Journal of Materials Chemistry B

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Article for Submission to Journal of Materials Chemistry B

Light-Induced Remodeling of Physically Crosslinked Hydrogels Using Near-IR Wavelengths

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Dynamic biomaterials utilize external stimuli to create well-defined physical properties with precise spatiotemporal control. This class of functional materials can be used in various biomedical applications including smart medical devices or model microenvironments for elucidating cellular or organotypic function.[1-4] Photoactive polymers are well-suited for functional biomaterials because network properties can be controlled using exogenous light.[5,6] Hydrogels are an important class of polymeric biomaterials for tissue engineering and drug delivery because they exhibit high mechanical compliance, modular chemical functionality, and the ability to load and release bioactive molecules and living cells.[7,8] Photoreconfigurable hydrogels have been used as light-sensitive biomaterials in biomedical applications including triggerable matrices for controlled drug release,[9-13] templates for three-dimensional biomolecular patterning[14,15] and programmable tissue scaffolds.[16-19] Hydrogels with

photocleavable moieties can elucidate the role of physicochemical cues in cell-material responses.[20,21] Light-induced uncaging is a prevalent strategy to confer photoreconfigurability within crosslinked hydrogels. Photolabile functional groups such as *o*-nitrobenzyl can be integrated within networks either as pendant groups or components of the polymer backbone to pattern hydrogels or control the crosslinking density.[2] Light-induced phase inversion is another strategy for in situ control of crosslink density in block copolymer networks. This mechanism has been recently demonstrated with UV-induced single-photon uncaging of ABA triblock copolymers composed of *o*-nitrobenzyl methacrylate groups.[22] Physically crosslinked gels offer advantages for prospective use in biomedical applications. They can form networks spontaneously from a single polymeric component. Physical crosslink domains can serve as depots to load and release bioactive compounds. Physically crosslinked hydrogels with photoreconfigurable nodes permit rapid and total network disintegration upon exposure to external light sources. Polymeric networks that utilize single-photon uncaging offer limited utility in biomedical applications because UV irradiation is tissue-opaque and can potentially cause DNA damage in cells.[23] Next-generation photodegradable biomaterials will benefit from light-sensitive chemistries that can be modulated using benign tissue-transparent wavelengths.[24,25] Two-photon uncaging of coumarin derivatives using near-IR wavelengths can release bioactive small molecules from both light-sensitive conjugates and micelles.[26,27] This work describes the synthesis and characterization of physically crosslinked hydrogel networks composed of triblock copolymers with pendant light-sensitive coumarin moieties, namely, [6-bromo-7-hydroxycoumarin-4-yl]methyl which has been reported with a large two-photon

uncaging cross section above 1 GM.[26] Mechanisms of light-induced disintegration and potential applications as functional surface coatings for medical implants are also discussed.

Light-sensitive linear amphiphilic ABA triblock copolymers are composed of hydrophilic poly(ethylene glycol) B blocks (PEG, M_w = 20 kDa) and hydrophobic photolabile poly([6-bromo-7-hydroxycoumarin-4-yl]methyl methacrylate) (PBHCMM) A blocks. Poly(methacrylic acid)-PEG-poly(methacrylic acid) (PMAA-PEG-PMAA) triblocks were prepared using atom transfer radical polymerization (ATRP). 6-bromo-4-chloromethyl-7-hydroxycoumarin was conjugated to methacrylic acids via esterification (**Figure 1**). ATRP synthesis of A blocks using PMAA precursors produces higher molecular weights and smaller PDI compared to coumarin methacrylate.[28,29] The average degree of polymerization (DOP) of PMAA blocks was 36 for each segment and the degree of esterification by coumarin was 50% as measured by ^{1}H NMR. The esterification efficiency of coumarin on PMAA blocks is lower compared to previous reports that conjugate *o*-nitrobenzyl halides to PMAA due to increased steric hindrance.[30,31] Longer reaction times can increase the degree of esterification to >75%. A degree of esterification of 50% was chosen to achieve a balance between robust mechanical properties and accelerated degradation rates of physically crosslinked hydrogels. Minimizing the amount of coumarin can also reduce the potential toxicity risks after uncaging.[32,33]

The rate of single-photon uncaging of poly(MAA-*co*-BHCMM)-PEG-poly(MAA-*co*-BHCMM) triblock copolymer was measured in solution using UV-vis spectroscopy (**Figure 2a**). Spectra of triblock copolymers (0.1 mg ml^{-1}, DMSO) exhibit a prominent absorbance peak at 330 nm. The intensity of this peak decreases with increased UV exposure (70% reduction in peak absorbance after 16 min). This reduction is attributed to the production of 6-bromo-7-hydroxy-

4-(hydroxymethyl)coumarin as a by-product of single-photon uncaging. Light-induced uncaging destabilizes physical crosslinks and leads to macroscopic disintegration of hydrogels through conversion of hydrophobic poly(MAA-*co*-BHCMM) blocks into water-soluble PMAA segments (Figure 1b). Self-assembled triblock copolymer hydrogels exhibit a swelling ratio of Q = 63.2 ± 3.4 by mass and retain structural integrity in water for several months at room temperature. Hydrolytic cleavage of ester bonds within ABA block copolymers is theoretically possible when hydrogel networks are incubated in aqueous conditions. Two potential cleavage sites include ester bonds that link the following functionalities: pendant coumarins with acrylic acid in A blocks; poly(MAA-*co*-BHCMM) A blocks and PEG B blocks in the polymer backbone. These hydrogels in aqueous environments exhibit frequency independent storage and loss moduli of *G′* = 139.72 ± 19.3 Pa and *G″* = 12.24 ± 1.3 Pa (**Figure S2**). These data suggest that the hydrogels are elastic and solid-like. The density of physical crosslinks inside hydrogels ρ is estimated to be 0.22 ± 0.11 x 10^{-3} mol m^{-3} using Equation (a).[34] This calculation result indicates that these coumarin-based triblock copolymers form loosely crosslinked networks compared with other reported physically crosslinked hydrogels.[35]

$$\rho = \frac{G'}{RTQ^{-1/3}} \qquad \text{(a)}$$

The normalized storage modulus $\overline{G}'$ is reduced to 25% after 1000 sec of illumination (Figure 2b). Temporal control of network disintegration was demonstrated by exposing hydrogels to intermittent UV irradiation. The reduction in *G′* is in concert with temporal patterns of UV irradiation. The value of $\overline{G}'$ in hydrogel networks continues to decrease in the dark after the initial UV exposure. The rate of decrease of *G′* in the dark is reduced as $\overline{G}'$ approaches 0.35.

Molecular by-products of uncaging may destabilize other physical crosslinks, thereby reducing $\overline{G}'$ with UV illumination.

Spatial selectivity in light-induced uncaging and disintegration of hydrogels was demonstrated by using photomasks (**Figure 3a).** The robust physical properties of hydrogels and the spatial selectivity of the uncaging process permit high feature fidelity (Figure 3a-ii). Fluorescent micrographs confirm that the uncaging mechanism correlates with microstructure fabrication (Figure 3a-iii). Exposing the surface to 15 min of continuous UV irradiation produces feature heights of approximately 50 μm (**Figure S3**). Well-defined microstructures can be fabricated using two-photon uncaging (Figure 3b) by leveraging non-linear absorption.[36] Insight into the uncaging mechanism could be inferred from a unique phenomenon observation immediately after raster scanning of the hydrogel. Time-lapse images (**Video S1**) capture a transient fluorescent signal (λ_{ex} = 740 nm, λ_{em} = 420 nm) that form near newly rastered voxels within hydrogels. The intensity of this fluorescent signal is larger than the background from the hydrogel. The signal decays steadily as the uncaged by-products diffuse away from the voxel (Figure 3b-iii). Transient fluorescent compounds are likely composed of newly liberated hydroxycoumarin molecules that do not self-quench.[26] These data support the proposed mechanism of hydrogel network dissolution based on light-induced uncaging and disruption of physical crosslinks. Z-stack images (**Figure S4 and Video S2**) illustrate the ability to fabricate microstructures contained within a 3D volume.

The proposed mechanism for hydrogel disintegration was further investigated by loading secondary reporter molecules into the networks. The time-dependent UV-vis spectra of by-products after single-photon uncaging of Rhodamine B-loaded hydrogels exhibit prominent

absorbance peaks at 540 and 330 nm, which are assigned to newly liberated Rhodamine B and coumarin, respectively (**Figure S5**). These signals increase together with exposure time, which suggests that the release of non-covalently associated Rhodamine B and the liberation of uncaged hydroxycoumarin are coupled events. The normalized absorbance intensity of freed Rhodamine B increases with irradiation time (**Figure 4a**). Intermittent UV irradiation produces release profiles that are monotonically increasing with periodic interruptions. Rhodamine B release from photodegradable hydrogels that were not irradiated was also measured. These control conditions demonstrate that Rhodamine B release is governed by light-induced disintegration of the physically crosslinked poly(MAA-*co*-BHCMM)-PEG-poly(MAA-*co*-BHCMM) hydrogel network. These data indicate the strong correlation between UV irradiation and Rhodamine B liberation. Disruption of physical crosslinks was further investigated through two-photon uncaging. Hydrogels were illuminated with a two-photon laser and the resulting absorption spectra are shown in Figure 4b. Samples were charged with fresh aqueous medium after exposure and incubated in the dark (1 h) to produce intermittent illumination patterns. Values for peak absorption suggest that the extent of uncaging is consistent for a prescribed irradiation energy. These data also indicate the positive correlation between release of Rhodamine B and two-photon induced physical crosslinks dissociation.

Efficient light-induced remodeling of physically crosslinked hydrogels at tissue transparent wavelengths suggests that implants composed of triblock copolymers can be non-invasively manipulated using light sources from outside the body. Potential applications may include matrices for smart controlled release systems or remotely activated functional surfaces. A potential application as a remotely cleanable surface is examined here using a model material

environment. Specifically, photodegradable hydrogel films are prepared into substrates that can be expunged of adherent microparticles. Fluorescent polystyrene (fPS) microparticles were embedded into the apical region of hydrogels during film preparation. Hydrogels with embedded microparticles were uncaged using single- or two-photon absorption as previously described (**Figure 5**). Single-photon uncaging was performed with a photomask to demonstrate the selectivity of light-induced surface cleaning. Homogeneously distributed fPS beads were selectively removed in the regions exposed to UV light (Figure 5b). Selectivity in fPS removal using two-photon uncaging was achieved by focusing near-IR laser illumination on the hydrogel surface. Hydrogels with embedded fPS microparticles dedicated for two-photon uncaging were rastered in circular geometries. The disintegrated regions were removed leaving the pristine portion of the gel (Figure 5c). Coumarin-based physically crosslinked hydrogels can potentially serve as medical device coatings that can be cleaned remotely using exogenous light.[37]

Light-induced remodeling of coumarin-based hydrogels offers several notable advantages as a photodegradable polymer network. Physical crosslinks can serve as reservoirs for non-covalent loading of small molecule payloads. Two-photon uncaging permits modulation of network properties at tissue-transparent wavelengths.[36] Finally, PMAA-PEG-PMAA triblock copolymers and coumarin-based by-products of uncaging are potentially less cytotoxic compared to by-products in other photocleavable materials.[39,40] Poly(MAA-*co*-BHCMM)-PEG-poly(MAA-*co*-BHCMM) hydrogels could be processed into functional coatings for medical implants or dynamic material microenvironments to elucidate cell-cell and cell-material interactions.

Experimental Summary

Poly([6-bromo-7-hydroxycoumarin-4-yl]methyl methacrylate)-based triblock copolymers were synthesized by first preparing PMAA-PEG-PMAA triblocks using ATRP. 6-Bromo-4-chloromethyl-7-hydroxycoumarin was conjugated to PMAA-PEG-PMAA using 1,8-diazabicycloundec-7-ene (DBU). Rheological measurements were conducted using a HR-2 rheometer with a UV illumination accessory (TA Instruments). Single-photon uncaging was performed as previously described.[20] Two-photon uncaging was performed using a LSM (Zeiss) equipped with a Ti: Sapphire laser (Coherent). All values reported as mean ± std. dev. unless otherwise stated. See Supporting Information for experimental details.

Keywords: block copolymers, hydrogel, near-infrared, photocleavable, self-assembly

Acknowledgements: Funding provided by the following organizations: the Berkman Foundation, the American Chemical Society Petroleum Research Fund (ACS PRF #51980-DNI7), the National Science Foundation (NIH; R21EB015165). NMR instrumentation at CMU was partially supported by National Science Foundation (CHE-0130903 and CHE-1039870). The authors also acknowledge the support from Molecular Biosensor & Imaging Center in Carnegie Mellon University.

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Figures

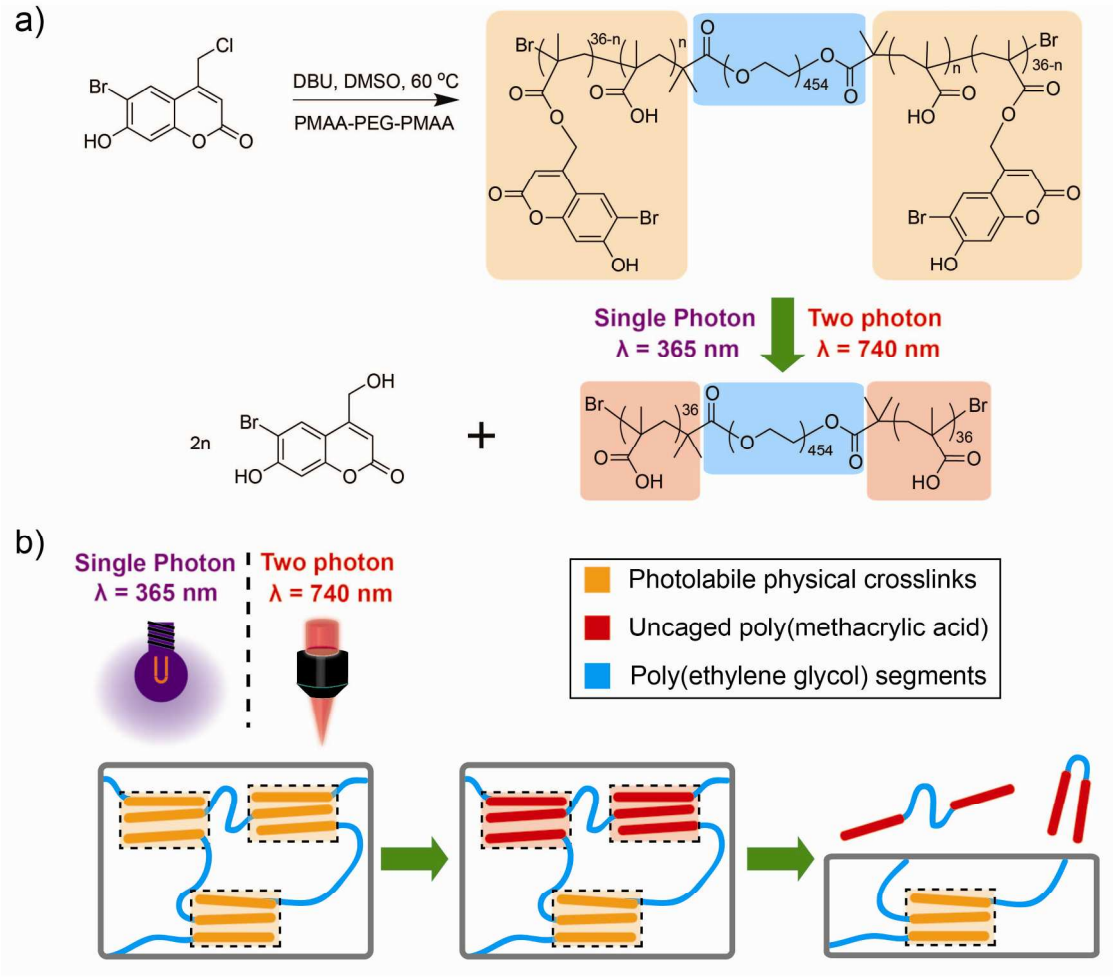

Figure 1. a) Synthesis and photodeprotection schemes for poly(MAA-*co*-BHCMM)-PEG-poly(MAA-*co*-BHCMM) ABA triblock copolymers is shown. Light-induced uncaging of PBHCMM segments yields water soluble PMAA-PEG-PMAA ABA triblock copolymers. b) A schematic illustration that describes the proposed mechanism of light-induced phase inversion and disintegration of physically crosslinked hydrogels.

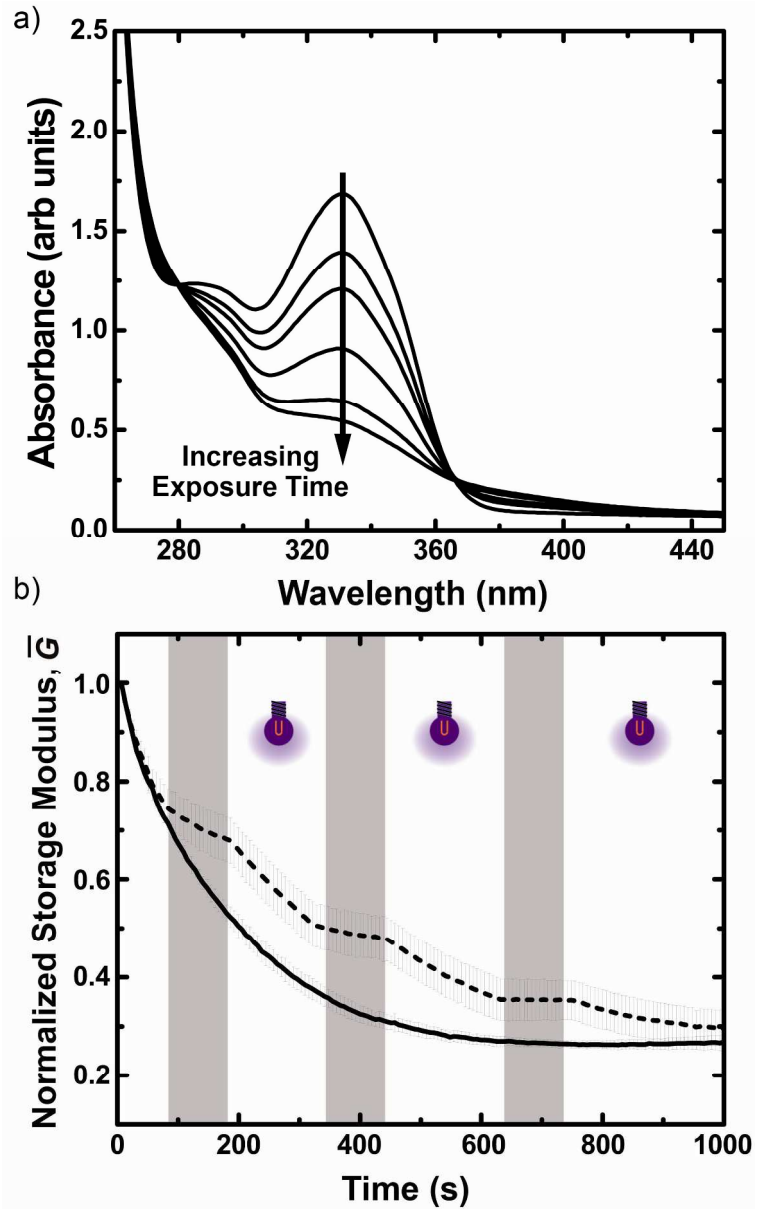

Figure 2. a) UV-vis spectra of poly(MAA-*co*-BHCMM)-PEG-poly(MAA-*co*-BHCMM) in DMSO solution before and after UV exposure. Uncaging is evident by the decrease of absorbance peak at 330 nm. b) The temporal evolution of normalized storage modulus *G'* is plotted for continuous (solid line) and intermittent (dashed line) exposure to UV irradiation. White (grey) vertical bars correspond to the presence (absence) of UV illumination.

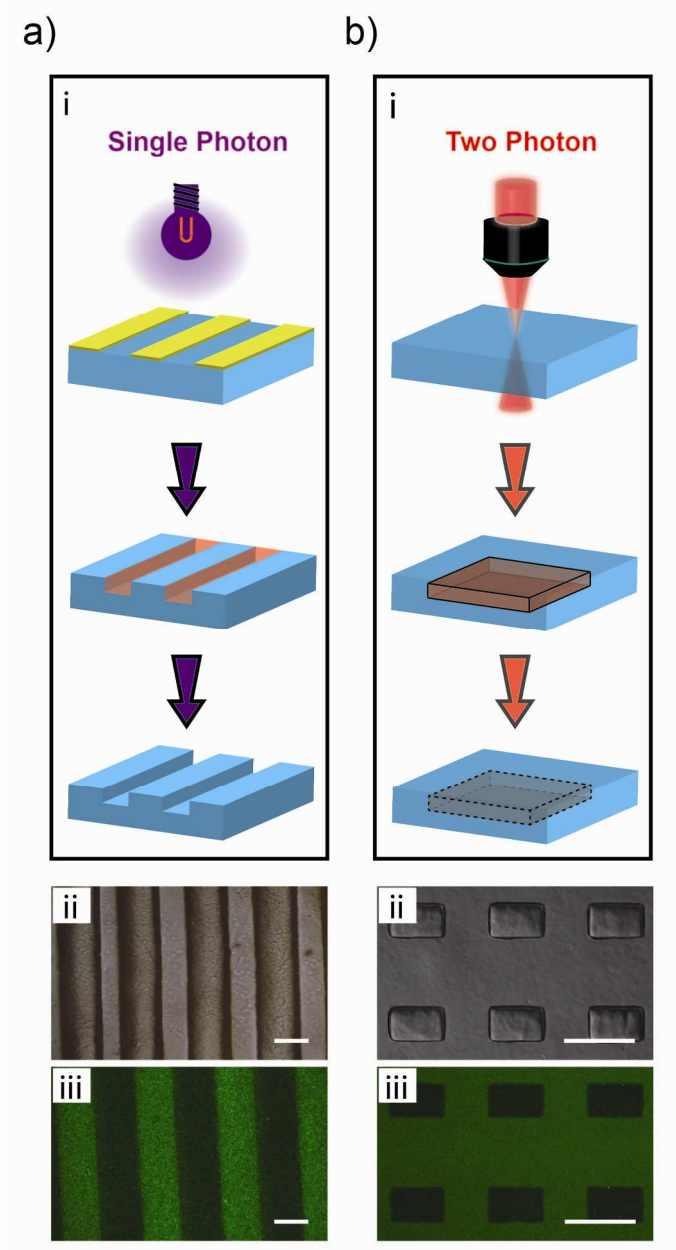

Figure 3. a-i) Microstructures are fabricated on hydrogel surfaces using UV photolithography and single-photon uncaging. Representative (ii) optical and (iii) fluorescent micrographs of the resulting microstructures are shown. b-i) A rectangular array of interior microstructures is fabricated using two-photon uncaging of poly(MAA-*co*-BHCMM)-PEG-poly(MAA-*co*-BHCMM) hydrogels. Representative structures are characterized by (ii) optical and (iii) fluorescent microscopy. Scale bars represent 100 µm in all panels.

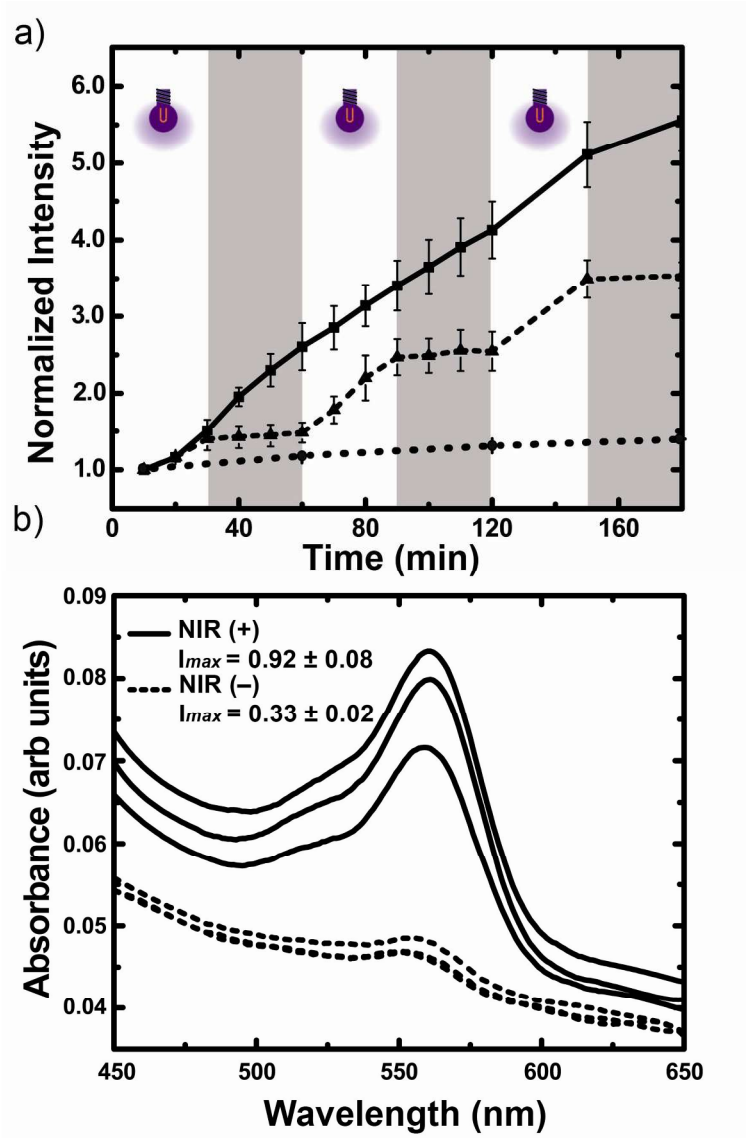

Figure 4. a) Normalized UV-vis absorbance intensity indicates that non-covalently bonded Rhodamine B associated with physical crosslinks is liberated after both continuous (solid lines) and intermittent (dashed lines) UV illumination. Control experiments measured the Rhodamine B release from hydrogels without illumination. These data confirm that that light-induced disintegration of crosslinked domains governs the release of Rhodamine B from the hydrogel networks. b) Absorbance spectra of aqueous solutions co-incubated with Rhodamine B loaded hydrogels (n = 3) after either two-photon laser raster scanning (solid lines) or no irradiation at near-IR wavelengths (dashed lines). Absorbance intensities at λ = 540 nm (A_{540}) were normalized using absorbance values measured at λ = 650 nm (A_{650}) to produce a normalized Rhodamine B intensity $I_{max} = A_{540}/A_{650}$. These data confirm that two-photon uncaging and network disintegration is the primary driving force for Rhodamine B release from the hydrogel.

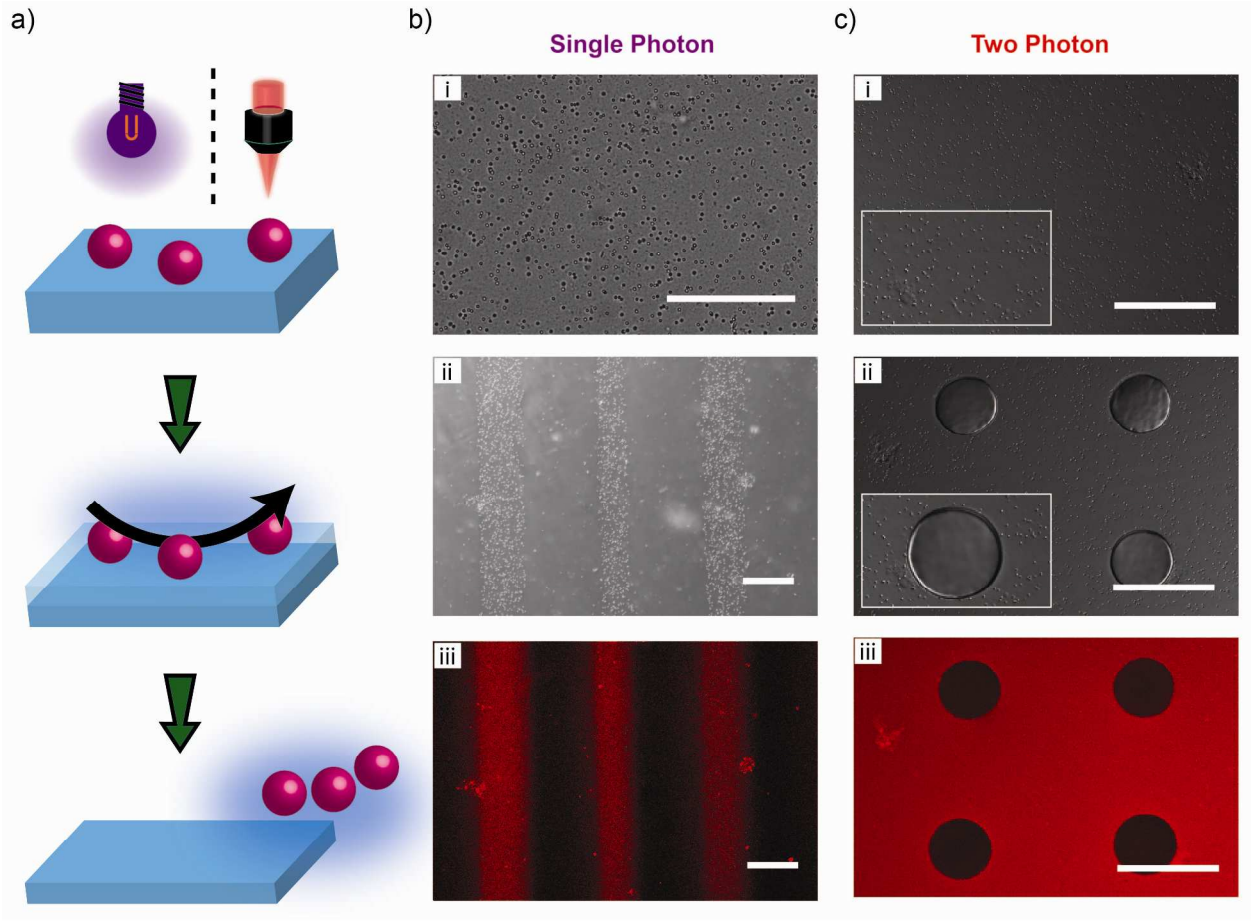

Figure 5. a) Photodegradable hydrogels are capable of remote cleaning. b-i) Fluorescently labeled polystyrene microparticles embedded within the apical surface are shed using a photomask and UV illumination. Selective particle removal is evident from both (ii) bright field optical and (iii) fluorescent microscopy. c) A comparable cleaning mechanism is shown for two-photon uncaging before (i) and after (ii & iii) raster scans. Inset: 1.5X additional magnification. Scale bars represent 100 µm.

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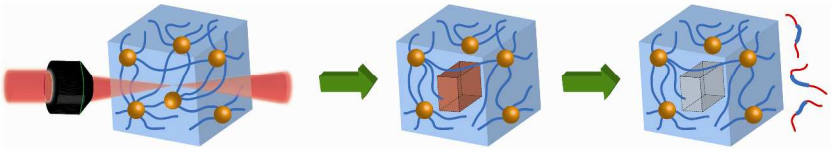

Light-Induced Remodeling of Physically Crosslinked Hydrogels Using Near-IR Wavelengths

Light-induced disintegration of physically crosslinked hydrogel networks has been demonstrated by selective photodeprotection of self-assembled triblock copolymers. Physical crosslinks can be disrupted through light-induced uncaging of coumarin-based blocks using single- and two-photon absorption mechanisms. Photodegradable hydrogels exhibit promising applications as biomedical materials that can be manipulated using light at tissue-transparent wavelengths.