influence of matrix stiffness on the cell behavior, the existing hydrogels can only mimic a static artificial matrix in vitro, which is inconsistent with the in vivo situation, where the ECM is highly dynamic.[112] The spatiotemporal changes in the matrix stiffness affect cell morphology, function, and fate. For instance, in response to an injury to the heart, cardiac fibroblasts are known to differentiate into active myofibroblasts, a hallmark of cardiac fibrosis, resulting in permanent scarring and enhanced ECM moduli.[121] The increasing ECM stiffness will further

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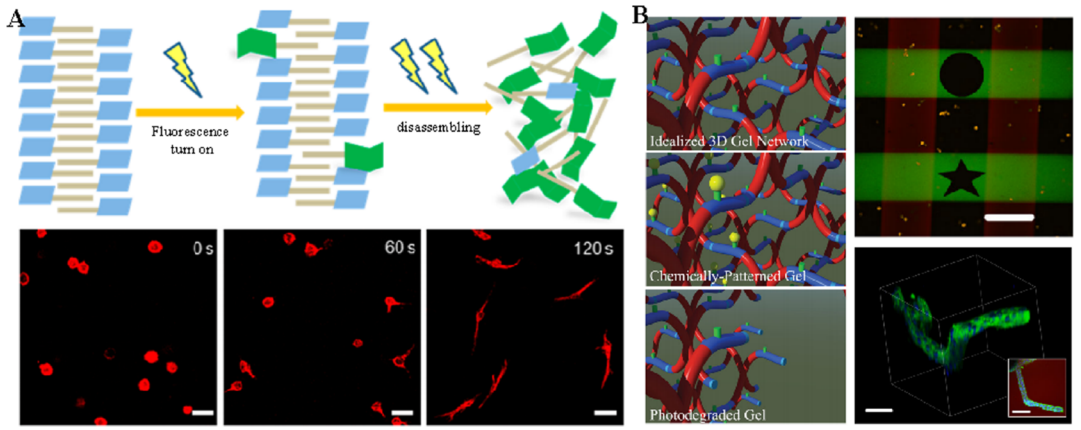

Figure 8. Photoclickable hydrogels applied in engineering the cell microenvironment. (A) Schematic of the photoclickable hydrogel structure change under light irradiation and the related morphology study of hMSCs encapsulated in gels with different times of UV exposure. (B) Schematic of the photoclickable hydrogel structure change via visible light-induced chemical patterning and UV-induced gel degradation. Both the patterns and related cell culturing results in 2D and 3D are shown below. Reprinted with permission from refs 100 and 129.

promote myofibroblast differentiation, which prolongs the existence of fibrosis through increases in the secretion of transforming growth factor β and α-smooth muscle actin. Thus, PRHs provide a strategy to achieve the change of ECM stiffness through spatiotemporally controlled cross-linking density or degradation via light irradiation. A photodegradable hydrogel composed of 4-bromomethyl-1-3-nitrobenzoic acid and PAA acryl hydrate, with dynamic changes in its stiffness has been developed.[122] A 20−30% decrease in the modulus can be achieved after UV irradiation. The softening of the hydrogel network was achieved by the removal of the NB group via UV exposure at a dose tolerated by the cells. The softening of hydrogels led to a decrease in the cell spreading area. Similarly, Anseth et al. developed a photodegradable monomer for synthesizing PEG-based hydrogels that degrades in response to light.[123] The effect of dynamic changes to the matrix stiffness on valvular interstitial cell (VIC) fibroblast-to-myofibroblast activation was clarified by irradiating samples with activated cells, decreasing the modulus, and inducing cell deactivation. These PRHs demonstrated the ability of both probing and directing cell function through dynamic changes in the mechanical properties of the substrate. Moreover, a PRH based on PEG diphotodegradable acrylate cross-linker was synthesized and used for probing whether MSCs can remember previous mechanical signals and whether these can be exploited to mechanically dose the cell.[124] It was found that, for hMSCs cultured on soft PEG hydrogels (~2 kPa), it was the previous culture time on stiff tissue culture polystyrene (~3 GPa) that determined the activation of the yes-associated protein, its transcriptional coactivator with a PDZ binding domain, and the preosteogenic transcription factor RUNX2 (Figure 7). This result suggests a temporal role for cellular mechanotransduction that involves the history of a cell's microenvironment.

Existing dynamic regulation of the mechanical cell microenvironment based on PRHs has been widely used for studying cell functions in 2D cell culture systems. However, in vivo, cells are embedded in a 3D ECM, assuming a stellate morphology, and only polarize from front to rear during migration.[125] Recently, a variety of studies have employed 3D hydrogel microenvironments to investigate the effect of mechanical cues on cell behaviors. For instance, a thiol−ene-functionalized PEG hydrogel was polymerized through UV light, with stiffness dynamically regulated by varying the irradiation time and monomer fraction.[126] hMSCs were then encapsulated in the PRHs, and cell aggregates formed after 6 days in culture. In another case, PEG-based hydrogel cross-linked with AZO could be reversibly softened and stiffened upon multiple light irradiation through photoisomerization of the incorporated AZO-containing cross-linker.[127] Upon irradiation with 365 nm light, isomerization to the AZO cis configuration leads to a softening of the hydrogel up to 0.1−0.2 kPa, which results in the high viability and a stellate morphology of the porcine aortic VICs encapsulated in the PRHs. Such PRHs provide a feasible way to noninvasively control the stiffness of the cell microenvironment, allowing for the investigation of cell behaviors related to dynamic changes in the stiffness of the PRH.

In vivo, the cell microenvironment is composed of spatiotemporal gradients of multiple physical (e.g., stiffness, porosity and stress/strain) and chemical (e.g., molecules) cues, which play important roles in regulating the cell function, especially for pathological processes such as tumorigenesis.[128] Therefore, it is important to fabricate 3D cell microenvironments with various gradient features in vitro. Although PRHs hold great potential for achieving such objects by the design of their molecular structures or by using opaque photomasks, several challenges still exist. For instance, most of the photochromes only respond to UV or visible wavelengths of light that may cause unwanted damage to the cells and have a limited penetration depth, restricting the dimensions of PRHs, especially the thickness of the PRHs. Additionally, it is still challenging to fabricate PRHs with desired mechanical cues (e.g., matrix stiffness) and biomechanical cues (e.g., adhesion ligand density and molecule gradient) that are well-coupled with each other, which is essential to generate functional tissue constructs using PRHs.

In addition, photoclickable technology provides functional hydrogels with surface sites or areas that can be precisely, spatially, and temporally regulated by lights. This photoclickable technology provides advanced control over the behaviors of cells in the hydrogel by modifying the mechanical and biochemical microenvironment. For instance, He et al. synthesized a novel photoclickable hydrogel, where the surface structure of the hydrogel in the specific area under light irradiation would degrade, and investigated different cell behaviors of living cells growing on the hydrogel (Figure

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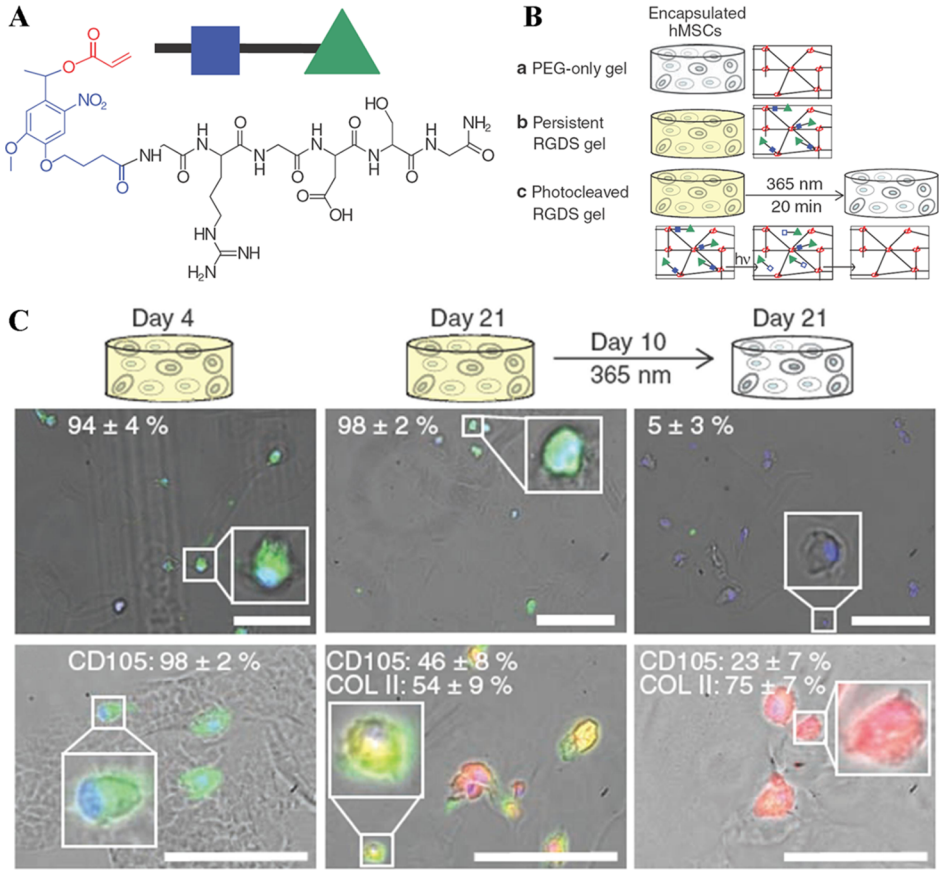

Figure 9. Photolabile biological molecule synthesis utilized for dynamic changes in microenvironment chemistry. (A) A biofunctional acrylic monomer was synthesized containing the adhesion peptide Arg-Gly-Asp-Ser (black) attached to the acrylate (red) and photolabile moiety (blue). (B) hMSCs were encapsulated in nondegradable PEG gels (b) with or (a) without photoreleasable Arg-Gly-Asp-Ser. (c) Presentation of Arg-Gly-Asp-Ser was temporally altered by photocleavage of Arg-Gly-Asp-Ser from the hydrogel on day 10 in culture. (C) Influence of dynamic microenvironment chemistry on the integrin expression and differentiation. Cells (blue) with photoreleasable Arg-Gly-Asp-Ser have decreased expression of the $\alpha_v\beta_3$ integrin (green) and increased expression of the chondrocyte marker (COL II red) by day 21 (right), indicating that the cells have responded to the removal of Arg-Gly-Asp-Ser. The scale bar is 100 μm. Reprinted with permission from ref 63.

8A).[129] The phototrigger underwent a rapid intermolecular photoclick ligation upon mild light irradiation, which disturbed the balance between the hydrophilic interaction and $\pi-\pi$ stacking of the self-assembled system and induced the disassembly of the hydrogel matrix. By cultivating channels with a photomask, the difference in the expression of differentiation markers of living C2C12 cells inside and outside the spatially defined channels could be investigated. DeForest and Anseth presented a system where the functionality and architecture of the hydrogel network are independently controlled by multiple wavelengths of light (visible and UV) to obtain biochemical and biophysical photopattern, click-based hydrogels (Figure 8B).[100] hMSCs were encapsulated in RGD and PHSRN gel-binding peptide ligands to demonstrate the effect of a variety of biomolecular cues on the functions of cells growing within the gel. With photoclick hydrogels regulated independently by multiple wavelengths, we expect a new photoclickable hydrogel, which has multiple functional sites or areas. When the sites or area on the hydrogel surface are discriminatively exposed to light channels, the desirable hydrogel modification can be realized with great ease.

3.2. Biochemical Microenvironment. Despite the mechanical properties of the ECM, various bioactive molecules, including functional proteins, growth factors, and therapeutic drugs, are also important factors in regulating the cell behavior. For instance, bioactive molecules, such as fibroblast growth factor, vascular endothelial growth factor, and intermedin, are necessary for angiogenesis and vasculogenesis in vivo.[130,131] Cellular behaviors such as adhesion, migration, proliferation, and differentiation are regulated by bioactive molecules such as adhesive ligands and growth factors. To mimic such a biochemical microenvironment using hydrogels, one of the most successful applications for promoting cell adhesion in cell-compatible hydrogels is to incorporate peptide-based analogues of native ECM components, such as RGD, YIGSR, and IKVAV, into the hydrogel matrix.[132] Significant advances have been achieved in regulating the cell biochemical microenvironment using PRHs. The common strategy is to modify the hydrogel network with bioresponsive functionalities.[133] For example, photoassisted modification of biological functional molecules (e.g., Arg-Gly-Asp peptides, collagen, and fibronectin) on the hydrogel surface can significantly enhance cell adhesion and spreading.[104,134] For a 3D hydrogel system, the chemical patterning of a hydrogel via a second interpenetrating network or a peptide tether has been demonstrated by diffusing chemical moieties into a hydrogel network and covalently linking these functionalities to the network by photocoupling or reaction with photolytically uncaged reactive groups.[135,136] Although these approaches can control the cell biochemical microenvironment, it is still difficult to modulate the chemistry

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of the hydrogel in real time by photoregulation of the hydrogel structure. Efforts have been made to produce photolytically degradable hydrogels whose chemical properties are temporally and spatially tunable upon light irradiation.[63] For example, an adhesive peptide (Arg-Gly-Asp-Ser) was incorporated into a PEG diacrylate hydrogel network. Upon UV irradiation, the modified peptide was cleaved from the polymer network and released into the solution. The peptide then diffused out of the hydrogel (Figure 9) to promote stem cell adhesion and chondrogenic differentiation. The lack of the adhesive peptide in the hydrogel network will trigger cell behavior changes. The viability and differentiation of hMSCs encapsulated in the PRH were well-studied, temporally and spatially, by regulating the peptide fraction by UV irradiation. In another study, two types of functional molecules, an ECM-derived adhesion peptide sequence (CRGDS and CYIGSR) and a photocleavable peptide sequence (KCGPQG), were grafted onto the PEG−norbornene monomer. Photoinitiated thiol−ene click chemistry was then employed to generate a PRH.[137] The aggregation and outgrowth of mESC-derived motor neurons with varying concentrations of the biochemical molecules in the hydrogels were further investigated through UV irradiation. Hydrogels with an elastic modulus below 350 Pa may provide a robust platform for axonal outgrowth. However, hydrogels with a modulus below 350 Pa were difficult to manipulate post gelation, making consistent imaging and observations impossible. These PRHs hold great potential for spatiotemporally regulating both the 2D and 3D cell microenvironments, leading to applications in fields ranging from tissue regeneration to drug release.

4. CONCLUSIONS AND FUTURE PERSPECTIVES

PRHs can respond to a beam of light by changing their mechanical properties (e.g., matrix stiffness, shape and size, and degradation) and chemical properties (e.g., surface hydrophilicity and bioactive molecule immobilization) in a real time and spatiotemporal manner, making them a versatile platform for mimicking the dynamic cell microenvironment in vivo. Fundamental questions about the complex inter-relationships between the mechanical and chemical properties of the microenvironment and cell functions are being elucidated. However, several challenges still need to be addressed.

Monitoring techniques for probing the structural and chemical changes in the hydrogels (e.g., porosity and ligand density) and the cell functions in a 3D culture system still need to be developed. Of these techniques, the emerging fluorescence resonance energy transfer (FRET)-based method holds great potential for studying the degradation of hydrogels induced by the encapsulated cells.[138] With confocal microscopy, the FRET-based technique can also quantify the cell−matrix interactions and the cell traction force. Moreover, other physical and chemical properties of PRHs are also important for biological applications. Recently, several methods have been developed for the characterization of PRHs. Li et al. have developed a noncontact method to characterize the mechanical properties of PRHs on the micro- and mesoscale through magnetic actuation.[139] Hong et al. used the dual-mode ultrasound elastography technique to test the material viscoelasticity by noninvasive creep testing.[140] To meet the demands of the dynamic processes of cell activities in the PRHs, the challenge is how to develop novel methods, which can achieve precise, spatiotemporal characterization of both PRHs and encapsulated cell behaviors.

Another challenge is how to use PRHs to mimic the stress/strain microenvironment spatiotemporally. This is important because in vivo tissues commonly experience large deformations under both physiological and pathological conditions. For instance, the solid tissues of hollow organs (e.g., the bladder) can routinely stretch up to 2.5 times their length, a nominal strain of 150%.[141] The alveoli can expand 1.5 times and 3.0 times larger than their volume in normal mice and emphysematous mice, respectively.[142] Existing methods to mimic the stress/strain microenvironment of cells are realized by the mechanical loading of hydrogels. These hydrogels are usually vulnerable to mechanical loading. Therefore, it is important to design functional hydrogels that can deform when exposed to light. Moreover, the dynamic regulation of mechanical and chemical properties of the hydrogel through light irradiation results in structural changes to the hydrogel network (e.g., porosity). Therefore, it is necessary to decouple the effect of these factors on cell function, especially in a 3D microenvironment. Mooney et al. developed an interpenetrating hydrogel composite based on alginate and collagen, which enables independent tuning of the mechanical properties of the hydrogel (e.g., stiffness and viscoelasticity) by changing the divalent cation concentration.[143−145] Such hydrogels may be modified with photoresponsive ligands and used for mimicking the cell microenvironment in the future. Through these approaches, the collection of methods for quantifying the changes in the cell and hydrogel properties are growing toward a better understanding of the dynamic relationship between cells and their microenvironment.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: danbai@xjtu.edu.cn (D.B.).
*E-mail: minlin@mail.xjtu.edu.cn (M.L.).

ORCID

Feng Xu: 0000-0003-4351-0222
Min Lin: 0000-0002-3259-1955

Author Contributions

⊥Y.D. and G.J. contributed equally.

Notes

The authors declare no competing financial interest.

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