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Photodegradable Supramolecular Hydrogels with Fluorescence Turn-On Reporter for Photomodulation of Cellular Microenvironments

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Supporting Information

ABSTRACT: Photodegradable hydrogels that allow 3D encapsulation of cells are important biomaterials to modulate cellular microenvironments with temporal and spatial resolution. Herein we report a photodegradable hydrogel formed by the self-assembly of short peptides modified with a novel phototrigger. The phototrigger is a biaryl-substituted tetrazole moiety that, upon mild light irradiation, undergoes rapid intramolecular photoclick ligation to form a highly fluorescent pyrazoline moiety. Short peptides linked with a tetrazole-containing moiety, Tet(I) or Tet(II), are able to self-assemble into hydrogels, among which the Tet(I)-GFF and Tet(II)-GFRGD gels show good mechanical strength and biocompatibility for 3D encapsulation and prolonged culture of live cells. The phototriggered tetrazole-to-pyrazoline transformation generates a highly fluorescent reporter and induces the disassembly of the hydrogel matrix by disturbing the balance between hydrophilic interaction and π-π stacking of the self-assembled system. Photomodulation of cellular microenvironments was demonstrated not only for the cells grown on top of the gel but also for stem cells encapsulated inside the hydrogels.

Supramolecular hydrogels that respond sensitively to external stimuli have emerged as "smart" biomaterials.[1] The responses of the supramolecular hydrogels can be induced by disturbing the delicate balance between hydrophobic and hydrophilic interactions of the self-assembled hydrogelators,[2] based on which various stimuli-responsive supramolecular hydrogels have been reported.[3−5] Biocompatible photoresponsive hydrogels are important biomaterials for temporal and spatial control of cellular microenvironments.[6,7] Although photodegradable polymeric hydrogels have been developed for photomodulation of the microenvironments of encapsulated cells,[7] it remains a challenge to construct supramolecular hydrogels with excellent cytocompatibility, good mechanical strength, and sensitive photoresponses to encapsulate live cells and serve as 3D culture media.

Several types of photoresponsive moieties have been used as the photoswitch or phototrigger in the construction of photoresponsive hydrogels. Photoisomerizable moieties have been integrated into hydrogelators as photoswitches to induce reversible photoresponses of the hydrogelators.[8] Synthetic peptides or polymeric backbones have been modified with photoremovable caging functionalities to trigger photodegradation or formation of hydrogels in an irreversible manner.[7,9] Recently, rapid photoinduced ligation of tetrazole with alkenes has emerged as an interesting bio-orthogonal photoclick reaction.[10] Due to the excellent biocompatibility and rapid reaction kinetics of this photoclick reaction, we tried to use it in the construction of biocompatible supramolecular hydrogels with sensitive photoresponses for photomodulation of cellular microenvironments. Here we report novel photoresponsive small-molecule hydrogels formed by short peptides linked with a tetrazole-based phototrigger to disturb the self-assembled hydrogel matrix and induce photodegradation.

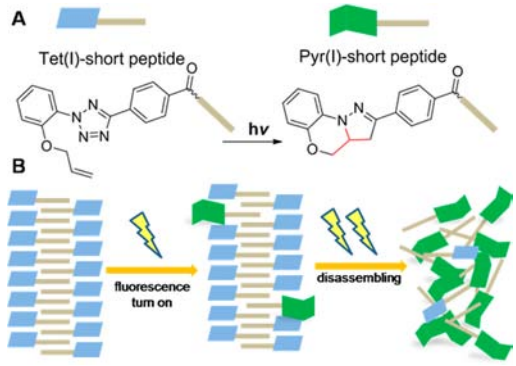

Figure 1. (A) Components of the photoresponsive hydrogelator. (B) Photoresponse of the self-assembled hydrogels.

Figure 1 illustrates the molecular design of the photoresponsive hydrogelator. We used a biaryl-substituted tetrazole with an *o*-allyloxy group on the *N*-phenyl ring (Tet) to link with the N-terminal of synthetic short peptides. Tet has been reported to undergo rapid intramolecular photoclick ligation, leading to the fluorescent pyrazoline cycloadduct (Pyr).[11] Pyr has a slightly tilted tricyclic ring system, which may interrupt the previous π-π stacking among the aromatic Tet moieties. Since a delicate balance between the hydrophilic interaction and π-π stacking promotes the self-assembly of short peptide hydrogelators linked with aromatic moieties such as Fmoc, naphthalene, or pyrene,[12] it is possible for the aromatic Tet-linked short peptides to self-assemble into fibrous network promoted by the balance of π-π stacking of Tet and hydrophilic interaction of the peptide

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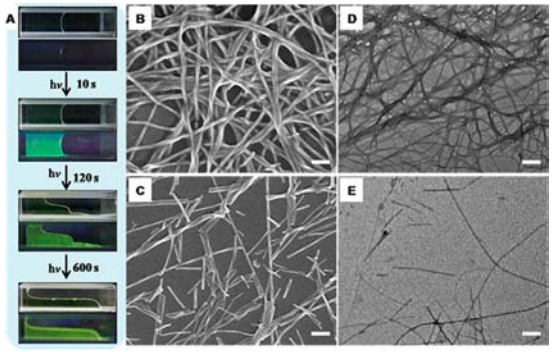

Figure 2. (A) Optical pictures of Tet(I)-GFF gel before and after different times of UV exposure (an 8 W hand-held UV lamp emitting at 302 nm) taken under ambient light and with a UV lamp, respectively. (B,C) SEM images of the Tet(I)-GFF gel before (B) and after (C) 2 min UV exposure. (D,E) TEM images of the Tet(I)-GFF gel before (D) and after (E) 10 min UV exposure. Scale bar = 200 nm.

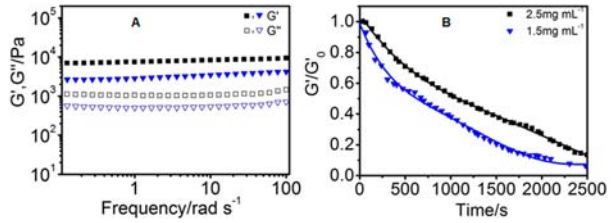

Figure 3. (A) Dynamic frequency sweep of the Tet(I)-GFF gel at 2.5 (squares) and 1.5 mg/mL (triangles) at 1% strain. (B) In situ rheology data of the Tet(I)-GFF gel at different time points upon light irradiation; data were plotted as the relative value of the storage modulus G' to the initial storage modulus G'_0 before light irradiation. The hydrogel samples were subjected to irradiation by reflected light from an omnicure lamp with a 302 nm filter.

backbone. Moreover, the photoinduced Tet-to-Pyr transformation might interrupt the π-π stacking and induce disassembly of the hydrogel matrix, which was also reported by a fluorescence turn-on response due to the formation of the Pyr fluorophore.

We first tried to find an appropriate short peptide sequence that, upon linkage with Tet(I), was able to form supramolecular hydogel at low concentration under physiological pH. Preliminary tests using several peptide sequences with 2−5 amino acid residues, including FF, RGD, GAGAS, etc., to link with Tet(I) showed promising hydrogelation ability of the Tet(I)-linked short peptides (Table S1, Supporting Information). Similar to the short peptides linked with Fmoc, naphthalene, or pyrene,[12] the Tet(I)-linked short peptides easily self-assembled into a fibrous network (Figure S1). Upon addition of one glycine residue in Tet(I)-FF that was insoluble at neutral pH, the resulting Tet(I)-GFF was found to gel water within a wide pH range. At pH ∼6.4, Tet(I)-GFF was able to gel water at concentration as low as 0.4 mg/mL, which was comparable to that of supergelators.[13] At pH 7, Tet(I)-GFF formed clear and stable hydrogel with the lowest concentration ∼0.8 mg/mL.

The photoresponse of the Tet(I)-GFF gel was then tested using a hand-held UV lamp (8 W) emitting at 302 nm as a mild light source. In aqueous solution, Tet(I)-GFF undergoes fast intramolecular photoclick ligation, and complete transformation from Tet(I)-GFF to Pyr(I)-GFF takes <2 min (Figure S2). The hydrogel formed by Tet(I)-GFF in phosphate-buffered saline (PBS) at 0.8 mg/mL was then irradiated by the mild UV light, and its response was recorded (Figure 2A). Fluorescence turn-on response was observed in the hydrogel matrix within 10 s upon light irradiation, indicating the formation of Pyr fluorophore. Photodegradation of the hydrogels did not appear as fast as the fluorescence turn-on response. However, the gel began to collapse within 3 min of UV exposure, and a complete gel-to-solution phase transition was observed within 10 min of UV exposure (see video in the Supporting Information).

We next confirmed that the photoresponse of the Tet(I)-GFF gel was due to the transformation of Tet(I) to Pyr(I). A control hydrogel formed by Fmoc-GFF showed no response upon light irradiation under the same conditions (Figure S3). The transformation of Tet(I)-GFF to the highly fluorescent Pyr(I)-GFF in the gel matrix upon different irradiation times was monitored using HPLC, UV, and fluorescence spectroscopy (Figures S4 and S5). It is estimated that a 3% conversion from Tet(I)-GFF to Pyr(I)-GFF within 10 s was enough to illuminate the gel matrix, while ∼25% and 44% conversion of Tet(I)-GFF in the gel matrix were needed for the collapse and complete phase transition of the gel, respectively, according to the HPLC quantification (Figure S4). It is noteworthy that the fluorescence intensity of the irradiated gel did not show a constant increase with increasing amount of Pyr(I)-GFF, which might be explained by the self-quenching of the Pyr fluorophore formed in the condensed gel matrix.

Photoinduced secondary structure and microstructure changes of the Tet(I)-GFF gel were also observed. Circular dichroism spectra of the gel suggested a continuous decrease in the β-sheet secondary structure with increasing UV exposure time (Figure S6). The freeze-dried sample of the Tet(I)-GFF gel before UV irradiation showed an entangled fibrous network under scanning electronic microscopy (SEM), while the sample of the Tet(I)-GFF gel after 2 min UV irradiation showed broken fibers (Figure 2B,C). Transmission electronic microscopy (TEM) characterization of the sample directly from the gel before and after 10 min UV exposure also showed the degradation of the fibrous network in the gel matrix (Figure 2D,E).

Dynamic frequency sweep of the gels formed by different concentrations of Tet(I)-GFF (1.5 mg/mL and 2.5 mg/mL) showed that the storage modulus (G') of the gels was at the kilopascal level (Figure 3A), which is relatively high for common small-molecular hydrogels[2] and is comparable to those of polymeric hydrogel biomaterials for 3D encapsulation of cells.[14] After different times of exposure to the 8 W hand-held UV lamp, the Tet(I)-GFF gel showed dramatic decrease of G', and the rheology data of the gel after 10 min of irradiation were no longer like those of gels (Figure S7). We also used a rheometer that allows *in situ* photoirradiation with reflected light from an omnicure lamp with a 302 nm filter on the hydrogel tested and dynamic data acquisition to monitor the continuous time course of the G' change of the same gel sample upon light irradiation. As shown in Figure 3B, a continuous decrease on the storage modulus of the hydrogels with photoirradiation was observed, which indicated the gradual degradation of the gel matrix upon light irradiation.

By far, Tet(I)-GFF has emerged as a photoresponsive hydrogelator with superior hydrogelation property and fast responses to mild light irradiation. Meanwhile, MTT tests showed that Tet(I)-GFF was cyto-compatible with different cells, even at high concentrations (Figure S8). Therefore, it is possible for us to explore the potential of the Tet(I)-GFF gel as a

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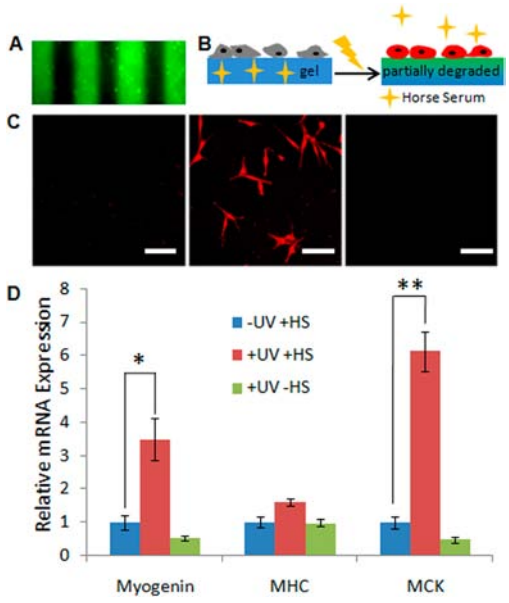

Figure 4. (A) 2D photopatterned fluorescent channels on the Tet(I)-GFF gel. (B) Schematic illustration of photomodulation on cellular microenvironments in a 2D manner through photocontrollable release of horse serum. (C) Fluorescent images of C2C12 cells cultured 48 h on top of different Tet(I)-GFF gels: left, HS-containing gel; middle, HS-containing gel after 2 min UV exposure; right, HS-free gel after 2 min UV exposure. The UV light source was the 8 W hand-held UV lamp emitting at 302 nm. The cells were fixed and immunostained with rabbit polyclonal anti-MyoD antibody, followed with Cy3-labeled goat anti-rabbit IgG secondary antibody. Scale bar = 100 μm. (D) Relative mRNA level of the differentiation markers Myogenin, MHC, and MCK in C2C12 cells with different microenvironments. Data are shown as mean ± SD. Asterisks denote statistically significant differences (*p < 0.05, **p < 0.01) between conditions.

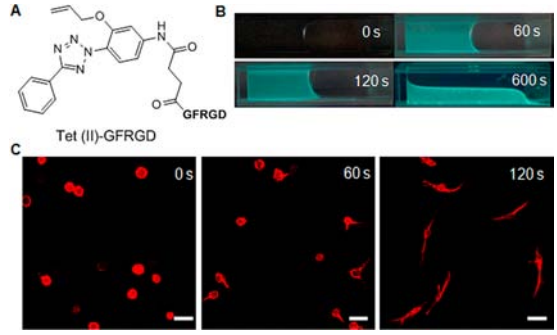

Figure 5. (A) Chemical structure of Tet(II)-GFRGD. (B) Fluorescent pictures of Tet(II)-GFRGD gel upon 0, 60, 120, and 600 s UV exposure. (C) Morphology of hMSCs encapsulated in TetII-GFRGD gels with different times of UV exposure (0, 60, and 120 s under the 8 W hand-held UV lamp emitting at 302 nm) after 36 h of 3D culture. The images were acquired under confocal laser scanning microscopy after fixing and staining the cells. Scale bar = 50 μm.

photodegradable cell culture medium to modulate cellular microenvironments through light irradiation.

Photomodulation of cellular microenvironments using the Tet(I)-GFF gel was first explored in a two-dimensional (2D) manner. It was convenient to pattern different channels on the Tet(I)-GFF gel through a user-defined photomask, and the channels could be distinguished with the fluorescent reporter (Figure 4A). We then used C2C12 cells cultured on top of the photoresponsive hydrogels as the model system because their differentiation is known to be induced by horse serum (HS).[15] HS was trapped inside the Tet(I)-GFF gel, and its release from the gel matrix to the culture medium was modulated through photodegradation of the gel matrix (Figure 4B). We were able to monitor the differentiation behavior of C2C12 cells cultured on different gels by fluorescent microscopy using immunostaining of MyoD protein, which is a differentiation marker (Figure S9). C2C12 cells cultured on top of HS-containing Tet(I)-GFF gels without and with UV irradiation for 2 min showed distinct differentiation behavior (Figure 4C, left and middle panels, respectively). Control cells grown on top of HS-free Tet(I)-GFF gel with UV irradiation showed low levels of differentiation (Figure 4C, right panel), which confirmed that the photomodulation was indeed based on the phototriggered release of HS. Furthermore, we used RT-PCR quantification to compare the relative mRNA level of other differentiation markers such as Myogenin, myosin heavy chain (MHC), and muscle creatine kinase (MCK) in the C2C12 cells cultured for 48 h on top of different gels. As shown in Figure 4D, C2C12 cells cultured on top of HS-containing gel with UV irradiation showed much higher levels of expression of these differentiation markers. The results suggest that it is possible to use Tet(I)-GFF gel containing various functional proteins to photomodulate the microenvironments of cells cultured on top of the gel surface.

Hydrogels with spatially controlled features have been considered as important biomaterials to encapsulate stem cells and investigate basic questions in stem cell behavior.[6,7,14] Thus, we explored the potentials of the Tet(I)-GFF gel for 3D cell culture and photomodulation of the cellular microenvironment in a 3D manner. We found that gels formed by TetI-GFF above 1.5 mg/mL in PBS were mechanically strong enough to encapsulate cells for several days with culture media on top of the gel surface. We then mixed human mesenchymal stem cells (hMSCs) with a viscous solution of Tet(I)-GFF in PBS (1.5 mg/mL). Rapid gel formation within 5 min made it possible to encapsulate the hMSCs inside the gel matrix (Figure S10). The stem cells also showed high viability when encapsulated in the Tet(I)-GFF gel (Figure S11). We were also able to cultivate fluorescent channels inside the hMSCs-encapsulated gel and observe the stem cells inside and outside the photomodulated channels with spatial resolution (Figure S12).

The presence of bioactive short peptide sequences such as IKVAV and RGD in cell microenvironments was reported to have a significant influence on the embedded neuron cells or stem cells.[7,16] Our initial efforts to form a RGD-containing hydrogelator gave Tet(I)-GFRGD, which was able to form an opaque gel at 4.0 mg/mL under neutral pH (Table S1). We then modified the linker between the Tet moiety and GFRGD to get Tet(II)-GFRGD, whose structure is shown in Figure 5A. Tet(II)-GFRGD was able to form a transparent hydrogel at physiological pH with concentration as low as 0.9 mg/mL. The Tet(II)-GFRGD gel also showed sensitive photoresponses, including fluorescence turn-on and photodegradation (Figure 5B), due to the fast intramolecular photoclick reaction leading to Pyr(II)-GFRGD (Figure S13). Characterization and cytocomptatibility tests similar to those described before (Figures S14−S18) showed the promise of the Tet(II)-GFRGD gel as a photodegradable 3D hydrogel for hMSCs encapsulation.

We next encapsulated the hMSCs inside Tet(II)-GFRGD gels (2.5 mg/mL) for 3D culture and tested the potential of using photoirradiation to change the 3D microenvironment for the embedded cells. The hMSC-encapsulated Tet(II)-GFRGD gel

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without UV exposure was quite stable for prolonged 3D culture over a week. After 2 min UV exposure, the same hMSC-encapsulated Tet(II)-GFRGD gel became less durable for long-time 3D culture, but it still allowed a time-course investigation on the embedded cells for more than 108 h (Figure S19). We observed a distinct morphology change of the hMSCs after 36 h culture inside the UV-exposed gel from those cultured inside the intact gel. Figure 5C shows the hMSCs 3D cultured for 36 h in the intact Tet(II)-GFRGD gel and in the gels subjected to 1 and 2 min UV irradiation. Spreading behavior of the hMSCs in the gel with 2 min UV exposure was very obvious, while the stem cells in the gel with 1 min UV exposure showed the initial spreading after 36 h of 3D culture. The different morphology of the hMSCs indicated that the time-dependent photodegradation of the hydrogel matrix had a significant influence on the spreading behavior of the encapsulated stem cells, which is consistent with the distinct behavior of hMSCs 3D cultured inside the polymeric hydrogels with photopatterned channels.[14]

In summary, we demonstrate the first example of using a bioorthogonal photoclick reaction to modulate the self-assembly of a small-molecular hydrogel and realize a sensitive photoresponse. Using biaryl-substituted tetrazole to modify short peptides gives supramolecular hydrogelators Tet(I)-GFF and Tet(II)-GFRGD that self-assemble into hydrogels at low concentrations under physiological conditions. The rapid intramolecular photoclick reaction of the tetrazole moiety turns on fluorescence of the hydrogel matrix instantly and then gradually disturbs the self-assembly of the hydrogelator and induces photodegradation of the supramolecular hydrogels. The potential of this new type of photoresponsive peptides in constructing photodegradable supramolecular biomaterials is demonstrated by the photomodulation of the microenvironment of C2C12 cells cultured on top of the gel or hMSCs encapsulated inside the gel. The additional fluorescence turn-on response of the gels makes them even more attractive as smart biomaterials for spatially defined modulation on cellular microenvironments. Investigation into precise control of the photopatterned channels and different biological behavior of the live cells inside and outside the spatially defined channels is under way in our group using this novel type of supramolecular hydrogel.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplemental figures, synthetic schemes, experimental procedures, characterization of all new compounds, and video showing the gel-to-solution phase transition. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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