



Cite this: *J. Mater. Chem. C*, 2023, 11, 2826

Received 10th December 2022,  
Accepted 23rd January 2023

DOI: 10.1039/d2tc05270a

rsc.li/materials-c

## Construction of gel networks via [2+2] photocycloaddition

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Gel networks could be achieved *via* the photocycloaddition and cross-linking of olefin moieties in branched polymers. The formed cyclobutane unit made the bulky gel photoreversible, which shows potential for promising applications in self-healing, drug release and extracellular matrices. Related research on gel networks formed *via* [2+2] photocycloaddition was retraced and the potential utilization of visible light catalysis was also conceived.

The [2+2] photocycloaddition of two C=C bonds is a versatile tool to access carbocyclic compounds.<sup>1–7</sup> In the reaction, one olefin is excited to its first excited singlet state or successively to its first excited triplet state, and then two new bonds are formed at both carbon atoms of excited olefins by another olefin to obtain cyclobutane products in a single operation.

For its convenience, [2+2] photocycloaddition plays a predominant role in total synthesis.<sup>8–12</sup> Moreover, it also attracts materials scientists' attention in the field of photoresponsive polymer gels<sup>13–18</sup> owing to its intrinsic reversible property controlled by different wavelengths of UV light or by high temperatures. Usually, a longer wavelength of UV light causes the cycloaddition of olefins, while a shorter wavelength and high temperature induce reversion (Scheme 1).<sup>19,20</sup>

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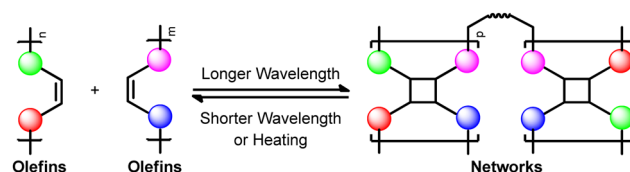
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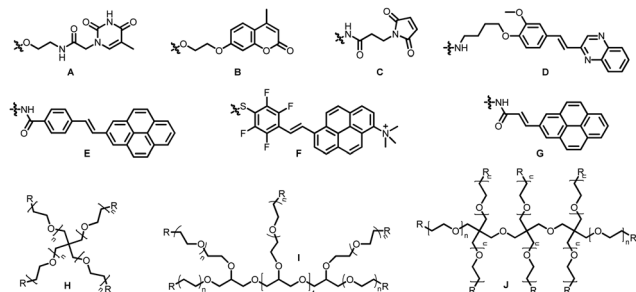
Since gel networks built by full-fledged chemical covalent cross-linking or physical noncovalent interactions<sup>21–32</sup> have been widely used in the field of biomaterials science and biomedical materials,<sup>33–37</sup> the investigation of the new type of network *via* [2+2] cycloaddition is still on the way, and some seminal work emerged one after another, most of which were focusing on the modification of the monomer structure, reaction condition and biocompatibility. In this perspective, we will discuss the construction of gel networks *via* [2+2] photocycloaddition and the possible applications in biology, and present a brief future direction about the topic.

In 2013, Yang and coworkers<sup>38</sup> demonstrated a novel multi-responsive hydrogel based on polyacrylamide-functionalized thymine derivatives (Fig. 1A). Under the irradiation of 365 nm wavelength light, a polyacrylamide-based hydrogel was obtained *via* the [2+2] photocycloaddition of two thymine monomers linked by a polyacrylamide chain. Furthermore, the gel transformed to a solution because of the thymine dimer's photocleavage upon UV-light irradiation ( $\lambda_{\text{max}} = 240 \text{ nm}$ ).

Zhong, Xing and co-workers<sup>39</sup> reported a photoreversible self-healing and cytocompatible hydrogel system. The photo active unit is polyacrylate-modified coumarin (Fig. 1B), which induced cycloaddition to generate gels and exhibited excellent self-healing ability at wavelengths of 365 nm and 254 nm. Besides, it possesses high mechanical strength after healing for 60 min with a stress intensity of  $2 \times 10^5 \text{ Pa}$ , and the



Scheme 1 Construction of gel networks via 2+2 photocycloaddition.



**Fig. 1** Reactive substrates and branched linking polymers. (A) Thymaine moiety; (B) Coumarin moiety; (C) Maleimide moiety; (D) Styrylquinoxaline moiety; (E) Styrylpyrene moiety; (F) 1-trimethylammonium-6-pentafluorystyryl-pyrene moiety; (G) Arylamidylpyrene moiety; (H) Four-arm PEGs; (I) Eight-arm PEGs reported by Frisch, Truong, Kowollik; (J) Eight-arm PEGs reported by Kowollik group.

elongation at break is over 96%. Furthermore, a poly amidoamine crosslinker is used to enhance cell compatibility and the referred gels would regulate bone marrow stromal cell differentiation.

Schlierf and co-workers<sup>40</sup> utilized a two-photon [2+2] cycloaddition reaction of maleimide groups (Fig. 1H, 4R = 4C) to modify surfaces with precision in the submicrometer scale, and optimal conditions of maximizing the structure at minimal energy deposition for potential make it possible to access bottom-up hydrogel structures with fiber dimensions below 350 nm, mimicking aspects of the extra-cellular matrix, which is a promising method from single-cell *in situ* encapsulation to micrometer precision incorporation of signaling molecules for the differentiation of cells.

Truong, Fosite and co-workers<sup>41</sup> reported photolabile hydrogels utilizing the reversible [2+2] photocycloaddition by 4-arm PEG-SP ( $M_w = 20\,000\text{ g mol}^{-1}$ , SP = styrylpyrene) (Fig. 1H, 4R = 4E), and the cross-linked gel could maintain its morphology up to a concentration of 8 wt% (4 mM) in water with a storage modulus value of  $6.8 \pm 0.8\text{ kPa}$ . Remarkably, a low concentration ( $c = 1\text{ mM}$ ) does not affect the cross-linking process, and the formed gel gives a  $G'$  value of  $0.5 \pm 0.1\text{ kPa}$  upon UV light excitation at 340 nm. The network collapses since the cleavage of the cyclobutane unit in the network and lower concentration would cause a higher degradation efficiency and effective repeatable photocuring–photodegradation. However, photodegradation shows less efficiency at subsequent cycles at higher concentrations.

Besides, the obtained gels were compatible in a PBS buffer solution for one week at 37 °C. They can encapsulate human mesenchymal stem cells, which would apply to disease progression and tissue regeneration.

Later, based on the above-mentioned task, Frisch, Kowollik and Truong reported a four-arm PEG-AP (Fig. 1H, 4R = 4G, AP = arylamidylpyrene) and a hybrid with styrylpyrene (Fig. 1H, 4R = 2E + 2G)<sup>42</sup> to undergo [2+2] cycloaddition to get the indicated cross-linked polymers under irradiation at 455 nm. The hybrid PEG consisting of two arylamidylpyrene and two styrylpyrene is mainly detected. Then, four-arm-SP-AP-PEG

stiffens and shrinks at  $\lambda_{\text{max}} = 455\text{ nm}$  and  $\lambda_{\text{max}} = 420\text{ nm}$  one after another but shows no harm to cells.

In 2020, Walther and coworkers in collaboration with Frisch and Kowollik designed a new kind of photodynamic crosslinking unit, 1-trimethylammonium-6-pentafluorystyryl-pyrene,<sup>43</sup> which can perform [2+2] photocycloaddition upon visible light irradiation at 470 nm in water. Besides, A pentafluoro moiety makes molecule modular and easy access to the functionalization by thiolated macromolecules *via* nucleophilic aromatic substitution. Thus, a four-arm-PEG-SH-modified qStyPy, named sPEG-qStyPy ( $M_n = 23\,110\text{ Da}$ ) (Fig. 1H, 4R = 4F) in the original article, was synthesized after brief investigation of qStyPy in water. The association of given sPEG-qStyPy is based on reversible supramolecular interactions *via* hydrophilic ammonium and hydrophobic styrylpyrene. The former enables homogeneous distribution upon irradiation of a fiber-coupled 470 nm LED. The cross-linked hydrogels are elastic and viscous in accordance with the storage value  $G'$  and the loss modulus  $G''$ , and the stiffness of the hydrogels increased smoothly as the irradiation time reached 20 h. The amplitude sweep shows an elastically cross-linked network with a yield point at a strain amplitude of  $\gamma_y \approx 250\%$  and a flow point at  $\gamma_y \approx 590\%$ , and the frequency sweep suggests sacrificial supramolecular cross-links *via* sticky- $\pi$ - $\pi$  interactions playing a key role in the network apart from the covalent crosslinks by [2+2] cycloaddition. However, the positive charge and extended  $\pi$ -system make it possible to intercalate into DNA. The potential toxicity needs further investigation.

Frisch, Truong, Kowollik and co-workers reported [2+2] photocycloaddition upon the green light illumination of a halochromic system dependent on a styrylquinoxaline unit,<sup>44</sup> and the “on” and “off” state of the process could be switched by adjusting the pH of the system. Further investigations indicated that the water solution gives higher conversion and a longer activation wavelength than acetonitrile under indicated conditions. Then, eight-arm PEG-functionalized styrylquinoxaline ( $20\,000\text{ g mol}^{-1}$ ) (Fig. 1I, 8R = 8D) was synthesized to undergo [2+2] cycloaddition between 400 nm and 510 nm, in which hydrogels were formed with a range of mechanical properties and pH responsiveness.

The light of  $\lambda = 420\text{ nm}$  and  $450\text{ nm}$  present the most effective gelation rate among multiple irradiation processes at different wavelengths under the same irradiation intensity. If one of the two parallel tubes of polymer solution ( $c = 5\text{ mM}$ , thickness = *ca.* 1 cm) was irradiated at 425 nm or 405 nm, and the other at 455 nm, the thickness of the obtained gels is 0.2 cm and 1 cm after 1 h of irradiation respectively. Cell viability studies of hydrogels containing encapsulation fibroblasts (L929) highlight the possibility of the gel in cell biology.

Besides, the decreased pH would slow down the crosslinking rate and could entirely suppress the process at pH = 1, yet the reactivity recovered when the pH was adjusted to neutral or basic. In addition, an enlarged concentration of the polymer brings an exponential increase in stiffness from 1 mM to 10 mM, giving the modulus values from 0.5 kPa to 40 kPa.

On the basis of the above-mentioned achievement, the Kowollik group intensively developed a  $\lambda$ -orthogonal photochemical

system<sup>45</sup> for the design of hydrogels, based on a halochromic styrylpyrido[2,3-*b*]pyrazine moiety (SPP) and a non-halochromic acrylamidylpyrene function (AAP), and its stiffness can be independently adjusted by the green light or blue light (Fig. 1J, 8R = 8D and 8R = 4D + 4G).

The photoreactivity of SPP is pH dependent, thus the acidic environment will inhibit cycloaddition. By using a spiropyran-based photoacid generator with an appropriate absorption wavelength, the activation wavelength of the SPP moiety was limited to the green light region (520–550 nm), thereby realizing the photoactivation of the AAP group.

Above all, [2+2] cycloaddition processes are indispensable in each published work, and play a key role in cross-linking *via* the formation of multiple cyclobutane units; otherwise, it is unable to form gel networks.

## Summary and outlooks

In the synthesis of natural products and some cage-like compounds, [2+2] photocycloaddition chemistry has achieved great success for decades. Because of its convenience to construct two bonds concurrently with high efficiency, [2+2] photocycloaddition is increasingly emerging as a versatile tool for the construction of gel networks. The reversible process makes it photoresponsive with promising applications of self-healing materials, actuators, drug release, and extracellular matrices, though drawbacks accompanied the merits. For example, most of the gelation processes of cycloaddition are conducted upon UV light excitation, which is harmful to human bodies. However, the reaction conditions were not invariable; it all depends on the excited-state energy of distinct olefins, some  $\pi$ -extended and heteroatom-modified molecules could react at a red-shift wavelength. Though these subsequently developed strategies can fill the gap, the functional groups might be limited and toxic to tissues and the reversible reaction upon directly irradiation might be inefficient since the cleavage of carbon-carbon single bonds need high energies, which made the other functional groups fragile. In addition, the isomers of Head-to-Head, Head-to-Tail, *syn*, *anti*, *cis* and *trans* would bring difficulties to characterizations and poor reproducibilities to the transformations.

With the development of photochemistry, [2+2] cycloaddition upon visible light catalysis<sup>46–51</sup> of two olefins can occur mildly and smoothly *via* single-electron transfer and energy transfer.<sup>52–71</sup> Compared with the traditional [2+2] photocycloaddition, visible light-induced photocatalytic reactions occur under extremely mild conditions, with most reactions proceeding at room temperature and highly reactive radical initiators were hardly used. The light source is typically a commercial household light bulb rather than the specialized equipment required for processes employing high-energy UV light. Additionally, since most organic molecules generally do not absorb visible light, there are little potential possibilities for deleterious side reactions that might arise from the direct photoexcitation of the substrate itself. Finally, catalyst loadings in photocatalysis may be at a very low level.

Under the circumstances, the biocompatible and bio-applicable photocatalysis process was further investigated *in vitro* and in cellulo.<sup>72–82</sup> However, photocatalyzed [2+2] reactions were not developed in this field, nor the formation of gel network, probably due to the difficulties in the removal of the photocatalyst and some potential additives. As a result, the inductions of bio-compatible catalysis and leaving groups in the system would be crucial and green process of visible light photocatalyzed [2+2] will take a good effect in the construction of gel networks and the applications in biomedical science.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

This research was financially supported by Guangdong Basic and Applied Basic Research Foundation (No. 2019A1515110216) and Post-doctorate Scientific Research Fund for staying at Shenzhen (K21217531).

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