ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 6175–6177

www.rsc.org/chemcomm

COMMUNICATION

A novel mixed-component molecular hydrogel system with excellent stabilities†

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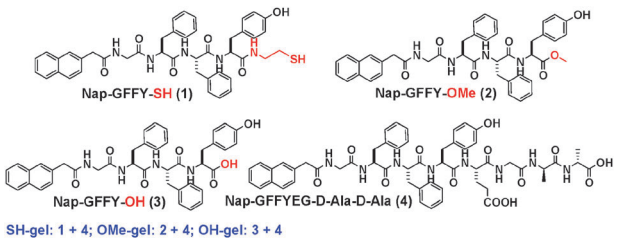
Received 1st April 2012, Accepted 28th April 2012
DOI: 10.1039/c2cc32348f

We report a novel mixed-component molecular hydrogel system with excellent stabilities against dilution and enzyme digestion.

Molecular hydrogels have attracted extensive research interests recently because of their great potential for tissue engineering,[1] mineration of organic and inorganic materials,[2] drug delivery,[3] *etc*. Molecular hydrogels are formed by the self-assembly of small molecules (molecular hydrogelators) and those composed of one kind and two kinds of molecular hydrogelators are called single-component and two-component molecular hydrogels, respectively. Compared with single-component molecular hydrogels, two-component ones possess more functionality, tunability, and control.[4] However, the examples of two-component molecular hydrogels are much fewer than single-component ones and it is difficult to rationally design a two-component hydrogel. Indeed, there is a relatively easy approach to produce not very strictly two-component molecular hydrogels, mixed-component ones.

Mixed-component hydrogels are formed by mixing a gelator with a functional molecule with similar chemical structure to the gelator. The introduction of functional molecules into the nanofibers formed by gelators will give novel properties and functionalities of gels. Several groups have conducted pioneering works in this field: Stupp's group reported a mixed-component nanofiber system with good control of the density of functional groups,[5] Gasiorowski and Collier have developed a mixed-component hydrogel as an immune adjuvant,[6] and both groups have demonstrated that functional molecules present at the surface of self-assembled nanofibers exhibit much higher activities than those in the solution phase;[7] Ulijn's group also introduced a bioactive RGD ligand into the self-assembled fibers formed by a short peptide, thus promoting cells spreading and proliferation in gels;[8] Nilsson's group demonstrated that co-assembly of a C-terminal PEG-functionalized gelator and the gelator resulted in gels with higher rigidities and better solvolytic stabilities.[9] Though several groups have demonstrated the better properties of mixed-component gels than single-component ones, the simple mixing strategy cannot guarantee the full incorporation of functional molecules into self-assembled nanofibers because the functional molecules are not the requisite building blocks for the formation of self-assembled structures and some of the functional molecules might be in soluble form. In this study, we report a novel strategy to prepare a mixed-component hydrogel system with most of the functional molecules being incorporated into nanofibers, thus leading to a better stability of the functional molecules against proteinase K digestion in the mixed-component gel.

The development of the mixed-component hydrogels was based on our recent observations.[10,11] We had reported that Nap-GFFY-ss-EEE could be converted to **1** (Scheme 1) by dithiothreitol (DTT) and we observed the formation of molecular hydrogels during the conversions.[11] However, the gels were not stable because more and more Nap-GFFY-ss-EEE was converted to the hydrophobic **1**—the gels could only last for less than one hour and would change into precipitates after one hour.[11] We also found that bovine serum albumin (BSA) could stabilize the gels of **1** for more than 2 months because BSA had a hydrophobic pocket that could interact with **1** and help to stabilize hydrophobic self-assembled nanofibers of **1**.[11] The phenomenon of gel formation at low conversion percentages of Nap-GFFY-ss-EEE suggested that Nap-GFFY-ss-EEE could also co-assemble with **1** and stabilize the hydrophobic self-assembled nanofibers of **1**, which stimulated us to use Nap-GFFYEG-D-Ala-D-Ala (**4** in Scheme 1) to stabilize gels of **1**. The reason for us choosing **4** was due to the fact that



Scheme 1 Chemical structures of peptides used for molecular hydrogelations.

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† Electronic supplementary information (ESI) available: Synthesis and characterization of Nap-GFFYEG-D-Ala-D-Ala, dynamic strain/time sweeps, and procedures to prepare the gels and determine the stability of compounds against proteinase K digestion. See DOI: 10.1039/c2cc32348f

dipeptide D-Ala-D-Ala was the terminal peptide on the bacterial cell wall and the self-assembled fibers bearing it might mimick the bacterial cell wall for the study of antibiotics–bacterial cell wall interactions. We envisioned that the mixed-component gels formed by compounds **1** and **4** would possess excellent stability against dilution because **4** is a requisite building block for the formation of stable nanofibers and **4** would be predominately located in self-assembled nanofibers but not in the solution phase.

We firstly tested whether the addition of **4** could stabilize gels of **1** or not. The results shown in Fig. S4 (ESI†) indicated that **4** could stabilize the gel and the minimum percentage of **4** to form a stable gel for more than one week was about 25 wt% to the total amount of compounds **1** and **4**. We then prepared a mixed-component gel containing 70 wt% of **1** and 30 wt% of **4** (SH-gel, Fig. 2) for the test. We also prepared two other mixed-component gels formed by incorporation of **4** into gels Nap-GFFY–COOMe (**2**, OMe-gel) and Nap-GFFY–COOH (**3**, OH-gel) as controls.[12]

In order to test the stability of **4** in self-assembled nanofibers in gels, we added equal volumes of phosphate buffer saline (PBS, pH = 7.4) on the top of gels in small vials and measured the released percentages of **4** from gels. The results in Fig. 1A indicate that **4** kept releasing from OMe-gel and OH-gel at similar speeds and about 25% of it got released from gels to upper buffer solutions after 108 hours. The release speed of **4** from SH-gel was much lower than those from OMe-gel and OH-gel. And the release of **4** from SH-gel reached a balance after 72 hours (less than 5% of **4** got released). These observations indicated that more **4** was entrapped in the matrix of SH-gel than the other two gels. The phenomenon partially demonstrated our design that most of **4** would be incorporated into self-assembled nanofibers in SH-gel because it is a requisite building block for stable 3D self-assembled networks.

We then added proteinase K to the gels to test the stability of **4** against enzyme digestion. The results in Fig. 1B show that the cleavage percentage of **4** increased rapidly in the first 2 hours and reached a balance after about 12 hours in three gels. However, the cleavage percentages of **4** were different in three gels at 24 hours time point—about 48, 61, and 70% of **4** got cleaved by the enzyme in SH-gel, OMe-gel, and OH-gel, respectively. More than 80% and nearly 100% of **4** were cleaved in solutions of it after 1 hour and 4 hours, respectively (Fig. S6, ESI†). The cleavage percentage of **1** in SH-gel was also much lower than that of compounds **2** and **3** in their corresponding gels (Fig. S7, ESI†). These results demonstrated that the stability of both the bioactive molecule and gelator against proteinase K digestion could be enhanced in our stable mixed-component SH-gel, which was crucial for its future applications in tissue engineering and regenerative medicine. We then went back to characterize three mixed-component gels by several techniques such as rheology, TEM, and fluorescence.

The rheological measurements (Fig. 2) indicated that both the storage modulus (G′) and loss modulus (G″) of three mixed gels showed weak frequency dependency from 0.1 to 100 rad s^{-1}. And the G′ value of three gels was about ten times larger than their corresponding G″ value. Both results suggested the formation of 3D elastic networks and true gels. The G′ values of three gels followed the order of SH-gel > OMe-gel > OH-gel

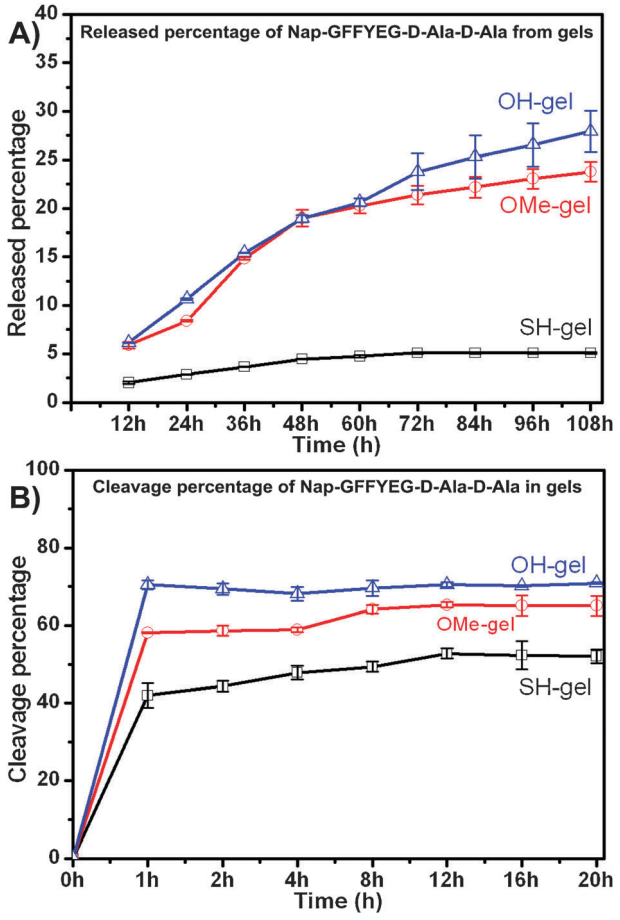

Fig. 1 (A) Released percentage of Nap-GFFYEG-D-Ala-D-Ala from gels and (B) cleavage percentage of Nap-GFFYEG-D-Ala-D-Ala in gels (30 wt% of **4** in all gels, total concentration of gelators is 1.0 wt%).

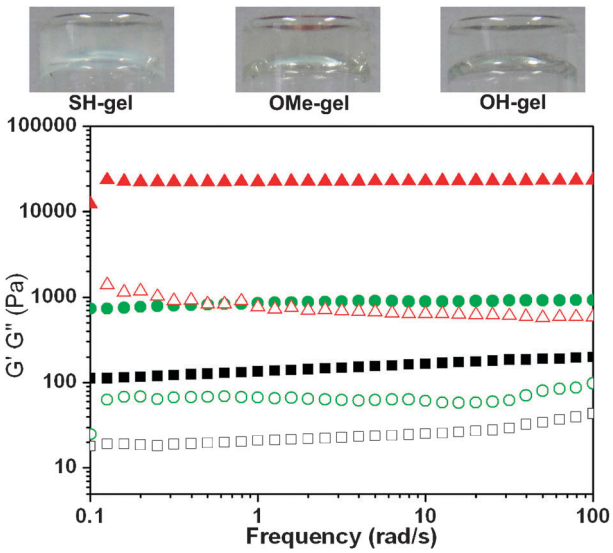

Fig. 2 Optical images of gels containing 1.0 wt% of gelators (compound **4** was 30 wt% to total amounts of compounds **1** and **4**, compounds **2** and **4**, and compounds **3** and **4** for SH-gel, OMe-gel, and OH-gel, respectively) and dynamic frequency sweep of gels (triangles: SH-gel, circles: OMe-gel, and squares: OH-gel, filled symbols: G′ and open symbols: G″).

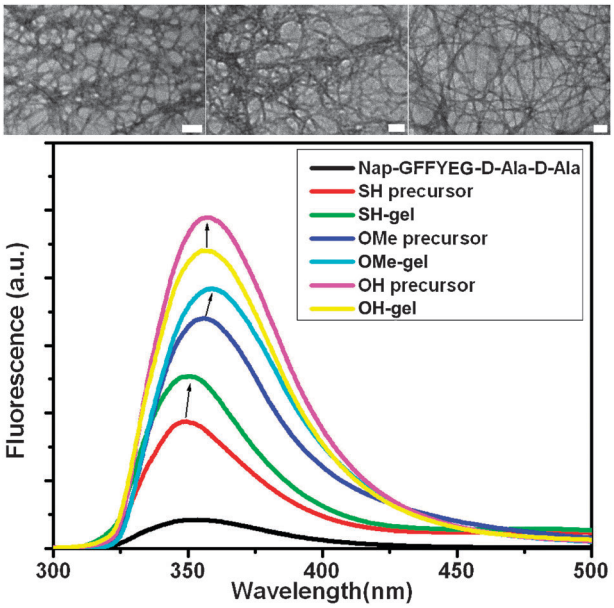

Fig. 3 TEM images of the gels (from left to right: SH-gel, OMe-gel, and OH-gel, scale bars represent 100 nm) and emission spectra of solutions of precursors and gels (λ_{exc} = 272 nm).

and were about 24 000, 850, and 120 Pa for SH-gel, OMe-gel, and OH-gel, respectively. The order of G′ values of three gels was the same as that of cleavage percentages of **4** in gels, suggesting that larger G′ values (higher mechanical strength) may account for the better stability of **4** in gels against the enzyme digestion.

The transmission electron microscopy (TEM) images in Fig. 3 showed networks of fibers in gels. The size of nanofibers was 20–40, 20–40, and 25–30 nm in SH-gel, OMe-gel, and OH-gel, respectively. The fibers in SH-gel and OMe-gel formed larger bundles than those in OH-gel, which was consistent with the observations in Fig. 2 that the G′ values of SH-gel and OMe-gel were larger than that of OH-gel.

We also obtained the emission spectra of solutions of **4** and precursors of gelators (Nap-GFFY-ss-EEE for compound **1**, Nap-GFFpY-OMe for **2**, and Nap-GFFpY-OH for **3**) and three mixed-component gels. As shown in Fig. 3, there were slight red shifts for emission spectra of gels, compared with the emission spectra of their corresponding precursor solutions. The red shift wavelength number was small and it was about 2.5, 2.7, and 0 nm for SH-gel, OMe-gel, and OH-gel, respectively. Since the emission spectra of gels were recorded at the same concentration of gelators, the lower intensity of the emission peak suggested more efficient aromatic–aromatic stacking in self-assembled fibers. The intensity of peaks followed the order of SH-gel < OMe-gel < OH-gel, which was consistent with the result of reverse order of G′ values of three gels.

In summary, we have introduced a novel mixed-component molecular hydrogel system with excellent stability against dilution and proteinase K digestion. Though we only incorporated Nap-GFFYG-D-Ala-D-Ala into self-assembled nanofibers of Nap-GFFY–SH, other bioactive molecules with chemical structures of Nap-GFFYG-hydrophilic bioactive molecules could also be used to generate stable mixed-component molecular hydrogels. Since the activity of functional molecules could be enhanced when present at the surface of self-assembled nanostructures,[7] our novel mixed-component self-assembled nanofibers will have great potential for tissue engineering, cancer therapy, and regenerative medicine.

This work is supported by NSFC (31070856 to Yang's group and 81171371 to Liu's group).

Notes and references

1 V. Jayawarna, M. Ali, T. A. Jowitt, A. E. Miller, A. Saiani, J. E. Gough and R. V. Ulijn, *Adv. Mater.*, 2006, **18**, 611; M. Zhou, A. M. Smith, A. K. Das, N. W. Hodson, R. F. Collins, R. V. Ulijn and J. E. Gough, *Biomaterials*, 2009, **30**, 2523; J. P. Jung, J. V. Moyano and J. H. Collier, *Integr. Biol.*, 2011, **3**, 185; Y. F. Tian, J. M. Devgun and J. H. Collier, *Soft Matter*, 2011, **7**, 6005.

2 B. T. Li, L. M. Tang, L. Qiang and K. Chen, *Soft Matter*, 2011, **7**, 963; Y. J. Wang, L. M. Tang and J. Yu, *Prog. Chem.*, 2009, **21**, 1312; X. J. Liu, W. Zhuang, B. Z. Li, L. M. Wu, S. B. Wang, Y. Li and Y. G. Yang, *Chem. Commun.*, 2011, **47**, 7215; Y. Li, L. F. Bi, S. B. Wang, Y. L. Chen, B. Z. Li, X. L. Zhu and Y. G. Yang, *Chem. Commun.*, 2010, **46**, 2680; M. M. Zhang, S. T. Sun, X. D. Yu, X. H. Cao, Y. Zou and T. Yi, *Chem. Commun.*, 2010, **46**, 3553.

3 F. Zhao, M. L. Ma and B. Xu, *Chem. Soc. Rev.*, 2009, **48**, 883; L. Q. Chen, J. C. Wu, Y. W. Lihui, T. M. Shu, M. Xu, M. M. Zhang and T. Yi, *Langmuir*, 2009, **25**, 8434; S. Sutton, N. L. Campbell, A. I. Cooper, M. Kirkland, W. J. Frith and D. J. Adams, *Langmuir*, 2009, **25**, 10285; J. Nanda and A. Banerjee, *Soft Matter*, 2012, **8**, 3380.

4 A. R. Hirst and D. K. Smith, *Chem.–Eur. J.*, 2005, **11**, 5496; S. Manna, A. Saha and A. K. Nandi, *Chem. Commun.*, 2006, 4285; A. Saha, S. Manna and A. K. Nandi, *Chem. Commun.*, 2008, 3732; J. P. Jung, A. K. Nagaraj, E. K. Fox, J. S. Rudra, J. M. Devgun and J. H. Collier, *Biomaterials*, 2009, **30**, 2400; X. D. Xu, C. S. Chen, B. Lu, S. X. Cheng, X. Z. Zhang and R. X. Zhuo, *J. Phys. Chem. B*, 2010, **114**, 2365.

5 M. J. Webber, J. Tongers, M. A. Renault, J. G. Roncalli, D. W. Losordo and S. I. Stupp, *Acta Biomater.*, 2010, **6**, 3.

6 J. Z. Gasiorowski and J. H. Collier, *Biomacromolecules*, 2011, **12**, 3549.

7 G. A. Silva, C. Czeisler, K. L. Niece, E. Beniash, D. A. Harrington, J. A. Kessler and S. I. Stupp, *Science*, 2004, **303**, 1352; S. M. Standley, D. J. Toft, H. Cheng, S. Soukasene, J. Chen, S. M. Raja, V. Band, H. Band, V. L. Cryns and S. I. Stupp, *Cancer Res.*, 2010, **70**, 3020.

8 V. Jayawarna, S. M. Richardson, A. R. Hirst, N. W. Hodson, A. Saiani, J. E. Gough and R. V. Ulijn, *Acta Biomater.*, 2009, **5**, 934.

9 D. M. Ryan, T. M. Doran and B. L. Nilsson, *Chem. Commun.*, 2011, **47**, 475.

10 C. H. Ren, Z. J. Song, X. M. Chen, W. T. Zheng, L. Wang, D. L. Kong and Z. M. Yang, *Chem. Commun.*, 2011, **47**, 1619.

11 D. X. Li, H. M. Wang, D. L. Kong and Z. M. Yang, *Nanoscale*, 2012, **4**, 3047.

12 H. M. Wang, C. H. Ren, Z. J. Song, L. Wang, X. M. Chen and Z. M. Yang, *Nanotechnology*, 2010, **21**, 225606.