Short-peptide-based molecular hydrogels: Novel gelation strategies and applications for tissue engineering and drug delivery

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FEATURE ARTICLE

Short-peptide-based molecular hydrogels: novel gelation strategies and applications for tissue engineering and drug delivery

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Molecular hydrogels hold big potential for tissue engineering and controlled drug delivery. Our lab focuses on short-peptide-based molecular hydrogels formed by biocompatible methods and their applications in tissue engineering (especially, 3D cell culture) and controlled drug delivery. This feature article firstly describes our recent progresses of the development of novel methods to form hydrogels, including the strategy of disulfide bond reduction and assistance with specific protein–peptide interactions. We then introduce the applications of our hydrogels in fields of controlled stem cell differentiation, cell culture, surface modifications of polyester materials by molecular self-assembly, and anti-degradation of recombinant complex proteins. A novel molecular hydrogel system of hydrophobic compounds that are only formed by hydrolysis processes was also included in this article. The hydrogels of hydrophobic compounds, especially those of hydrophobic therapeutic agents, may be developed into a carrier-free delivery system for long term delivery of therapeutic agents. With the efforts in this field, we believe that molecular hydrogels formed by short peptides and hydrophobic therapeutic agents can be practically applied for 3D cell culture and long term drug delivery in near future, respectively.

1. Introduction

Molecular hydrogels consist of three dimensional (3D) self-assembled networks and water have showed big potential in fields of cell culture,[1–11] cancer therapy,[12–14] minerations of organic and inorganic materials,[15–19] drug delivery,[20–28] and regenerative medicine.[4,29–31] Small molecules (molecular hydrogelators), usually with molecular weight lower than 2000, self-assemble into 3D networks by external stimulus to form molecular hydrogels.[32–40] Up to now, there are several widely reported systems of molecular hydrogelators including

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Zhimou Yang received his BS from Nanjing University in 2001. He obtained his PhD in 2006 from the Hong Kong University of Science and Technology under the supervision of Professor Bing Xu. Before starting his independent research at Nankai University in March 2009, he was a post-doctoral fellow with Prof. Matthew Bogyo at Stanford Medical School. His research interests focus on molecular hydrogels of therapeutic agents (especially anti-cancer drugs) and short peptides and hydrogels based on protein–peptide interactions.

sugar-based compounds,[41,42] peptides,[5,12,39,40,43–47] amino acid derivatives,[48,49] *etc.*[50–52] Among these systems, peptide-based molecular hydrogelators attracted the most extensive research efforts probably due to their versatile synthetic pathway, excellent gelation ability, good biocompatibility, and easily manipulated bioactivity.[38,40,53,54] Zhang's group have conducted pioneering works in the development of peptide-based hydrogels and their applications in cell encapsulation,[31,55] stem cell differentiation,[3,4] and delivery of therapeutic agents such as growth factors,[56] *etc.*[57–59] The Schneider and Pochan groups reported on β-heparin peptide hydrogels for cell culture,[1,9] drug delivery,[25] heavy metal removal,[60] *etc.*[22,61] The Stupp and Hartgerink groups also demonstrated peptide amphiphiles were well suitable for controlled differentiation of stem cells,[6,62] biomaterations of inorganic materials,[63] and cancer therapy.[14,64] Recently, the Collier group focused on the immune response of peptide-based self-assembled systems.[65–67] They have shown that peptide-based self-assembled systems could be used as immune adjuvants and they also introduced the application of peptide-based hydrogels in cell transplantation and regenerative medicine.[68,69]

Besides the long-peptide-based (more than 10 amino acids) hydrogels developed by above-introduced groups, short-peptide-based ones have also been widely studied by several groups. The Gazit group firstly observed that aromatic dipeptide (FF) could self-assemble into nanotubes.[70] They have demonstrated that many Fmoc-protected short peptides could form self-assembled structures and hydrogels.[71–74] The Xu and Ulijn groups also conducted pioneering works in aromatic short-peptide-based hydrogelators, especially enzymatic formation of hydrogels of aromatic short peptides.[5,12,38–40,53,75] The Adams and Zhang groups reported novel methods of self-hydrolysis and enzyme-catalyzed hydrolysis of chemicals to trigger the formation of short-peptide-based hydrogels.[32,76] Both groups have applied short-peptide-based hydrogels for controlled drug delivery.[20,77,78] The Nilsson group studied the relationship between chemical structure and the self-assembly ability of short-peptide-based hydrogelators.[79–82] Our group has also focused on the development of short-peptide-based hydrogels and their applications in tissue engineering and drug delivery system in the past several years. This feature article introduces our progresses in novel methods for hydrogelations, application of hydrogels in tissue engineering, and hydrogels of hydrophobic compounds including hydrophobic therapeutic agents for drug delivery.

2. Novel methods for molecular hydrogelations

The development of biocompatible methods to trigger the formation of molecular hydrogels is crucial for their applications in cell encapsulation, delivery of thermo- or pH-sensitive components such as proteins and drugs, *etc.* Actually, the development of biocompatible methods to form hydrogels keeps attracting research interest in the field of molecular self-assembly. Until now, several biocompatible methods such as enzymatic triggers,[12,38–40,53] metal ion induction,[83–85] and light irridiation[37,42,86] have been reported by several groups. We believed that the development of these biocompatible methods would push forward the practical application of molecular hydrogels. Our lab has also developed two methods for hydrogelation, which will be discussed in the following sections.

2.1 Disulfide bond as a cleavable linker

We had used a disulfide linker to connect a molecular hydrogelator and a hydrophilic part.[87] Our design was based on a recent work from the Nilsson group.[33] Stimulated by the Nilsson group who used the strategy of disulfide reduction to form molecular hydrogels through the transformation of a cyclic peptide to the linear peptide, we synthesized **2** with a cleavable disulfide linker (Fig. 1). Compound **2** was well soluble in aqueous solutions due to the presence of hydrophilic penta-peptide of Glu-Glu-Arg-Gly-Asp (EERGD). Upon the addition of reductants such as dithiothreitol (DTT), tris(2-carboxyethyl)phosphine (TCEP), and glutathione (GSH), the disulfide bond could be cleaved, releasing the molecular hydrogelator of Nap-GFFYE-s (**1**) and forming a molecular hydrogel. This process would guarantee the rapid formation of homogeneous hydrogels for cell encapsulations and controlled drug delivery. GSH was a biocompatible cellular component. Therefore, hydrogels formed by GSH were suitable for 3D cell culture and the results will be reported in near future.

2.2 Using specific protein–peptide interactions for hydrogelation

Specific protein–peptide interactions have been used to cross-link polymer chains bearing peptides for hydrogelation.[88–93] Polymeric hydrogels formed by protein–peptide interactions have been used for cell encapsulations and controlled delivery of bioactive macromolecules.[88–90] Stimulated by these results and the method of metal ion-triggered molecular hydrogelations,[83–85] we have used a specific protein–peptide interaction to enhance the interaction between self-assembled nanofibers, thus leading to more crosslinking points between the nanofibers and molecular hydrogels.[94] As shown in Fig. 2, compound **3** could self-assemble into stable solutions of nanofibers but not hydrogels due to the weak interaction between self-assembled nanofibers. The addition of a rationally designed tetrameric protein of ULD-TIP-1 could enhance the inter-fiber interactions, increase cross-linking points between fibers, and form molecular hydrogels. The mechanical property of the resulting hydrogels could be manipulated by several means, including using different peptides with different affinities to the protein, changing the concentration of the cross-linker (ULD-TIP-1), and varying the concentration of peptides. The hydrogels formed by this method were thixotropic,

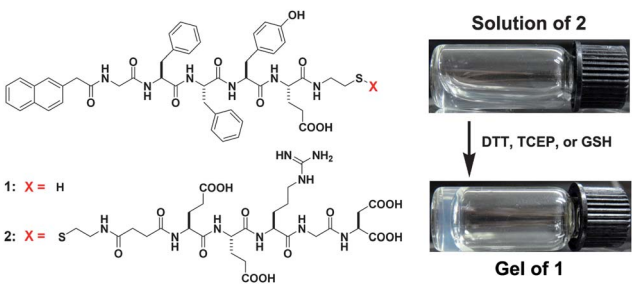

Fig. 1 Chemical structures of the precursor of the gelator (**2**) and the molecular gelator (**1**) and optical images to show hydrogelation by the addition of reductants to PBS buffer solutions (pH = 7.4) of **2** (0.4 wt%, 2.42 mM).

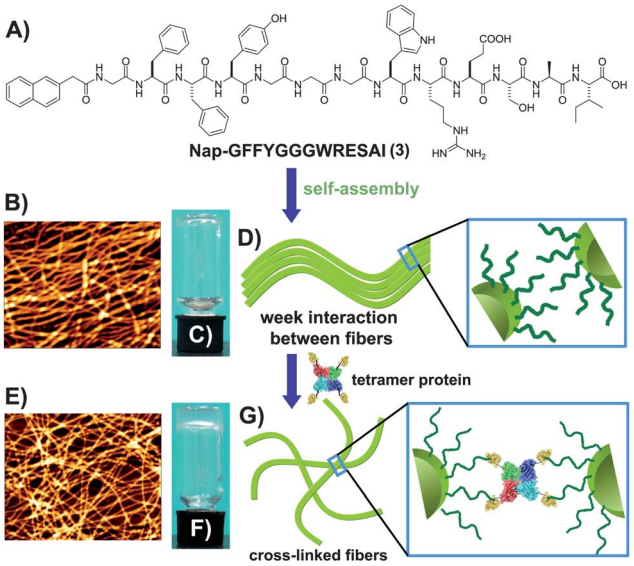

Fig. 2 Using a specific protein–peptide interaction to enhance interactions between self-assembled fibers thus leading to molecular hydrogelation: (A) the chemical structure of Nap-GFFYGGGWRESAI (**3**: non-gelator) with a possible self-assembly ability, (B, C, and D) compound **3** could self-assemble into nanofibers that lack strong interactions between fibers in aqueous solutions, thus resulting in fiber networks with a low density of cross-linking points, and (E, F, and G) the addition of our rationally designed fusion protein (ULD-TIP-1) could enhance the interactions between fibers, thus leading to a 3D fiber network with high density of cross-linking points and hydrogelation (the scale bars in (B and E) represent 500 nm, the small balls in insets of (D and G) represent hydrophilic part of **3** (GGGWRESAI), and the images of (D and G) do not represent the exact length-scale and structure of the components).

which meant that they could be converted into homogeneous solutions by external forces such as vortexing and pipetting. The hydrogels also possessed a rapid recovery property, indicating that the viscous solution formed by external forces could convert back to hydrogels rapidly. The hydrogel systems formed by specific protein–peptide interactions were homogeneous and suitable for encapsulation of cells and other thermo-/pH-sensitive components. This novel method to form molecular hydrogels will have applications such as using bioactive small molecules as drug derivatives in nanomedicine therapy and 3D cell culture or drug delivery in our future work.

3. Molecular hydrogels for tissue engineering

Peptide-based molecular hydrogels, especially those of long peptides, could provide suitable environments for cell growth, division, and differentiation. For example, the Zhang group and the Stupp group have demonstrated that peptide-based hydrogels could guide the differentiation of stem cells.[3,6] One challenge that remains in this field is a method to separate cells from gels post culture. Our lab opted to apply short-peptide-based molecular hydrogels formed by biocompatible methods for 3D cell culture, stem cell controlled differentiation, and cell delivery.[95,96] Responsive molecular hydrogels for the recovery of cells post culture were also attractive for us.[97]

3.1 A hydrogel for stem cell differentiation

Collagen is the most abundant protein in mammals, making up about 30% of the overall body protein content. In order to mimic collagen nanofibers, our lab have synthesized a serial of short peptides bearing collagen repeating tripeptide of Gly-Xaa-4-Hyp (GXO, X was Lys (K), Glu (E), Ser (S), Ala (A), or Pro (P)). However, these short peptides with only one repeating unit of GXO failed to mimic the collagen triple helix structure. Reports have showed that peptides with at least four repeating units of GXO could mimic collagen triple helix structures.[98,99] Therefore, we have synthesized a long peptide hydrogelator of Nap-GFFYGGKOGEOGKOGSO (**4** in Fig. 3A). Though it could not form collagen triple helix either, gels of **4** were thixotropic, which would facilitate the separation of cells cultured on them.

We tested the differentiation of murine embryonic stem (mES) cells cultured on hydrogels of **4**. Hydrogels of **4** supported the growth of mES cells on them (Fig. 4A–E). The cells could be isolated from gels for further analysis by gentle pipetting for several times and then centrifugation. More importantly, hydrogels of **4** could selectively enhance *Flk1* expression in differentiated mES cells (Fig. 4G and H). Flk1-positive cells derived from ES cells had been demonstrated as vascular progenitors supporting the formation of vascular system. Thus, hydrogels of **4** had great potential for the preparation of a pure population of Flk1-positive cells, which could be used for the study of vascular formation.

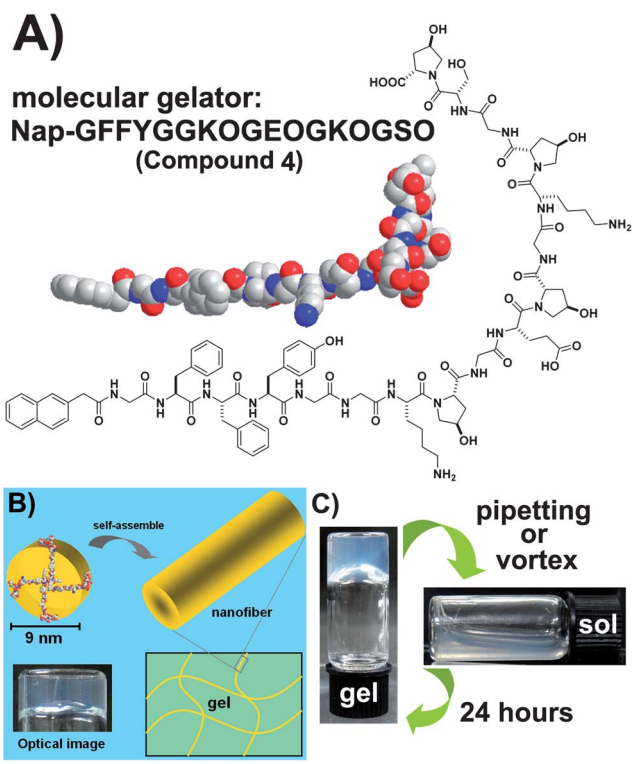

Fig. 3 (A) Chemical structure of Nap-GFFYGGKOGEOGKOGSO (**4**), (B) a proposed molecular model of nanofibres and an optical image of the gel containing 2 wt% of **4**, and (C) gels of **4** were thixotropic.

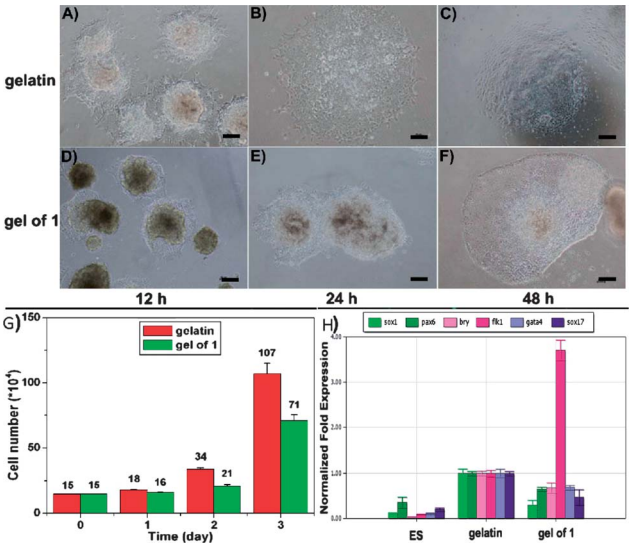

Fig. 4 The morphology of EBs on gelatin and hydrogel surfaces at different time points: (A–C) shows gelatin groups, and (D–F) shows hydrogel groups; (A) and (D) at 12 hours, (B and E) at 24 hours, and (C and F) at 48 hours after plating on gelatin or hydrogel surfaces (the scale bars represent 100 μm); (G) proliferation of differentiated mES cells on gelatin and hydrogel surfaces. The total cell number in a single well of the 24-well plate was shown. (H) The gene expression of differentiated mES cells on gelatin and hydrogel surface after 72 hours' culture.

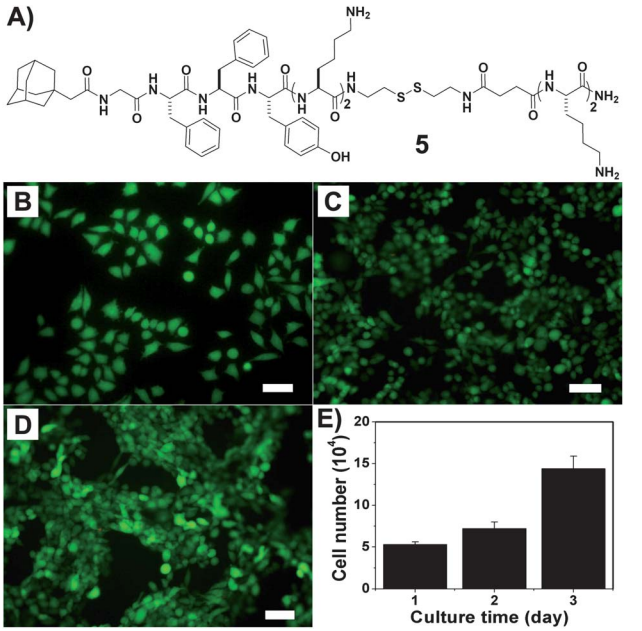

Fig. 5 (A) chemical structure of the molecular hydrogelator of a adamantane-peptide (Ada-GFFYKK-ss-KK, **5**), Live/dead assay of NIH 3T3 cells cultured on gel of **5** at (B) day 1, (C) day 2, and (D) day 3. (live cells are shown in green and dead ones in red, the scale bars in (A), (B), and (C) represent 50 μm), and (E) proliferation rate of NIH 3T3 cells on gel of **5** determined by a MTT assay.

3.2 Responsive hydrogels for cell culture

Though cells cultured on thixotropic hydrogels could be separated from cell–gel constructs, responsive molecular hydrogels that could convert to clear solutions by external stimulus would be beneficial for the full recovery of cells post culture. We have developed a series of short peptides with a capping group of adamantane that could form hydrogels by disulfide bond reduction. Among these peptides, there was one peptide of Ada-GFFYKK-ss-KK (**5**, Fig. 5A) whose gel could be converted to a clear homogeneous solution by the addition of methyl-β-cyclodextrin due to the complexation between adamantane and the β-cyclodextrin derivative. This observation suggested the potential application of gels of **5** for recovery of cells post culture. As shown in Fig. 5B–D, NIH 3T3 cells attached and grew well on the top of gels of **5**. The cell number kept increasing during the three days' culture period (Fig. 5E). After three days' culture, 3T3 cells could be collected from cell–gel constructs by the addition of methyl-β-cyclodextrin, following centrifugation and washing processes.[97] However, this hydrogel was not suitable for 3D 3T3 cell culture. We are developing hydrogel systems suitable for 3D cell culture and cell collection post culture.

3.3 Surface modification of hydrophobic nanofibers

Aliphatic polyesters are thought to be well-suited for biomedical applications due to their biocompatiblity and biodegradability. Many materials fabricated from them have been approved by the FDA for biomedical applications. However, they are limited in scope due to their hydrophobic properties and lack of functional groups for modification post fabrication. We have developed a simple and versatile strategy for surface modification of nanofibers of poly(3-caprolactone) (PCL) prepared by the electrospinning method.[100] Upon contact with a solution of gelator (Nap-FFGRGD, **6** in Fig. 6), compound **6** could self-assemble to form a thin layer of structure at the surface of PCL nanofibers probably due to the 'surface-induced hydrogelation' reported by the Tiller group.[101,102] Due to the presence of a hydrophilic and bioactive tripeptide of Arg-Gly-Asp (RGD) on **6**, the hydrophilicity of the nanofibers was greatly improved, indicated by the

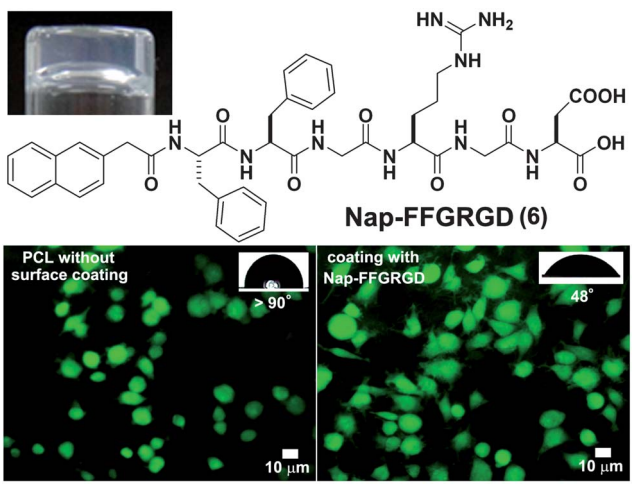

Fig. 6 Chemical structure of Nap-FFGRGD (compound **6**), an optical image of the gel formed by a heating–cooling cycle in PBS buffer solution (pH = 7.4, concentration of compound **6** = 0.5 wt%) and fluorescence images of MSCs (FDA staining) cultured on PCL films before and after surface coating.

water contacting angle decreasing from larger than 90 degrees to about 48 degrees. After post modification, the materials could accelerate cell attachment and promote cell proliferation. A detailed study about the relationship between the morphology of surface-induced self-assembly structures and the chemical structure of the peptides, the pH value of the coating buffer, and the structure of the PCL nanofibers will be reported by us in due course.

3.4 Anti-degradation of proteins

Recombinant proteins frequently suffer from the degradation by protease, which hinders their practical applications. We have developed a simple strategy to improve the stability of a recombinant complex protein by incorporation into a molecular hydrogel of Nap-GFF (**7** in Fig. 7). From the crystal structure of **7**, it adopted a totally anti-parallel dimer structure, thus facilitating efficient molecular stacking in self-assembled structures. Based on this observation, we have synthesized many efficient short-peptide-based hydrogelators of derivatives of Nap-GFF. The complex protein, [Membrane-associated guanylate kinases p55 subfamily member 6 (PDB MPP6), residues 1-106AA complexed with Mals3], was not stable in buffer solutions at room temperature probably due to the presence of residual proteases. After 2 days in solution it would totally decompose. Its stability could be improved by incorporation in the gel of **7**; there was no obvious degradation of the complex protein being observed in the gel of **7** at room temperature after 48 hours and about 58% of the protein remained after 7 days. The results indicated that hydrogels of **7** not only had the potential to be developed into promising biomaterials for long term storage of recombinant proteins at room temperature, but also would be useful for the delivery of proteins to treat different diseases.[103]

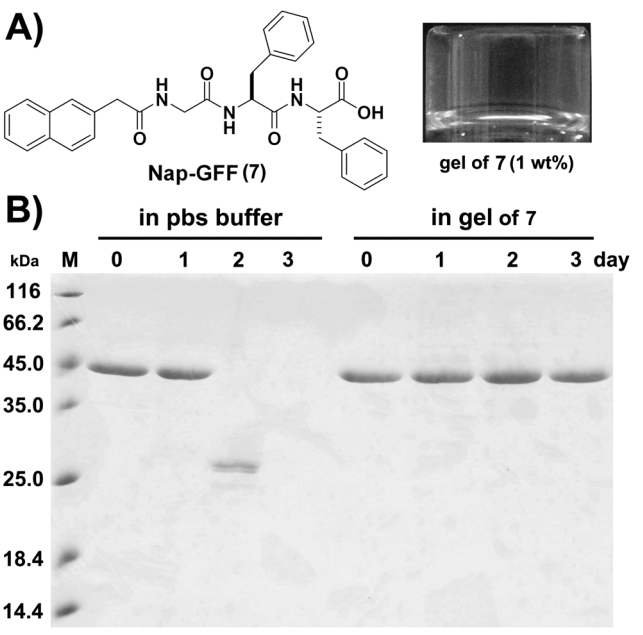

Fig. 7 (A) The chemical structure of Nap-GFF (**7**) and an optical image of gel with 1 wt% of **7** in PBS buffer (pH = 7.4) and (B) the scanning image of SDS-PAGE gel electrophoresis of recombinant MPP6 complex protein in the PBS buffer solution (pH = 7.4) and in gel of **6** at day 0–3.

4. Molecular hydrogels for drug delivery

Molecular hydrogels have been widely used as carriers for the delivery of therapeutic agents.[12,25,27,28,64] Therapeutic agents can be physically entrapped in the matrix of self-assembled nanofibers in hydrogels or be covalently bond to hydrogelators *via* hydrolysable bonds.[20,23–27,57,104] The encapsulated therapeutic agents can then be released from the gels *via* hydrolysis of hydrolysable chemical bonds, diffusion, and enzymatic degradation of the gels.[20,23–27,57,104,105] Besides using molecular hydrogels as carriers for the delivery of therapeutic agents, molecular hydrogels of therapeutic agents or derivatives of therapeutic agents have been reported as novel carrier-free self-delivery systems.[106] Our lab focuses on the development of molecular hydrogels of hydrophobic therapeutic agents, especially those of anti-cancer drugs (hydrophobic anti-cancer drugs themselves and their derivatives).[13,48,107,108] These hydrogels could only be formed by the hydrolysis process and we have made a series of hydrogelators of taxol, which will be discussed in the following sections.

4.1 Molecular hydrogels of hydrophobic compounds

Amphiphilic molecules can form extensive hydrogen bonds and hydrophobic interactions. Up to now, most molecular hydrogelators are amphiphilic molecules. We thought that some hydrophobic molecules could also form hydrogels. In order to form hydrogels of hydrophobic compounds, there were two crucial factors: hydrophilic derivatives of hydrophobic gelators and the hydrolysis process. Water soluble hydrophilic derivatives of hydrophobic gelators firstly formed homogeneous stable aqueous solutions. Upon the hydrolysis processes releasing hydrophobic gelators, the hydrophobic gelators and their hydrophilic derivatives co-assemble into 3D nanostructures supporting the formation of hydrogels. As shown in Fig. 8, Fmoc-tyrosine methyl ester (**9**) was highly hydrophobic with a solubility of lower than 0.01 mg mL^{-1} and could not form hydrogels by the processes of heating–cooling cycles or pH adjustment. Hydrogels of **9** could only be formed by a hydrolysis process catalyzed by phosphatase and the resulting hydrogels were stable over a wide range of pH values (there was no obvious change for 48 hours (*e.g.* swelling or dissolution upon contacting 1 equal volume of a water solution at pH values from 0 to 9.0 at 25 °C). The hydrophobic nanofibers of **9** were probably stabilized by the residual **8** with a similar chemical structure to **9**. The Xu group also reported a similar hydrogel system of a

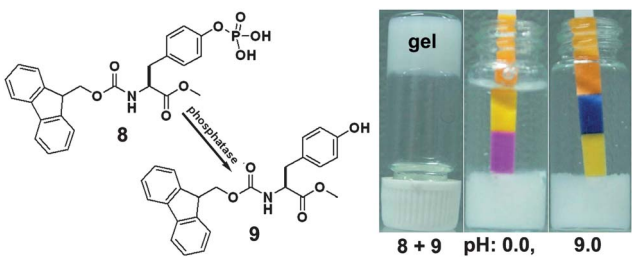

Fig. 8 Molecular hydrogels of a hydrophobic compound (Fmoc-Tyr-OMe, **9**) formed by phosphatase; the resulting hydrogels were stable over a wide range of pH values.

hydrophobic amino acid derivative. These anisotropic hydrogels could only form by a hydrolysis process catalyzed by bases or esterase and were stable at pH 0–14.[109] These results on molecular hydrogels of hydrophobic compounds extended the scope of molecular hydrogelators and might lead to novel hydrogel systems for controlled delivery of therapeutic agents.

4.2 A BSA-stabilized molecular hydrogel of a hydrophobic peptide

In the hydrogel described in above section, the hydrophobic nanofibers of **9** were probably stabilized by its precursor (**8**). In the absence of hydrophilic **8**, it was impossible to form stable 3D networks for hydrogelation. There was an alternative approach to stabilize self-assembled nanofibers of hydrophobic compounds—with the assistance of proteins. As shown in Fig. 9, Nap-GFFY-s (**11**) could be produced from its precursor of Nap-GFFY-ss-EEE (**10**) by disulfide bond reduction. We observed the formation of a hydrogel during the hydrolysis process because the nanofibers of **11** could be stabilized by **10**. However, the hydrogels were not stable after several hours because most of **10** has been converted to **11** by reductants. In the presence of the bovine serum albumin (BSA), the stability of hydrogels could be greatly improved and hydrogels were stable at room temperature for more than two months. The observation indicated that BSA could stabilize the hydrophobic self-assembled nanofibers of **11** probably because BSA had a hydrophobic pocket allowing the interaction to hydrophobic compounds.[110] The development of protein-stabilized hydrogel systems would also extend the scope of hydrogelators and might give novel functionality to molecular hydrogels.

4.3 Molecular hydrogels of taxol derivatives

Many therapeutic agents are hydrophobic, such as the anticancer drugs taxol and camptothecin. They are usually formulated as concentrated solutions in organic solvents such as cremophor EL and needed to be diluted in aqueous solutions right before use.[111] Using molecular hydrogels as carriers to deliver these hydrophobic therapeutic agents or developing molecular hydrogels of these drug molecules may lead to novel delivery systems for practical use. The Xu group had reported a hydrogel system formed by a taxol derivative.[12] They connected taxol with a phosphorylated peptide of Nap-FFKpY *via* a hydrolysable ester bond. The resulting conjugate (**12** in Fig. 10)

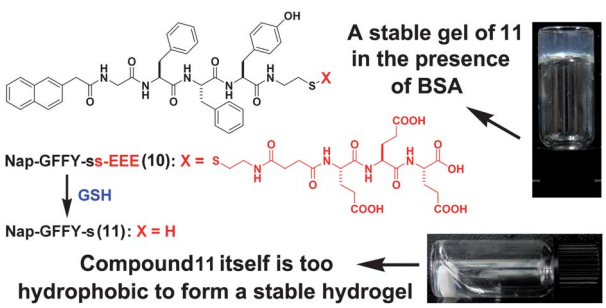

Fig. 9 A diagram to illustrate the conversion from **10** to **11** by GSH (compound **11** was very hydrophobic and could not form a stable hydrogel, while it could form a stable gel in the presence of BSA).

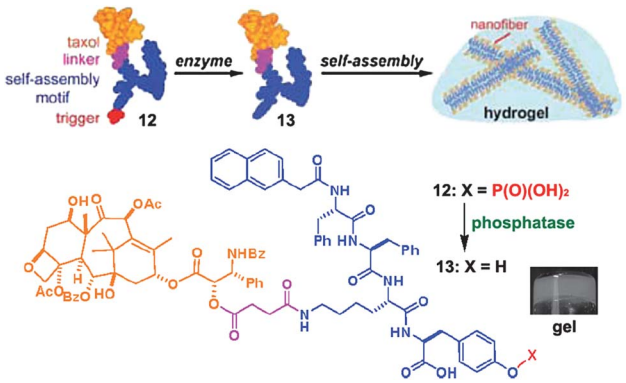

Fig. 10 Illustration for phosphatase-catalyzed formation of molecular hydrogels of a taxol derivative.

could be converted into a gelator (**13**) by phosphatase, thus leading to the formation of nanofibers and molecular hydrogels of taxol. Similar to our results, the hydrogels of taxol could only form by hydrolysis process but not heating–cooling cycle or pH adjustment. Remarkably, this hydrogel released taxol or a taxol derivative at a constant rate, which released 0.13% of **13** per hour in the 24 hours' experimental period. The release profile could be controlled by mixing gels of **13** with gels of Nap-FFKY at different ratios. For example, a mixed gel formed by treating a solution containing 0.6 wt% of **12** and 0.6 wt% of Nap-FFKpY with phosphatase could release taxol at a constant rate of 0.046% per hour. Taxol derivatives exhibited similar activities against cancer cells, suggesting their big potential as a long acting delivery systems for chemotherapy.

Stimulated by the report of Xu, our group rationally designed and synthesized a folic acid (FA)–taxol conjugate of FA-GpYK-taxol (**14** in Fig. 11). There were many FA derivatives with the self-assembly ability able to form stable tetramer structures. Therefore, we choose FA to assist the self-assembly of FA–taxol conjugates. Upon the addition of phosphatase, FA-GpYK-taxol was converted to FA-GYK-taxol that could self-assemble into molecular hydrogels. Unlike other hydrogels commonly with the morphology of nanofibers, gels of the FA–taxol conjugate showed a nanosphere morphology with uniform size of about 50 nm. The nanospheres stuck to each other to form a three

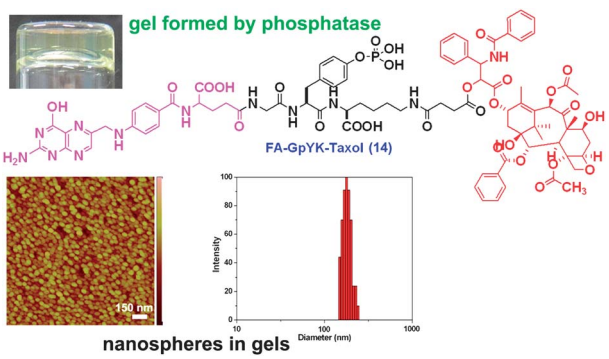

Fig. 11 Chemical structure of the FA-GpYK(taxol) (**14**), optical image of a hydrogel formed by phosphatase, nanostructure in the gel characterized by atomic force microscopy and dynamic light scattering result of solution of the gel.

dimensional network for hydrogelation. Since the gel was formed by nanospheres, it could be converted to a clear solution upon vortexing or shaking and the resulting solution would not change back to a gel. This interesting phenomenon will be studied in near future. The nanospheres were stable in different kinds of aqueous solutions including PBS buffer and cell culture DMEM medium with 10% of FBS at 37 °C. The weight percentage of taxol in nanospheres was 49.4%, which was much higher than that in most of other delivery systems. The nanospheres might be dispersed in solutions for intravenous administration. Taken advantage of the targeting effect of FA to tumor cells and the so called enhanced permeability and retention (EPR) effect of the nanospheres, the nanospheres might be enriched in tumor tissues, which could improve the efficiency and reduce side effects of taxol during chemotherapy. This was the first example of molecular hydrogel formed by a FA derivatives. Our group is synthesizing more FA derivatives and testing their gelation ability.[107]

To the best of our knowledge, all molecular hydrogels of therapeutic agents reported were based on a single drug molecule component. However, combination therapy by using more than one kind of drug is widely used in the clinic, especially in cancer chemotherapy. Combination chemotherapy could improve efficiency, reduce side effects, and overcome drug resistance during chemotherapy. Our lab has reported the first example of molecular hydrogel system based on two complementary anticancer drugs for chemotherapy (Fig. 12).[108] This hydrogel system was formed by the self-assembly of conjugates of dexamethasone and taxol–10-hydroxycamptothecin (HCPT). Since taxol and HCPT were modified through ester bonds, the hydrogels could release original drug molecules by ester bonds hydrolysis. Both kinds of drug molecules could be released from mixed gels. Dexamethasone is an anti-inflammatory and immunosuppressant agent. It is usually given alone or in combination with other anti-cancer drugs during chemotherapy. Our hydrogel system with two coexisting complementary drugs might be used as a co-delivery system for the treatment of cancer.

4.4 Molecular hydrogels of taxol itself

The above mentioned molecular hydrogels and most of reported molecular hydrogels of therapeutic agents were formed by derivatives of drug molecules. It took time and much cost to develop their molecular hydrogels into delivery systems for practical uses. It would be much easier for practical applications if molecular hydrogels of therapeutic agents themselves could be

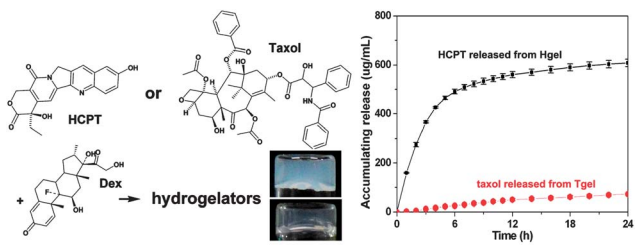

Fig. 12 The combination of HCPT–taxol and dexamethasone will lead to molecular hydrogelators and original drugs of HCPT and taxol can be released sustainably from the gels.

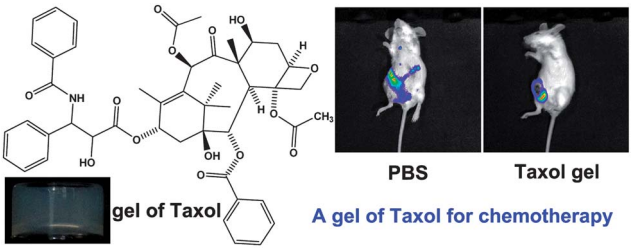

Fig. 13 Tumor growth inhibition and metastasis prevention by molecular hydrogels formed by taxol itself.

used without any modification. Actually, the only two FDA approval drugs (Lanreotide and Degarelix) based on molecular hydrogels were formed by therapeutic agents themselves.[112–114]

During the preparation of solution of taxol derivatives, we occasionally found that taxol itself could form molecular hydrogels by a hydrolysis process.[115] Therefore, we had designed and synthesized a taxol derivative of taxol-Succ-GSSG. The oxidized glutathione of GSSG could enhance the solubility of the taxol derivative. After keeping the clear solutions of taxol-Succ-GSSG in buffer solutions such as PBS (pH = 7.4) at room temperature or 37 °C for several hours (at least 4 hours), the clear solutions would change to hydrogels. Without this hydrolysis process, taxol could not form hydrogels by heating–cooling cycles or pH adjustment. The hydrogels formed by the hydrolysis process were injectable and could be administrated into solid tumors. As shown in Fig. 13, the administration of taxol hydrogels into 4T1 tumors could dramatically reduce the size of tumors and prevent the metastasis of cancer. We believed that molecular hydrogels of therapeutic agents themselves would have great potential for practical applications and our lab will report more examples of these molecular hydrogels in the near future.

5. Conclusions

This feature article mainly focuses on our efforts to develop novel biocompatible methods to trigger hydrogelation, apply molecular hydrogels for cell culture and tissue engineering, and study the potential of hydrogels of therapeutic agents for practical applications. Though we have gained promising results, there are still many challenges and shortcomings of our system remaining. Up to now, we have not developed even a hydrogel that can be used for both 3D cell culture and recovery of cells post culture; we have not evaluated the *in vivo* compatibility and stability of our hydrogel system; we have developed only one example of a molecular hydrogel of therapeutic agent itself. The potential of other hydrophobic drug molecules to form molecular hydrogels by the hydrolysis process also need to be evaluated; specific disease models such as the mouse breast cancer model and certain kind of rabbit/pig ophthalmic disease model must be chosen to study the potential of the hydrogel system of therapeutic agents for practical use; *etc.* We envision that self-assembled protein hydrogels may provide suitable environments for both 3D cell culture and cell recovery, which will be studied in our lab. We intend to develop more hydrogels from hydrophobic therapeutic agents themselves by the hydrolysis process or by protein-stabilized mechanisms. The long term goals of our lab

are developing hydrogels for practical applications in 3D cell culture, regenerative medicine, protein expression, and drug delivery.

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