Organic & Biomolecular Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Journal Name

ARTICLE

Multi-responsive Supramolecular Hydrogels Based on Merocyanine-peptide Conjugates

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Stimuli-responsive hydrogels are "smart" materials with diverse applications. We now report short peptide conjugates with merocyanine (MC) that are able to form stimuli-responsive hydrogels. Systematic investigation reveals that merocyanine is a highly effective promoter for the self-assembly of its oligopeptide conjugates. Hydrogels formed by MC-peptide conjugates showed responses towards light and heat, and their sol-gel phase transition could be manipulated by the reverse photochromism of the corresponding spiropyran moiety. Impressively, a **MCI**-RGD conjugate formed supramolecular hydrogel with responses to multiple stimuli, including visible light irradiation, pH change and the presence of Ca^{2+} ions. Erasable photo-lithographon the **MCI**-RGD hydrogel was demonstrated using visible light to write and heat-and-cool treatment to erase for multiple rounds without siginificant loss of sensitivity.

Introduction

Hydrogels with responses to external stimuli are known as "smart" materials[1] that have diverse applications in the engineering of biosensors,[2] controlled delivery of bioactive molecules,[3] tissue engineering,[4] etc. The construction of various stimuli-responsive hydrogels based on supramolecular interaction has been of great interest in the past decade.[5] Peptide conjugates are promising components in stimuli-responsive supramolecular hydrogels because of their ablility to mimic biologically functional peptides and to interact selectively with biological species.[6] For example, peptide conjugates containing D-Ala-D-Ala sequence were found to form hydrogels with responses to vancomycin due to the biomimetic ligand-receptor interaction.[7] Hydrogels containing biomimetic peptide scaffolds susceptible to protease cleavage were able to response to specific enzymes.[8] Hydrogel matrix coated with cell-adhesive peptides such as RGD is known to regulate the spreading or differentiation of cells.[9] Recently, we reported a supramolecular hydrogel formed by diaryl tetrazole modified GFRGD peptide, which has successfully been used to regulate the migration behavior of the stem cells encapsulated inside the hydrogel matrix.[10]

Photo-responsive hydrogels are special biomaterials whose functions are subject to spatio-temporally resoluted regulation through light irradiation.[11] The construction of supramolecular hydrogels that respond to light irradiation is therefore of current research interest.[10, 12-13] Photo-switches or photo-triggers integrated in various molecular hydrogelators include photo-removable caging functionalities,[13] photo-isomerizable moieties[14-16] and photo-linkable ligation substrates.[10] Pentapeptides conjugated with hydrophobic moieties such as fluorenylmethyloxycarbonyl (Fmoc), pyrene and naphthelene that possess aromatic-aromatic interactions have been reported to form nanofibers and supramolecular hydrogels.[17] However, none of these aromatic moieties was able to promote the self-assembling of the RGD tripeptide. We speculate that the self-assembly of RGD is possible when linked to aromatic moieties with stronger π-π interaction than those used in existing conjugated systems.

Merocyanine (MC) is the ring-open form of spiropyran (SP) and has a strong tendency to form aggregate-like structures.[18] The photo-isomerization between SP and MC has been applied to the construction of photo-switchable fluorophores,[19] light-driven proton pumps,[20] photo-responsive molecular engines[21] and other dynamic materials.[22] Our previous work demonstrated that the MC-D-Ala-D-Ala conjugate was able to form hydrogel with dual responses.[15] Here, we report our systematic investigation on the hydrogelation properties of MC-peptide conjugates. Our results demonstrated that MC is a highly effective promoter for the self-assembly of its oligopeptide conjugates (Fig. 1).

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

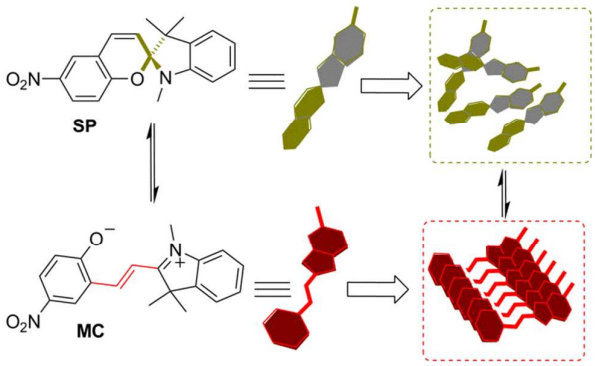
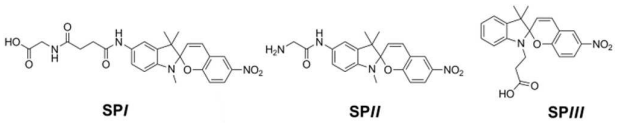


Fig. 1 The generation and self-assembly of merocyanines generated from the reverse photochrism of spiropyrans.

Results and discussion



Scheme 1 Chemical structures of **SP*I*-SP*III***.

To prepare MC-peptide conjugates, we started from the synthesis of SP derivatives **SP*I*-SP*III*** (Scheme 1), which contain linkers of various lengths with free carboxylic group or amine group for further conjugation to oligopeptides. These linkers were designed to avoid possible influence of amino acid side chain on the stacking of merocyanine moieties by separating SP building blocks and oligopeptides. Compounds **SP*I*** and **SP*II*** were synthesized by direct coupling of 5-nitro-salicylaldehyde to corresponding 2-methyleneindole derivatives, whereas **SP*III*** was prepared according to a previously reported method (Fig. S1†). [23] These SP building blocks were then conjugated to either the N-terminal (**SP*I*** and **SP*III***) or C-terminal (**SP*II***) of synthetic oligopeptides.

Reverse photochromism of spiropyrans leading to merocynines

The transformation of SP- to MC-peptide conjugates is crucial for their hydrogelation. UV irradiation is generally employed to initiate the transformation from SP to MC form;[24] however, it only exhibited limited efficiency for large scale transformation of MC-peptide conjugates in our system. Therefore, we firstly optimized the conditions for reverse photochromism of **SP*I*-SP*III*** in aqueous solution.

We found that the reverse photochromism of SP could be effectively promoted by heating. When a solution of **SP*I*** (0.1 mg/mL) in PBS buffer (pH 7.4) was heated from 20°C to 80°C, an increase of the absorbance at 502 nm was observed by UV-Vis spectrometry, indicating the accumulation of MC form (Fig. 2). Such conversion from **SP*I*** to **MC*I*** was further quantified by high-preformance liquid chromatography (HPLC). Results showed that the highest conversion of **SP*I*** to **MC*I*** was achieved at 70°C with about 80% of the compound in **MC*I*** form (Fig. S2D†). The ring-opening reaction was kinetically fast and the conversion to **MC*I*** was completed within 3 minutes (Fig. S3†). Continued increase of temperature to 90 °C did not drive the equilibrium further towards the MC form, but lead to degradation of **SP*I*** (Fig. S2B†, S2C†). The reverse transformation to **SP*I*** was realized by exposing **MC*I*** to sunlight for only 0.5 min. Such heat-light regulated isomerization was highly reversible and could be repeated more than 5 cycles (Fig. S4†). Similarly, compounds **SP*II*** and **SP*III*** exhibited similar reversible response to heat and light stimuli (Fig. S5-S8†).

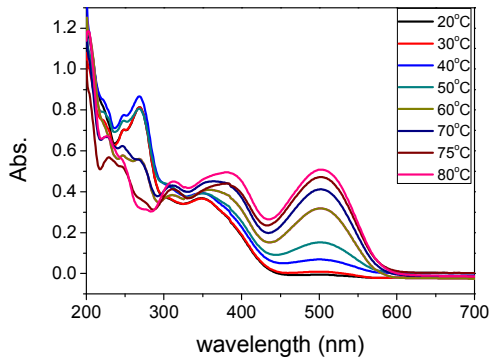
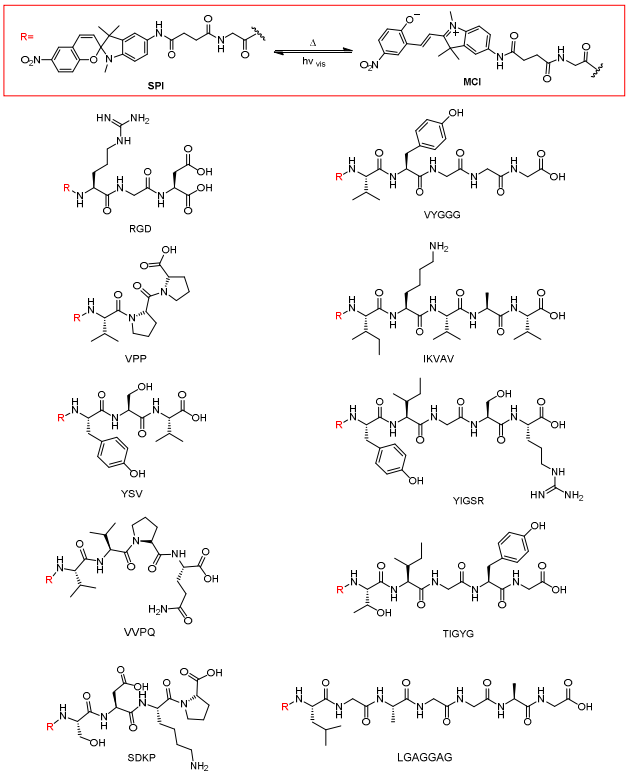


Fig. 2 Temperature-dependent changes in UV/Vis spectra of **SP*I*** (0.1 mg/mL) in PBS buffer, pH 7.4.

Hydrogelation of MC-peptide conjugates

Based on the efficient heat-induced transformation of SP to MC in aqueous solution, we proceeded to investigate the hydrogelation properties of various MC-dipeptide conjugates (Table S1†, S2†). Upon heating, the suspension of SP-dipeptides was turned into a dark red solution of MC-dipeptides. We then found that after cooling to room temperature slowly under appropriate pH, **MC*I*-** and **MC*II*-**dipeptide conjugates were able to self-assemble into hydrogels with fibrous network (Fig. S9-10†). Also, rheology data showed classic properties of hydrogel (Fig. S11-12†). However, none of the **MC*III***-dipeptide conjugates was able to self-assemble into hydrogel, indicating that the position on the MC moiety that oligopeptides were conjugated to played a important role to the hydrogelation ability of the resulting MC-peptide conjugates.



Scheme 2 Chemical structures of bio-functional oligopeptides conjugated with **SP*I* / MC*I***.

Encouraged by the success of MC-dipeptide hydrogelation, we proceeded to prepare **SP*I*** conjugates with a variety of bio-functional oligopeptides of 3-7 amino acids (Scheme 2). Among these oligopeptides, VPP is the milk casein derived tripeptide that modulates monocyte adhesion to vascular endothelium;[25] YSV is known as tyroservatide that inhibits tumor invasion and metastasis;[26] VYGGG exhibits inhibitory effect in binding monoclonal antibody $_{10}D_{11}$;[27] IKVAV and YIGSR are small laminin peptides that promote neurite outgrowth;[28] LGAGGAG has been reported to stimulate messenger RNA and cytokine production in fibroblast tissue cultures.[29] In a typical assay, aqueous suspensions of **SP*I***-peptide conjugates were first heated to homogenous solution by completing the transformation into **MC*I***-peptide conjugates. Followed by a slow cooling treatment, all **MC*I***-peptide conjugates formed dark-red hydrogels under such treatment at appropriate pH (Table 1), indicating that MC is a highly effective promoter for the self-assembly and gelation of oligopeptides. The resulting hydrogels disassembled and yielded yellow slurry when **MC*I*** was transformed back to **SP*I*** by intense visible light irradiation. The reversible self-assembly of **MC*I***-oligopeptide conjugates regulated by heat and light parallels to the transformation between **SP*I*** and **MC*I*** moieties, indicating that the π-π stacking interactions between planar MC moieties are the major driving force for the hydrogel assembly. Furthermore, results suggested that the optimal pH for gelation of **MC*I***-oligopeptide conjugates correlated with the hydrophobicity of peptide sequences, but barely relevant to the length of the oligopeptide attachments. For example, **MC*I***-IKVAV and **MC*I***-YIGSR, which were positively charged under neutral conditions, were able to gel water under neutral or slightly acidic pH. In contrast, MC conjugates composed of highly hydrophobic residues, such as **MC*I***-LGAGGAG, only formed hydrogel under strong acidic conditions (Table 1).

Table 1 Hydrogelation properties of **MC*I***-peptide conjugates.

Peptide Conjugates	pH[1]	C_{min}(mg)[2]
MC*I*-RGD	5.2	3.6
MC*I*-VPP	4.2	7.0
MC*I*-YSV	5.4	9.5
MC*I*-SDKP	4.6	8.1
MC*I*-VVPQ	4.0	6.5
MC*I*-IKVAV	6.0	4.0
MC*I*-YIGSR	6.8	8.3
MC*I*-TIGYG	5.2	2.9
MC*I*-VYGGG	4.6	3.2
MC*I*-LGAGGAG	3.0	3.4

[1]Optimal pH for hydrogelation; [2]minimum concentration for hydrogelation under the appropriate pH.

The light/thermal induced phase transformation between **SP*I***-RGD and **MC*I***-RGD was further analyzed by rheology and characterized by the viscoelastic properties of its resulting hydrogel (Fig. S13†). The value of the storage modulus (G') and the loss modulus (G'') slightly decrease with the increase of strain. The value of G' exceeded that of G'' by nearly 5 times, indicating that the sample was a hydrogel. Dynamic frequency sweep was employed to examine the **MC*I***-RGD hydrogel with the strain amplitude at 1%. The storage modulus and loss modulus increased when the frequency was raised from 0.1 to 100 rad/s. The value of G' was about four times larger than that of G'' in the whole range (0.1-100 rad/s), suggesting that gel was tolerant to external force. Upon visible light irradiation, the storage modulus of the **MC*I***-RGD hydrogel decreased dramatically from 300 Pa to 2.3 Pa (Fig. S13C†), indicating the hydrogel disassembly. Hydrogel could be formed again from the resulting aqueous suspension after heating/cooling treatment with the storage modulus back to the starting level. Such transformation could be repeated for up to 3 cycles without observable loss of gelation ability (Fig. S13C†).

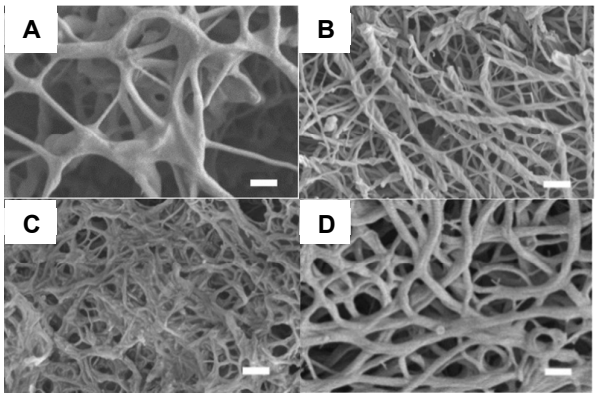
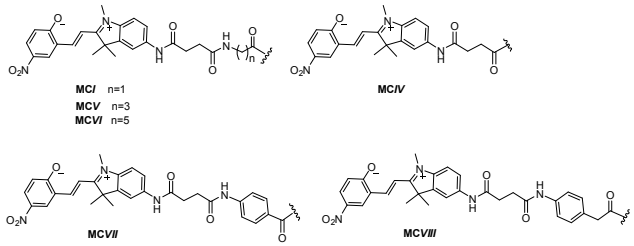


Fig. 3 The scanning electron micrographs of the hydrogels formed by (A) **MC*I***-RGD(4.0 mg/mL, pH 5.2), (B) **MC*I***-VVPQ(5.0 mg/mL, pH 4.2), (C) **MC*I***-YIGSR(10.0 mg/mL, pH 6.8), (D) **MC*I***-LGAGGAG(2.5 mg/mL, pH 3.0). Scale bar = 500 nm.

To further characterize the hydrogels generated from MC-oligopeptide conjugates, we employed scanning electron micrograph (SEM) to analyze the cryo-dried gel samples formed by the **MC*I***-oligopeptide conjugates. Results revealed that these hydrogel matrixes of **MC*I***-oligopeptides were constituted by fibrous network microstructures (Fig. 3). Interestingly, the nanofibers in the **MC*I***-VPP gel matrix are in right-handed helical micro-structure, which might be resulted from the high abundance of proline in the peptide sequence.



Scheme 3 Structures of MC moieties conjugated with RGD tripeptide.

Besides peptide sequences, the linkers between the MC moiety and oligopeptides also have influence on the hydrogelation abilities of the resulting conjugates. Tripeptide RGD was chosen as a model peptide to investigate such influence due to the ability of MC-RGD to assemble at low concentration (Table 1). In addition, RGD-bearing polymeric hydrogels have been used as biomaterials with multiple functions, which makes RGD sequence of particular interest.[30] Five MC-RGD conjugates with linkers of various lengths were synthesized (Scheme 3) and their gelation properties were characterized. Results suggested that the length of linkers between MC and oligopeptides did not significantly alter the gelation abilities of MC-oligopeptide conjugates. As shown in Table 2, RGD-conjugates with **MC*V***, **MC*IV***, and **MC*VI*** were all able to gel water at near neutral pH. On the other hand, increasing the hydrophobicity of RGD conjugates by the insertion of an aromatic ring in the linker jeopardized the gelation tendency of **MC*VII***-RGD and **MC*VIII***-RGD, leading to precipitation instead (Table 2).

Table 2 Hydrogelation properties of RGD conjugated with different MC moieties.

	pH < 4	4 < pH < 6	pH>6
MC*IV*-RGD	S	G	S
MC*V*-RGD	S	G	S
MC*VI*-RGD	S	G	S
MC*VII*-RGD	S	P	S
MC*VIII*-RGD	S	P	S

S=Solution; G=Gel; P=Precipitation

Multi-responsiveness of MCI-RGD hydrogel

Interestingly, **MC*I***-RGD hydrogel exhibited remarkable responsive property to a fourth signal, metal ions (Fig. 4). **MC*I***-RGD could not self-assembly into hydrogel by cooling treatment at pH 7.4; however, addition of Ca^{2+} ions gradually increased the viscosity of **MC*I***-RGD solution and eventually resulted in the formation of hydrogel when the amount of Ca^{2+} was 0.5 equivalent to the carboxyl groups in **MC*I***-RGD molecules (Fig. 4, Fig. S14†). SEM was employed to analyze the cryo-dried gel sample of this **MC*I***-RGD/Ca^{2+} hydrogel (Fig. S15†). Ca^{2+} induced hydrogel also showed 3-D fibrous network. To further establish the mode of the interaction between **MC*I***-RGD and Ca^{2+}, we analyzed the storage modulus as a function of [Ca^{2+}]/[COOH]. The storage modulus of **MC*I***-RGD solution was as low as 0.16 Pa without addition of Ca^{2+} at neutral pH, indicating it was at the liquid status. The storage modulus gradually increased with the addition of Ca^{2+} and reached its maximum when [Ca^{2+}]:[COOH]=1:1, indicating the completion of gelation. (Fig. S16†)Such Ca^{2+}-induced gelation can be reversed by the addition of EDTA, which is a chelator to Ca^{2+}. Soaking the Ca^{2+}-induced **MC*I***-RGD hydrogel with EDTA solution at neutral pH slowly extracted Ca^{2+} ions from the gel matrix and converted the hydrogel to a dark red solution (Fig. 4, Fig. S17†). This gel-to-solution transformation can be madulated reversibly by addition of $CaCl_2$ and EDTA for multiple times. Thus, **MC*I***-RGD is a multi-responsive hydrogel material that can be modulated by light, pH, heat and metal ion stimulis.

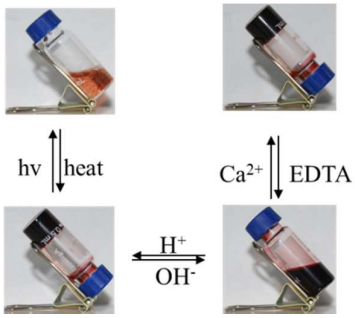


Fig. 4 Multi-responsiveness of the **MC*I***-RGD hydrogel.

Erasable photo-lithograph on the hydrogel

Inspired by its reversible response to light and heat, we employed **MC*I***-RGD hydrogel as an erasable photo-lithograph material. A typical operation procedure for recording and erasing characters using **MC*I***-RGD hydrogel is shown in Fig. 5. Through a photomask, pre-formed **MC*I***-RGD hydrogel (Fig. 5a) was exposed to visible light, which converted the exposed region into transparent liquid with yellowish color ("write process", Fig. 5b). The resulting image can be "erased" by homogenizing the hydrogel through a gentle heating/cooling treatment (Fig. 5b-c-d) and the resulting hydrogel is ready for further "writing". Such procedures can be performed repeatedly for multiple cycles without significant loss of sensitivity, which suggested the potential of MC-peptide hydrogels as photo-memorable materials.

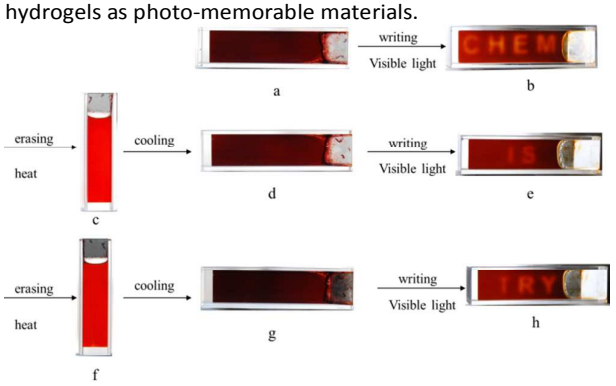


Fig. 5 MC*I*-RGD hydrogel can be employed as erasable photo-lithograph material.

Conclusions

We have investigated the hydrogelation of oligopeptides conjugated with merocyanine moieties at different positions and via different linkers. The MC-peptide conjugates were generated *in situ* from corresponding SP-peptide conjugates in aqueous solution via heat-induced reverse photochromism. All **MC*I***-peptide conjugates characterized in this report were able to gel water at appropriate pH to form photo-responsive hydrogels, indicating that MC had superior ability to promote the self-assembly of short peptides through proper conjugation. In the example of MC-RGD conjugates,the composition, instead of the length, of the linker between MC moiety and RGD pepitde has important influence on the hydrogelation properties of the conjugates. The hydrogel formed by **MC*I***-RGD demonstrated reversible, multi-responses to external stimuli including light, heat, pH, and Ca^{2+} ion. The potential application of this type of hydrogel as photo-memorable materials was demonstrated by several cycles of writing-erasing on the hydrogel formed by **MC*I***-RGD.

Experimental Section

General: All starting materials were obtained from commercial suppliers and used as supplied. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DPX 300 Spectrometer (^{1}H: 300 MHz; ^{13}C: 75 MHz). Chemical shifts were reported in δ (ppm) with respect to TMS as an internal standard. Coupling constants were reported in Hz with multiplicities denoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). MS was measured on Shimadzu LCMS-2020. HRMS was acquired on Agilent 6550 iFunnel Q-TOF LC/MS. UV-Vis spectra were acquired on PerkinElmer UV/Vis Spectrometer Lambda 35. Analytical HPLC analysis was carried out on Agilent 1200 LC with methanol-water as eluents. Preparative HPLC was carried out on Waters 2535 LC with methanol-water (0.1% TFA) as eluents.

Solid-phase peptide synthesis: All spiropyran conjugated N-terminal oligopeptides were synthesized through standard solid phase peptide synthesis protocol by using 2-chlorotrityl chloride resin (100~200mesh, ~1.0 mmol/g) and *N*-Fmoc-protected amino acids. The required oligopeptide was cleaved from resin with TFA and the crude peptide was purified by preparative reverse phase HPLC.

Tests on hydrogelation properties: Aqueous solution of the MC-peptides was prepared in a glass vial *via* heating the corresponding SP peptides in buffered solution. The vial was cooled to room temperature in dark and left undisturbed. The state was evaluated by the "stable-to-inversion of a test-tube" method. The minimum concentration for hydrogelation was measured by diluting the higher concentrated hot solution (usually 10.0 mg/mL) gradually until the solution failed to form stable hydrogel after cooling down in dark.

Microscopic study: SEM images were obtained on Hitachi S-4800 scanning electron microscope. Samples of xerogels for SEM were prepared by lyophilizing small amount of gel onto a silicon wafer. A thin layer of gold was sprayed on the samples for better image resolution.

Rheology: Rheological experiments were carried out on an HAAKE RheoStress 6000 rheometer (Thermo Scientific). All measurements were carried out in cone and plate geometry (19.992 mm diameter plate and 1º cone angle). Dynamic frequency sweep was used to examine the sample with setting the strain amplitude at 1%. The experiments were performed as a function of angular frequency (0.1-100 rad/s). Both storage modulus and loss modulus were plotted against angular frequency.

Erasable photo-lithograph on the hydrogel: In a glass vial *via* heating **MC*I***-RGD in buffered solution. Transfer the hot solution into a quartz cuvette and cool to room temperature in dark until the hydrogel formed.

1. **Writing:** Through a photomask, pre-formed **MC*I***-RGD hydrogel was exposed to visible light. After 2-5 min,

remove light source and photomask. Yellow word appeared on the hydrogel.

2. **Erasing:** Generally heating the quartz cuvette to about 70°C, the hydrogel gradually melt and formed a homogeneous red solution. Word on the hydrogel was erased. The quartz cuvette was cooled to room temperature in dark. With the formation of hydrogel, it could be used for next writing again.

Acknowledgements

Financial supports from the National Basic Research Program of China (No. 2014CB542300) and the National Natural Science Foundation of China (21372115, 21402091) is acknowledged. The authors are thankful for help from Prof. Yi Cao at Nanjing University on Rheology tests.

Notes and references

1 (a) E. F. Banwell, E. S. Abelardo, D. J. Adams, M. A. Birchall, A. Corrigan, A. M. Donald, M. Kirkland, L. C. Serpell, M. F. Butler and D. N. Woolfson, *Nat. Mater.*, 2009, **8**, 596-600; (b) F. Zhao, M. L. Ma and B. Xu, *Chem. Soc. Rev.*, 2009, **38**, 883-891; (c) X. D. Yu, L. M. Chen, M. M. Zhang and T. Yi, *Chem. Soc. Rev.*, 2014, **43**, 5346-5371; (d) X. Z. Yan, F. Wang, B. Zheng and F. H. Huang, *Chem. Soc. Rev.*, 2012, **41**, 6042-6065.

2 (a) D. W. P. M. Löwik, E. H. P. Leunissen, M. van den Heuvel, M. B. Hansen and J. C. M. van Hest, *Chem. Soc. Rev.*, 2010, **9**, 3394-3412; (b) T. Yoshii, S. Onogi, H. Shigemitsu and I. Hamachi, *J. Am. Chem. Soc.*, 2015, **137**, 3360-3365; (c) C. H. Ren, H. M. Wang, D. Mao, X. L. Zhang, Q. Q. Fengzhao, Y. Shi, D. Ding, D. L. Kong, L. Wang and Z. M. Yang, *Angew. Chem. Int. Ed.*, 2015, **54**, 4823-4827.

3 (a) S. C. Lee, I. K. Kwon and K. Park, *Adv. Drug Deliv. Rev.*, 2013, **65**, 17-20; (b) L. Qin, F. Xie, P. F. Duan and M. H. Liu, *Chem. Eur. J.*, 2014, **20**, 15419-15425; (c) S. P. Patil, H. S. Jeong and B. H. Kim, *Chem. Commun.*, 2012, **48**, 8901-8903.

4 (a) E. C. Wu, S. G. Zhang and C. A. E. Hauser, *Adv. Funct. Mater.*, 2012, **22**, 456-468; (b) S. Khetan, M. Guvendiren, W. R. Legant, D. M. Cohen, C. S. Chen and J. A. Burdick, *Nat. Mater.*, 2013, **12**, 458-465; (c) K. Vulic and M. S. Shoichet, *J. Am. Chem. Soc.*, 2012, **134**, 882-885.

5 (a) M. D. Segarra-Maset, V. J. Nebot, J. F. Miravet and B. Escuder, *Chem. Soc. Rev.*, 2013, **42**, 7086-7098; (b) X. W. Du, J. Zhou and B. Xu, *Chem. Asian J.*, 2014, **9**, 1446-1472; (c) W. Cao, X. L. Zhang, X. M. Miao, Z, M, Yang and H. P. Xu, *Angew. Chem. Int. Ed.*, 2013, **52**, 6233-6237; (d) Y. Zhang, B. Zhang, Y. Kuang, Y. Gao, J. F. Shi, X. X. Zhang and B. Xu, *J. Am. Chem. Soc.*, 2013, **135**, 5008-5011; (e) Z. F. Sun, Z. Y. Li, Y. H. He, R. J. Shen, L. Deng, M. H. Yang, Y. Z. Liang and Y. Zhang, J. *Am. Chem. Soc.*, 2013, **135**, 13379-13386; (f) M. Ikeda, T. Tanida, T. Yoshii, K. Kurotani, S. Onogi, K. Urayama and I. Hamachi, *Nat. Chem.*, 2014, **6**, 511-518; (g) M. Ikeda, T. Tanida, T. Yoshii and I. Hamachi, *Adv. Mater.*, 2011, **23**, 2819-2822; (h) D. Yuan, X. W. Du, J. F. Shi, N. Zhou, J. Zhou and B. Xu, *Angew. Chem. Int. Ed.*, 2015, **54**, 5705-5708; (i) S. Liu, A. M. Tang, M. L. Xie, Y. D. Zhao, J. Jiang and G. L. Liang, *Angew. Chem. Int. Ed.*, 2015, **54**, 3639-3642; (j) Z. F. Sun, F. C. Lv, L. J. Cao, L. Liu, Y. Zhang and Z. G. Lu, *Angew. Chem. Int. Ed.*, 2015, **54**, 7944-7948.

6 (a) A. Dasqupta, J. H. Mondal and D. Das, *RSC Adv.*, 2013, **3**, 9117-9149; (b) S. S. Babu, V. K. Praveen and A. Ajayaghosh, *Chem. Rev.*, 2014, **114**, 1973-2129; (c) S. Fleming and R. V. Ulijn, *Chem. Soc. Rev.*, 2014, **43**, 8150-8177.

7 (a) Y. Zhang, H. W. Gu, Z. M. Yang and B. Xu, *J. Am. Chem. Soc.*, 2003, **125**, 13680-13681; b) Y. Zhang, Z. M. Yang, F. Yuan, H. W. Gu, P. Gao and B. Xu, *J. Am. Chem. Soc.*, 2004, **126**, 15028-15029.

8 (a) K. M. Galler, L. Aulisa, K. R. Regan, R. N. D'Souza and J. D. Hartgerink, *J. Am. Chem. Soc.*, 2010, **132**, 3217-3223; (b) V. A. Kumar, S. Y. Shi, B. K. Wang, I-C. Li, A. A. Jalan, B. Sarkar, N. C. Wickremasinghe and J. D. Hartgerink, *J. Am. Chem. Soc.*, 2015, **137**, 4823-4830; (c) V. A. Kumar, N. L. Taylor, S. Y. Shi, N. C. Wickremasinghe, R. N. D'Souza and J. D. Hartgerink, *Biomaterials*, 2015, **52**, 71-78; (d) L. E. R. O'leary, J. A. Fallas, E. L. Bakota, M. K. Kang and J. D. Hartgerink, *Nat. Chem.*, 2011, **3**, 821-828; (e) R. J. Wade, E. J. Bassin, C. B. Rodell and J. A. Burdick, *Nat. Commun.*, 2015, **6**, 6639.

9 (a) T. T. Lee, J. R. García, J. I. Paez, A. Singh, E. A. Phelps, S. Weis, Z. Shafiq, A. Shekaran, A. del Campo and A. F. García, *Nat. Mater.*, 2015, **14**, 352-360; (b) J. Sun, D. Wei, Y. D. Zhu, M. L. Zhong, Y C. Zuo, H S. Fan and X. D. Zhang, *Biomaterials*, 2014, **35**, 4759-4768.

10 M. T. He, J. B. Li, S. B Tan, R. Z. Wang and Y. Zhang, *J. Am. Chem. Soc.*, 2013, **135**, 18718-18721.

11 (a) Y. Luo and M. S. Shoichet, *Nat. Mater.*, 2004, **3**, 249-253; (b) S. Khetan, J. S. Katz and J. A. Burdick, *Soft Matter.*, 2009, **5**, 1601-1606; (c) S. Khetan and J. A. Burdick, *Biomaterials*, 2010, **31**, 8228-8234; (d) K. M. C. Tsang, N. Annabi, F. Ercole, K. Zhou, D. J. Karst, F. Li, J. M. Haynes, R. A. Evans, H. Thissen, A. Khademhosseini and J. S. Forsythe, *Adv. Funct. Mater.*, 2015, **25**, 977-986.

12 T. Yoshii, M. Ikeda and I. Hamachi, *Angew. Chem. Int. Ed.*, 2014, **53**, 7264-7267.

13 (a) C. Maity, W. E. Hendriksen, J. H. van Esch and R. Eelkema, *Angew. Chem. Int. Ed.*, 2015, **54**, 998-1001; (b) J. K. Sahoo, S. K. M. Nalluri, N. Javid, H. Webb and R. V. Ulijn, *Chem. Commun.*, 2014, **50**, 5462-5464; (c) L. A. Haines, K. Rajagopal,B. Ozbas, D. A.Salick, D. J. Pochan and J. P. Schneider, *J. Am. Chem. Soc.*, 2005, **127**, 17025-17029; (d) T. Muraoka, C. Y. Koh, H. G. Cui and S. I. Stupp, *Angew. Chem. Int. Ed.*, 2009, **48**, 5946-5949; (e) S. Sur, J. B. Matson, M. J. Webber, C. J. Newcomb and S. I. Stupp, *ACS Nano*, 2012, **6**, 10776-10785.

14 (a) S. Matsumoto, S. Yamaguchi, S. Ueno, H. Komatsu, M. Ikeda, K. Ishizuka, Y. Iko, K. V.Tabata, H. Aoki, S. Ito, H. Noji and I. Hamachi, *Chem. Eur. J.*, 2008, **14**, 3977-3986; (b) H. Komatsu, S. Tsukiji, M. Ikeda and I. Hamachi, *Chem. Asian J.*, 2011, **6**, 2368-2375; (c) X. M. Li, Y. Gao, Y. Kuang and B. Xu, *Chem. Commun.*, 2010, **46**, 5364-5366; (d) G. F. Liu, W. Ji, W. L. Wang and C. L. Feng, *ACS Appl. Mater. Interfaces*, 2015, **7**, 301-307.

15 Z. J. Qiu, H. T. Yu, J. B. Li, Y. Wang and Y. Zhang, *Chem. Commun.*, 2009, 3342-3344.

16 Y. C. Huang, Z. J. Qiu, Y. M. Xu, J. F. Shi, H. K. Lin and Y. Zhang, *Org. Biomol. Chem.*, 2011, **9**, 2149-2155.

17 M. L. Ma, Y. Kuang, Y. Gao, Y. Zhang, P. Gao and B. Xu, *J. Am. Chem. Soc.*, 2010, **132**, 2719-2728.

18 (a) G. Berkovic, V. Krongauz and V. Weiss, *Chem. Rev.*, 2000, **100**, 1741-1754; (b) N. Tamai and H. Miyasaka, *Chem. Rev.*, 2000, **100**, 1875-1890.

19 (a) J. Yan, L. X. Zhao, C. Li, Z. Hu, G. F. Zhang, Z. Q. Chen, T. Chen, Z. L. Huang, J. T. Zhu and M. Q. Zhu, *J. Am. Chem. Soc.*, 2015, **137**, 2436-2439; (b) Q. K. Qi, J. Y. Qian, S. Q. Ma, B. Xu, S. X. A. Zhang and W. J. Tian, *Chem. Eur. J.*, 2015, **21**, 1149-1155.

20 (a) X. J. Xie, G. A. Crespo, G. Mistlberger and E. Bakker, *Nat. Chem.*, 2014, **6**, 202-207; (b) X. J. Xie and E. Bakker, *J. Am. Chem. Soc.*, 2014, **136**, 7857-7860.

21 Y. Kamiya and H. Asanuma, *Acc. Chem. Res.*, 2014, **47**, 1663-1672.
22 R. Klajn, *Chem. Soc. Rev.*, 2014, **43**, 148-184.
23 A. Fissi, O. Pieroni, G. Ruggeri and F. Ciardelli, *Macromolecules*, 1995, **28**, 302-309.
24 (a) I. Shimizu, H. Kokado and E. Inoue, *Bull. Chem. Soc. Jpn.*, 1969, **42**, 1726-1729; (b) I. Shimizu, H. Kokado and E. Inoue, *Bull. Chem. Soc. Jpn.*, 1969, **42**, 1730-1734; (c) E. Inoue, H. Kokado, I. Shimizu, H. Kobayashi and Y. Takahashi, *Bull. Chem. Soc. Jpn.*, 1972, **45**, 1951-1956.
25 K. Aihara, H. Ishii and M. Yoshida, *J. Atheroscler. Thromb.*, 2009, **16**, 594-603.
26 J. Jia, R. Lu, S. Qiu, H. Q. Li, X. C. Che, P. P. Zhao, M. J. Jin, H. X. Yang, G. Lin and Z. Yao, *Cancer Biol. Ther.*, 2005, **4**, 993-997.
27 E. Witkowska, A. Oriowska, J. Izdebski, J. Salwa, J. Wietrzyk and A. Opolski, *J. Pept. Sci.*, 2004, **10**, 285-290.
28 R. S. Bresalier, B. Schwartz, Y. S. Kim, Q. Y. Duh, H. K. Kleinman and P. M. Sullam, *Cancer Res.*, 1995, **55**, 2476-2480.
29 C. Spezzacatena, T. Perri, V. Guantieri, L. B. Sandberg, T. F. Mitts and A. M. Tamburro, *Eur. J. Org. Chem.*, 2002, **1**, 95-103.
30 (a) E. Alsberg, K. W. Anderson, A. Albeiruti, J. A. Rowley and D. J. Mooney, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 12025-12030; (b) F. Yang, C. G. Williams, D. A. Wang, H. Lee, P. N. Manson and J. A. Elisseeff, *Biomaterials*, 2005, **26**, 5991-5998; (c) J. A. Burdick and K. S. Anseth, *Biomaterials*, 2002, **23**, 4315-4323.