Wavelength-Selective Coupling and Decoupling of Polymer Chains via Reversible [2 + 2] Photocycloaddition of Styrylpyrene for Construction of Cytocompatible Photodynamic Hydrogels

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Supporting Information

ABSTRACT: Reversible photocycloaddition reactions have previously been employed in chemical cross-linking for the preparation of biomaterial scaffolds. However, the processes require activation by high-energy UV light, rendering them unsuitable for modification in biological environments. Here we demonstrate that the [2 + 2] photocycloaddition of styrylpyrene can be activated by visible light at $\lambda = 400-500$ nm, enabling rapid and effective conjugation and cross-linking of poly(ethylene glycol) (PEG) in water and under mild irradiation conditions ($I = 20 \text{ mW cm}^{-2}$). Notably, the reversion of the cycloaddition can be triggered by low-energy UV light at $\lambda = 340$ nm, which allows for efficient cleavage of the dimer adduct. Using this wavelength-gated reversible photochemical reaction we are able to prepare PEG hydrogels and modulate their mechanical properties in a bidirectional manner. We also demonstrate healing of the fractured hydrogel by external light triggers. Furthermore, we show that human mesenchymal stem cells can be encapsulated within the gels with high viability post encapsulation. This photochemical approach is therefore anticipated to be highly useful in studies of cell mechanotransduction, with relevance to disease progression and tissue regeneration.

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Photochemical reactions have been widely utilized to control chemical and physical properties of functional materials in a spatial and temporal manner. In particular, the photocleavages of 0-nitrobenzyl and coumarin have been employed to control the degradation of polyethylene glycol (PEG)-based hydrogel networks to probe cell–material interaction in a 3D environment or to trigger the release of therapeutics. Guvendiren et al. fabricated hyaluronic acid (HA)-based hydrogels that can be further cross-linked by light-induced radical polymerization and applied the stiffening to influence the differentiation of human mesenchymal stem cells (hMSC) cultured on these substrates. In a recent report, Rosales et al. combined both methacrylate photoradical cross-linking and 0-nitrobenzyl photocleavage approaches to confer bidirectional changes in mechanical properties of the HA hydrogel, extending the applicability of the light-responsive biomaterials in mechanobiological studies. While these strategies provide spatiotemporal changes in mechanical properties, such changes are irreversible and often generate reactive intermediates such as free radicals or nucleophiles which may participate in biochemical processes. Furthermore, stiffening of the substrate in many cases requires sequential addition of a photoinitiator and/or a cross-linker for light activation. Photosomerization reactions, such as the cis–trans conversion of azobenzene or open–closed ring transition of spiropyran, have been applied as internal photoswitches to modulate hydrogel properties. Although this approach eludes the use of external catalyst and minimizes alteration of the chemical environment within the matrix, the magnitudes of the mechanical changes are minor, with reported values in the 10–100 Pa range of the shear modulus. The photosomerization is also susceptible to thermal fatigue and photobleaching, causing the loss of photoresponsiveness over long periods of incubation typically required for biological studies.

The reversible [2 + 2] or [4 + 4] photocycloadditions are another set of photobased reactions that have been shown to be effective chemical approaches for the construction of light-responsive polymeric materials. These reactions start with the
photoactivation of an olefin into the excited state, which then forms an exciplex intermediate with a ground-state olefin molecule and decays into a cyclic structure. In many instances the reacting olefins are identical, and the process is known as dimerization. This chemistry is highly attractive for tuning the elasticity of biological substrates because both photo-cross-linking and photocleavage do not require additional catalyst, nor do they generate free radical or nucleophiles. The photo-dimerization of some functional groups such as coumarin, anthracene, cinnamoyl, and stilbene has therefore been employed in the fabrication of cross-linked polymer structure. However, most of the dimerization processes require activation by short-wavelength UV light, making them unsuitable to be carried out in biological media and in the presence of living cells. Barner-Kowollik and co-workers recently discovered that dimerization of an electron-rich anthracene group can be triggered by visible light at the wavelength of 410 nm. We were able to employ this reaction for bioorthogonal polymer cross-linking for preparation of bulk hydrogels and microgels and post-conjugation of bioactive molecules under mild irradiation conditions ($\lambda = 400–500$ nm and $I = 20$ mW cm$^{-2}$). Nevertheless, reversion of the anthracene dimer at $\lambda = 365$ nm for hydrogel degradation was unsuccessful. This is because both the anthracene group and its dimer absorb photons at $\lambda = 365$ nm, leading to a photostationary state under irradiation at this wavelength.

In the search for a reversible photocycloaddition of which the addition and cleavage can proceed efficiently at different wavelengths and under biologically benign irradiation conditions, we focused our attention to the diarylethylene derivatives. Kovalenko et al. reported, back in 1980, that replacement of one of the phenyl rings with a pyrene fragment resulted in styrylpyrene product having absorption extending into the visible-light region. Irradiation of the trans-styrylpyrene at $\lambda = 405$ nm in organic solvent led to the formation of both the cis-isomer and [2 + 2] dimer. Doi et al. recently reported that irradiation of the trans-carboxyl styrylpyrene group at $\lambda = 455$ nm in water did not cause isomerization, and the dimer was the dominant adduct, which can be efficiently reversed by irradiation at $\lambda = 340$ nm. In the work presented herein, we report the application of styrylpyrene photocycloaddition for polymer coupling and decoupling under mild irradiation of separated wavelengths. We further prepared hydrogels of which elasticity can be reversibly modulated by different wavelengths under mild irradiation conditions. We also investigated the utility of this chemical approach in a light-induced healing process and encapsulation of hMSCs.

We first synthesized the styrylpyrene carboxylic acid which can be attached to the hydroxyl or amine end group of a polymer chain via carbodiimide coupling. In this work we modified the hydrophilic PEGs with an amine end group via EDC/NHS coupling and first studied the photoreactivity of linear methoxy-PEG-styrylpyrene (molar mass of ca. 2000 g mol$^{-1}$) in aqueous solution (Figure 1a). UV-vis spectrum of 1 displays three absorption bands with the maximum absorbance ($\lambda_{\text{max}}$) of 237, 305, and 385 nm. Compared with general stilbene compounds, the absorption band with $\lambda_{\text{max}} = 385$ nm is bathochromically shifted by 50–70 nm. This is caused by the expanded \(\pi\)-conjugated system from the pyrene structure, which lowers the energy required for the \(\pi\rightarrow\pi^*\) transition. Irradiation of 1 with visible light (400–500 nm) led to a rapid decrease of the \(\pi\rightarrow\pi^*\) absorption bands and a corresponding change in color of the polymer solution (Figure 1b and 1c). Concurrently, we observed an increase of several absorption bands at around 244, 270, 281, 336, and 353 nm, indicating the formation of a photocycloaddition product. Two isosbestic points at 285 and 255 nm were also observed during the irradiation process.

Irradiation of the solution containing dimer product (2) with UV light (340 nm) resulted in rapid conversion of 2 to polymer 1, as indicated by the UV–vis spectra (Figure S9). Both cycloaddition and reversion processes follow first-order kinetics (Figure 1d) with the cleavage having a faster rate than the...
cycloaddition under similar irradiation intensity (20 mW cm\(^{-2}\)). Furthermore, quantum yields of the cycloaddition and reversion, measured using a chemical (potassium ferrioxalate) actinometer reference,\(^3\) are 8.9 \(\times\) 10\(^{-4}\) and 6.7 \(\times\) 10\(^{-3}\), respectively. The fast photo cleavage of dimer 2 may be due to the high absorptivity of this compound at \(\lambda = 340\) nm. In addition, the fluorescent intensity of polymer 1 in solution reduced significantly under visible-light irradiation and could be reversed by UV-light irradiation (Figure S10). The decrease in fluorescent intensity is due to the loss of the absorption band at around 300 nm, and indicates that the reaction proceeded via cycloaddition, not isomerization and photooxidation.\(^2\) Notably, the photocoupling and -decoupling can be repeated over many cycles—in our experiments we undertook 6 cycles of irradiation of alternative wavelengths between 400 and 500 and 340 nm (Figure 1e), with minimal photobleaching or photochemical oxidation of the styrylpyrene group.

To confirm the structural change of the MeO-PEG-styrylpyrene under light irradiation, we performed proton NMR and size exclusion chromatography (SEC) analysis of polymer 1 at the concentration of 1 mM during the time course of the irradiation process. We observed the disappearance of the chemical shifts at around 8.1 ppm, which correspond to the styryl alkene protons, alongside with the appearance of the chemical shifts at 4.4, 5.1, and 5.9 ppm, which are attributed to the protons from the cyclobutyl adduct (Figure 2a). SEC traces of polymer under different irradiation times also show a clear shift to higher molecular weight, indicating the presence of the polymer–polymer coupling product (Figure 2b). It is noteworthy that the low molecular weight shoulder from the polymer product after 10 min of irradiation is not from the incomplete photoaddition but due to the presence of the starting MeO-PEG-OH (conversion of MeO-PEG-OH to MeO-PEG-NH\(_2\) was 92.5%, Figure S4). Similar to UV–vis experiments the dimer product 2 can be reversed back to starting polymer 1 by irradiation with UV light at 340 nm, which is evident in NMR spectra and SEC data (Figures S11 and S12).

To utilize the reversible photocycloaddition of the styrylpyrene in polymer cross-linking for preparation of photolabile hydrogels, we prepared 4-arm PEG-styrylpyrene with the molecular weight of ca. 20 000 g mol\(^{-1}\) (3, Figure 3a) using a synthetic procedure similar to the synthesis of linear MeO-PEG-styrylpyrene. The degree of styrylpyrene conjugation was ca. 92% (Figure S5). Due to the hydrophobic nature of the styrylpyrene group, polymer 3 is fully soluble in water up to a concentration of 8 wt % (4 mM). At concentrations higher than 8 wt %, the solution becomes highly viscous, and some insoluble polymer fractions remain in solution even in the absence of external stimulus. This low water solubility can be a potential drawback when utilizing the photochemical cycloaddition of styrylpyrene in biomaterial applications. Nevertheless, we were able to prepare an aqueous solution of 3 at various concentrations to examine the formation of the cross-linked network by rheological tests. Rapid gelation, as indicated by the crossover between storage modulus (\(G'\)) and loss modulus (\(G''\)), was observed when the polymer solution (\(c = 4\) mM) was subjected to visible light (400–500 nm) irradiation at \(I = 20\) mW cm\(^{-2}\) (Figure 3b). The kinetics of gelation can be tuned by changing the intensity of the light irradiation (Figure S13), and the cross-linking is highly efficient even at the intensity of 5 mW cm\(^{-2}\). We also examined the cross-linking under irradiation at defined wavelengths and found that the process was not efficient at \(\lambda = 365\) nm and \(\lambda \geq 420\) nm (Figure S14). This is correlated to the UV–vis absorption of the PEG-styrylpyrene and suggests that the irradiation wavelength for efficient [2 + 2] cycloaddition of the end group is in the range of 390–410 nm.

Complete dimerization, marked by a storage modulus reaching a plateau, happened within 5 min of visible-light irradiation at \(I = 20\) mW cm\(^{-2}\). The \(G'\) value for 8 wt % gel was 6.8 ± 0.2 kPa, which decreased with decreasing concentration of the polymer in the gelling solution (Figure 3c). Notably, cross-linking still happened efficiently at \(c = 1\) mM with the equilibrium \(G'\) value of 0.5 ± 0.1 kPa. Previous reports on photocycloaddition of stilbene- or anthracene-conjugated polymers\(^5,46\) indicate that dimerization does not proceed in diluted conditions because the lifetime of the excited molecules is too short to interact with the ground-state molecules.\(^41\) Our results suggest that the photoexcited styrylpyrene is stable enough for interaction with a partner group from the other polymer chain end. The high cross-linking efficiency at low concentration may also be due to the bulky nature of the styrylpyrene group which prevents intramolecular interaction of the PEG chain ends. This photochemical reactivity can be highly advantageous in polymer synthesis such as the preparation of amphiphilic block copolymers\(^40\) utilizing different reactivity of the stilbene derivatives.

Treatment of the cross-linked polymer solution with UV light (340 nm, \(I = 20\) mW cm\(^{-2}\)) led to the photocleavage of the cyclobutyl group and rapid degradation of the hydrogel, characterized by the decrease in storage modulus (Figure S15 and Figure 3d). The degree of degradation is highly dependent...
on the polymer concentration, with higher degradation efficiency at lower polymer concentrations. In particular, hydrogel with $c = 1$ mM showed a 95% loss of the $G'$ value, while only 7.5% loss of the $G'$ value was observed for hydrogel with $c = 4$ mM. This may be because the styrylpyrene also absorbs light at $\lambda = 340$ nm; thus, a high concentration of the reacting group leads to faster formation of the photostationary state.

The wavelength-gated photocuring and photodegradation could be repeated several times for hydrogel at low polymer concentration ($c = 1$ mM, Figure 3f). At higher concentration, the photodegradation is less efficient at subsequent cycles ($c = 3$ mM, Figure S16). This is because the polymer solution was under static conditions and photo-cross-linking also induced polymer chain entanglements which enhance the establishment of a photostationary state under UV-light irradiation. Nevertheless, our experiment demonstrates that the modulus of the hydrogels can be tuned in a bidirectional manner using either visible light or UV light. Taking advantage of the reversibility of this photoreaction, we undertook experiments in which a fractured hydrogel was subjected to UV-light irradiation followed by visible-light irradiation (Figure 3e) to induce self-healing. The broken parts were seen to adhere together, confirming the healing process. Treatment of the fractured gel with only UV- or visible-light irradiation did not cause healing of the material. Hydrogels were also observed to be stable in PBS pH 7.4 solution at 37 °C for 7 days, with no noticeable change in the gel mass.

To assess the utility of the photochemical reactivity of styrylpyrene in biomaterials engineering, we carried out encapsulation of hMSCs within the gel network under visible-light illumination. Live/dead staining after 24 h postencapsulation shows high cell viability (92.7% ± 3.1%, Figure 4), indicating that the cross-linking process and the chemical components of the hydrogel are nontoxic to hMSCs. Treatment of cell-laden gel ($c = 2$ mM) with irradiation at $\lambda = 340$ nm did not cause any noticeable cell death (Figure S17). At lower concentration ($c = 1$ mM), hydrogels could be completely degraded under UV-light irradiation to release cells that display cell viability and morphology similar to those cultured on a tissue culture plate. Because the hydrogel is composed of only PEG with no cell attachment sequence, the...
viability of encapsulated cells decreased significantly after 3 days of culture (Figure S18). Since the styrylpyrene group can be efficiently conjugated via carbodiimide coupling, we expect that cell binding ligands may be modified to contain this group for incorporation into the PEG gels for prolonged cell culture in mechanotransduction study.

In conclusion, we report the wavelength-gated coupling and decoupling of polymer via reversible photocycloaddition of the styrylpyrene end group. The coupling can be undertaken under irradiation of visible light and low intensity, making it highly applicable for biomaterials engineering. The process can be efficiently reverted using UV light at $\lambda = 340$ nm. We demonstrate the utility of this photochemical process in the preparation of biocompatible hydrogels with mechanical properties that can be photomodulated. Furthermore, the reversibility of this reaction can be used for light-induced self-healing of hydrogel materials. We believe this photochemical reaction will be highly useful in bioengineering applications, particularly in the field of mechanobiology for studies of cell–materials interaction and tissue regeneration.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacrolett.7b00099.

Experimental procedures, NMR spectra of the synthesized compounds, additional UV–vis spectra, fluorescence spectra, additional rheology data, and cell culture conditions (PDF)

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The manuscript was written through contributions of all authors.

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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