Journal of Materials Chemistry

Cite this: J. Mater. Chem., 2012, 22, 16933

www.rsc.org/materials

PAPER

Molecular hydrogelators consist of Taxol and short peptides/amino acids†

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Received 9th April 2012, Accepted 26th June 2012
DOI: 10.1039/c2jm32203j

Injectable molecular hydrogels hold big potential for the local delivery of anti-cancer drugs for chemotherapy. We recently reported on a molecular hydrogelator of two complementary anti-cancer drugs of dexamethasone and Taxol. In this study, we study in detail the structure–gelation property of Taxol derivatives. We found that even the conjugates of Taxol and an amino acid such as glutamic acid, serine, and arginine with hydrophilic side chains were efficient molecular hydrogelators. The six gels reported in this paper were characterized by rheology and TEM and the release profile of Taxol from gels were also studied. Gels reported in this study possessed high drug (Taxol) loading percentages in self-assembled nanofibers, exhibited constant and sustained release of Taxol from gels, and were injectable. Our results demonstrated that Taxol derivatives were efficient gelators and the injectable gels formed by them might be developed into local delivery systems for chemotherapy.

1. Introduction

Cancer is one of the leading causes of death in the world. Up to now, surgery in combination with systemic chemotherapy is still the most effective way to save cancer patients and prolong their life.[1] However, the clinically used anti-cancer drugs such as Taxol and camptothecin are limited due to their low water solubility and the systemic chemotherapy will cause serious adverse side effects to patients. In order to improve the solubility of hydrophobic anti-cancer drugs and reduce side effects of systemic chemotherapy, many drug delivery systems composed of nano-sized vehicles and anti-cancer drugs have been reported and some of them have been used in clinic.[2,3] For example, nanoparticles of albumin can improve the solubility of Taxol and the resulting Abraxane (protein-bound paclitaxol) has been approved by the FDA for the treatment of metastatic breast cancers. The Wang group also designed a dual pH-sensitive nanoparticle system for the highly selective delivery of the anti-cancer drug to cancer cells including cancer stem cells.[3] Besides these successful examples of drug delivery systems, molecular hydrogels have attracted considerable attention for the delivery of anti-cancer drugs in recent years.[4] The self-assembled nanofibers in molecular hydrogels can be used as carriers for the delivery of hydrophobic anti-cancer drugs.[5] What's more, molecular hydrogels of anti-cancer drug derivatives have been reported with high drug loading capacities and are carrier-free drug delivery systems, which may further push forward the practical application of molecular hydrogels for chemotherapy.[6–8]

Molecular hydrogels,[9] formed by the self-assembly of small molecules,[10] have shown large potentials in fields of drug delivery,[11] 3D cells culture,[12] *etc.* The first example of a molecular hydrogel formed by a derivative of a clinically used anti-cancer drug was reported by Xu and co-workers.[6] The gels were formed by the conjugate of Taxol and a short peptide derivative of Nap-FFKY and it exhibited sustained release profiles of Taxol/Taxol derivatives. Stimulated by their results, our group has combined Taxol and the tumor targeting folic acid in a molecular hydrogelator that can self-assemble into nanospheres by the enzyme of phosphatase.[7] Recently, we reported on molecular hydrogels of two synergetic drugs, dexamethasone (Dex) and Taxol/camptothecin.[8] The preliminary results showed that the above reported molecular hydrogelators exhibited similar efficacies to inhibit cancer cell growth to original anti-cancer drugs and the gels can sustain the release original anti-cancer drugs or drug derivatives. However, preparing precursors of these molecular hydrogelators of anti-cancer drugs requires multiple synthetic steps, which leads to low yields and might hinder their practical applications. Thus, it will be beneficial to develop simpler molecular hydrogelators of anti-cancer drugs by easy synthetic pathways with high yields. In this paper, we reported several novel molecular hydrogelators consisting of Taxol and short peptides/amino acids. We found that even conjugates of Taxol and an amino acid with a hydrophilic side chain were efficient molecular hydrogelators.

2. Experimental

2.1 Materials and general methods

Fmoc-amino acids were obtained from GL Biochem (Shanghai). Taxol was purchased from Baoman Biotechnology (Shanghai).

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† Electronic supplementary information (ESI) available: characterization of the compounds, rheology, congress curve of cell inhibition, and optical images of gels post injection. See DOI: 10.1039/c2jm32203j

Succinic acid was obtained from Sigma. All the other starting materials were obtained from Alfa. Commercially available reagents and solvents were used without further purification, unless noted otherwise.

The synthesized compounds were characterized by ^{1}H NMR (Bruker ARX-400) using DMSO-d_6 as the solvent and ESI-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System. HPLC was conducted at LUMTECH HPLC (Germany) system using a C18 RP column with MeOH (0.05% of TFA) and water (0.05% of TFA) as the eluents. TEM samples were prepared as follows: a copper grid coated with a thin layer of carbon was dipped into the hydrogel. 10 seconds later, 20 μL of doubly distilled H_2O was used to wash the copper grid three times. A 6 μL solution of Uranyl acetate was used to stain the sample for 5 seconds, the staining solution was removed by a filter paper, and the copper grid was kept in a desiccator for about 30 minutes. The prepared sample was performed at the JEM100CXII system, operating at 100 kV. LC-MS was conducted at the LCMS-20AD (Shimadzu) system, HR-MS was performed at the Agilent 6520 Q-TOF LC/MS.

2.2 Synthesis of the peptide

All the peptides were prepared by standard solid-phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding Fmoc-protected amino acids with side chains properly modified. The first amino acid was loaded onto the resin at about 0.7 mmol g^{-1} of resin. After loading the first amino acid to the resin, the capping regent (DCM : MeOH : DIPEA = 17 : 2 : 1) was used to ensure that all the active sites of the resin were protected.A solution of 20% piperidine in DMF was used to remove the Fmoc group, next the Fmoc-protected amino acid was coupled to the free amino group using HBTU as the coupling reagent. The growth of the peptide chain followed the established Fmoc SPPS protocol. The crude peptides were collected using the TFA-mediated cleavage method: the peptide derivative was cleaved using 95% of trifluoroacetic acid with 2.5% of TIS and 2.5% of H_2O for 30 minutes. 20 mL per gram of resin of ice-cold diethyl ether was then added to cleavage the reagent. The resulting precipitate was centrifuged for 10 min at room temperature at 10 000 rpm. Afterward the supernatant was decanted and the resulting solid was dissolved in DMSO for HPLC separation. HPLC was used to separate the peptides.

2.3 Preparation of different precursors

100 mg (0.10 mmol) of Taxol-SA was dissolved in 10 mL of dichloromethane (DCM), followed by 1.1 equiv. (13.2 mg, 0.115 mmol) *N*-hydroxysuccinimide (NHS) and 25.9 mg (0.126 mmol) of *N*,*N*′-dicyclohexylcarbodiimide with a catalytic amount of 4-dimethylamiopryidine were added. After being stirred at room temperature for 3 h, the solution was filtered by a filter paper to remove the precipitation. The filtrate was evaporated under reduced pressure to yield a white power, which was used directly for the next step. After the white powder obtained above was dissolved in 5 mL of *N*,*N*-dimethylformamide, 1.3 equiv. of corresponding peptide or amino acid was then added with 3 equiv. *N*-diisopropylethylamine (DIPEA). The resulting reaction mixture was stirred overnight and the title products were purified by reverse phase HPLC.

2.4 Characterization of precursors of gelators

Dex-K(Taxol)E-ss-EE. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97–8.06 (m, 4H), 7.85–7.93 (m, 3H), 7.72–7.76 (m, 1H), 7.65–7.69 (m, 2H), 7.55–7.61 (m, 1H), 7.47–7.52 (m, 2H), 7.44–7.46 (m, 3H), 7.30 (d, J = 10.28, 1H), 7.18–7.20 (m, 1H), 6.21 (s, 1H), 6.23 (d, J = 10.20, 1H), 6.01 (s, 1H), 5.79–5.85 (m, 1H), 5.51–5.60 (m, 2 H), 5.33–5.42 (m, 3H), 5.16 (s, 1H), 5.02–5.07 (m, 1H), 4.90–4.94 (m, 2H), 4.76–4.81 (m, 1H), 4.64 (s, 1H), 4.25–4.31 (m, 1H), 4.09–4.17 (m, 5H), 3.98–4.04 (m, 2H), 3.57–3.59 (m, 1H), 3.40–3.44 (m, 1H), 3.17 (m, 1H), 2.84–3.06 (m, 3H), 2.72–2.78 (m, 3H), 2.60–2.68 (m, 4H), 2.22–2.39 (m, 16H), 2.08–2.19 (m, 4H), 1.88–2.00 (m, 3H), 1.70–1.82 (m, 7H), 1.58–1.67 (m, 4H), 1.48–1.54 (m, 7H), 1.23–1.36 (m, 6H), 0.99–1.03 (m, 6H), 0.88 (s, 2H), 0.78 (d, J = 6.74, 3H), HR-MS: calcd M^+ = 2176.82, obsvd $(M + H)^+$ = 2177.8274.

Ac-K(Taxol)E-ss-EE. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98–8.00 (m, 2H), 7.85 (d, J = 7.63, 2H), 7.72–7.75 (m, 1H), 7.64–7.69 (m, 2H), 7.55–7.59 (m, 1H), 7.44–7.51 (m, 6H), 7.20–7.21 (m, 1H), 6.30 (s, 1H), 5.82–5.87 (m, 1H), 5.54–5.59 (m, 2H), 5.34–5.43 (m, 2H), 4.91–4.93 (m, 2H), 4.65 (s, 1H), 4.09–4.15 (m, 1H), 3.99–4.02 (m, 2H), 3.59 (d, J = 6.58, 1H), 3.52 (s, 1H), 3.33 (s, 11H), 2.95–2.99 (m, 2H), 2.79–2.81 (m, 4H), 2.59–2.68 (m, 2H), 2.29–2.37 (m, 2H), 2.24 (s, 3H), 2.11 (s, 3H), 1.79–1.86 (m, 1H), 1.55–1.76 (m, 9H), 1.49–1.54 (m, 5H), 1.20–1.27 (m, 3H), 1.00–1.14 (m, 10H), HR-MS: calcd M^+ = 1744.63, obsvd $(M + H)^+$ = 1745.6378.

Taxol-K(ac)E-ss-EE. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94–8.00 (m, 3H), 7.84–7.86 (m, 2H), 7.72–7.78 (m, 2H), 7.65–7.69 (m, 2H), 7.54–7.59 (m, 1H), 7.44–7.52 (m, 6H), 7.19–7.22 (m, 1H), 6.30 (s, 1H), 5.80–5.85 (t, 1H), 5.52–5.57 (t, 1H), 5.42 (d, J = 6.99, 1H), 5.36 (d, J = 8.65, 1H), 4.90–4.94 (m, 2H), 4.65 (s, 1H), 4.25–4.31 (m, 1H), 4.11–4.22 (m, 4H), 3.99–4.04 (m, 2H), 3.59 (d, J = 6.97, 1H), 3.35–3.52 (m, 10H), 3.24–3.34 (m, 4H), 2.96–3.01 (m, 2H), 2.72–2.80 (m, 4H), 2.60–2.67 (m, 2H), 2.30–2.47 (m, 2H), 2.24–2.34 (m, 14H), 2.11 (s, 3H), 1.87–2.00 (m, 3H), 1.77 (m, 10H), 1.55–1.66 (m, 2H), 1.50 (s, 4H), 1.33–1.37 (m, 2H), 1.22–1.24 (m, 2H), 1.00–1.03 (m, 6H). HR-MS: calcd M^+ = 1744.63, obsvd $(M + H)^+$ = 1745.6368.

Taxol-E-ss-EE. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99 (d, J = 7.55, 2H), 7.86 (d, J = 7.27, 2H), 7.72–7.76 (m, 1H), 7.64–7.69 (m, 2H), 7.54–7.59 (m, 1H), 7.44–7.52 (m, 6H), 7.18–7.22 (m, 2H), 6.21 (s, 1H), 5.81–5.85 (t, 1H), 5.53–5.58 (t, 1H), 5.42 (d, J = 6.82, 1H), 5.34–5.37 (m, 1H),4.90–4.93 (m, 1H), 4.66 (s, 1H), 4.25–4.30 (m, 1H), 4.11–4.19 (m, 3H), 3.99–4.04 (m, 2H), 3.58–3.60 (m, 2H), 3.41–3.57 (m, 9H), 3.28–3.32 (m, 4H), 2.74–2.78 (m, 4H), 2.60–2.67 (m, 2H), 2.45–2.47 (m, 2H), 2.21–2.34 (m, 14H), 2.11 (s, 3H), 1.78–2.00 (m, 8H), 1.50–1.73 (m, 7H), 1.00–1.03 (m, 5H), HR-MS: calcd M^+ = 1574.52, obsvd $(M + H)^+$ = 1575.5303.

Taxol-R-ss-EE. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (d, J = 7.56, 2H), 7.71–7.77 (m, 1H), 7.64–7.70 (m, 1H), 7.46–7.60(m, 8H), 7.19–7.21 (m, 2H), 6.29 (s, 1H), 5.80–5.85 (t, 1H), 5.51–5.55 (t, 1H), 5.42 (d, J = 7.27, 1H), 5.34–5.36 (d, 1H), 4.92 (d, J =

7.86, 2H), 4.64 (s, 1H), 4.24–4.30 (m, 1H), 4.07–4.20 (m, 3H), 3.99–4.05 (m, 2H), 3.56–3.59 (m, 1H), 3.06–3.08 (m, 2H), 2.72–2.81 (m, 4H), 2.61–2.68 (m, 2H), 2.20–2.35 (m, 12H), 2.11 (m, 3H), 1.85–1.99 (m, 2H), 1.61–1.82 (m, 8H), 1.40–1.52 (m, 7H), 0.99–1.05 (m, 6H), HR-MS: calcd $M^+ = 1601.58$, obsvd $(M + H)^+ = 1602.5892$.

Taxol-S-ss-EE. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97–7.80 (m, 3H), 7.86 (d, $J = 7.64$, 2H), 7.24–7.76 (m, 1H), 7.64–7.69 (m, 2H), 7.55–7.59 (m, 1H), 7.45–7.52 (m, 6H), 7.18–7.21 (m, 1H), 6.30 (s, 1H), 5.81–5.86 (t, 1H), 5.53–5.58 (t, 1H), 5.42 (d, $J = 7.00$, 1H), 5.34–5.37 (m, 2H), 4.90–4.93 (m, 1H), 4.25–4.31 (s, 1H), 4.08–4.22 (m, 3H), 3.99–4.04 (m, 2H), 3.58–3.60 (m, 1H), 3.49–3.54 (m, 2H), 3.29–3.37 (m, 4H), 2.73–2.77 (m, 4H), 2.60–2.68 (m, 3H), 2.20–2.34 (m, 12H), 2.09–2.14 (m, 3H), 1.88–2.01 (m, 3H), 1.75–1.83 (m, 5H), 1.55–1.74 (m, 3H), 1.50 (s, 3H), 0.97–1.04 (m, 6H), HR-MS: calcd $M^+ = 1532.51$, obsvd $(M + H)^+ = 1533.5204$.

2.5 Preparation of hydrogels

5 mg of a precursor of the gelator was dissolved in 0.49 mL of PBS buffer solution (pH = 7.4). 4.0 equiv. of glutathione (GSH) in 10 μL of PBS buffer (pH = 7.4, adjusted by Na_2CO_3) was then added to the above solution. A gel would form after the solution was kept at room temperature (22–25 °C) or 37 °C for 30 minutes.

2.6 Determination of release profile of Taxol from gels

0.25 mL of gels (1.0 wt%, 8 hours after the addition of GSH) was treated with 0.25 mL of fresh PBS buffer solutions (pH = 7.4). 0.2 mL of the upper buffer solution was taken out to run LC-MS and 0.2 mL of fresh PBS buffer solution was added back each time. The areas of the peaks in LC-MS spectra were used to determine the percentage of Taxol from their corresponding gels. The experiment was conducted in 3 parallel experiments. The experiment was conducted at 37 °C.

2.7 Rheological measurement

All rheological experiments were performed at 37 °C ± 0.1 °C in different modes (dynamic time sweep, dynamic frequency sweep, dynamic strain sweep) using an AR 1500ex rheometer (TA company, America) by 40 mm parallel plates. To minimize evaporation, a solvent trap was employed and a low viscosity mineral oil was applied around the sample. Time sweep experiment data were collected at 1.0 rad s^{-1} frequency and 1.0% strain. Dynamic frequency sweep experiments were performed in the range of 0.1–100 rad s^{-1} at 1.0% strain. To study the injectable properties of the hydrogel, time sweep experiments were performed at 1.0 rad s^{-1} frequency and 1.0% strain after the hydrogel had been injected through a 1 mL syringe to the rheometer.

2.8 Determination of IC_{50} values

The cytotoxicity of different peptides and the IC_{50} values of Taxol, Taxol-succ, precursors of gelators, and gelators were measured by the MTT cell viability test. The HepG2 cells were seeded in 96-well plates at a density of 2000 cells per well with a total medium volume of 75 μL and incubated for 24 hours. 25 μL of the solutions containing serials of concentrations of above compounds were then added into the cells. 72 hours later, we replaced the medium with fresh medium supplemented with 15 μL of MTT reagent (5 mg mL^{-1}). After another 4 hours, the medium containing MTT was removed and DMSO (100 μL per well) was added to dissolve the formazan crystals. The optical density of the solution was measured at 490 nm, using a microplate reader (Bio-RAD iMarkTM, America). Cells without the treatment of the compounds were used as the control. The experiment was repeated for 3 times. The cell viability percentage was calculated by the following formula: The cell viability percentage (%) = $OD_{sample}/OD_{control} \times 100\%$. Prism 5.0 software was used to calculate the IC_{50}.

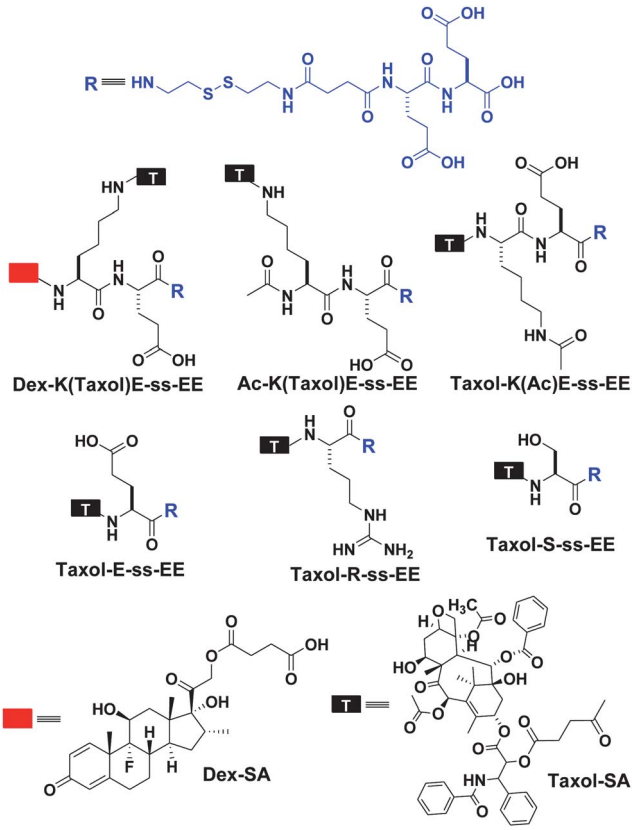
3. Results and discussion

3.1 Gelation ability

Our previous results indicated that Dex-FFFK(Taxol)E-ss-EE (Dex, F, K, E, and -ss- represented dexamethasone, phenylalanine, lysine, glutamic acid, and disulfide linker, respectively) could form hydrogels both at room temperature and 37 °C after being converted to Dex-FFFK(Taxol)E-s by glutathione (GSH).[8] However, the structure of Dex-FFFK(Taxol)E-ss-EE is very complicated and the resulting hydrogels are only stable for about two weeks. Therefore, we opt to refine the chemical structure of Dex-FFFK(Taxol)E-ss-EE to develop structurally simpler molecular hydrogelators of Taxol.

We firstly removed tripeptide of FFF from Dex-FFFK(Taxol)E-ss-EE to make Dex-K(Taxol)E-ss-EE (Scheme 1). We observed the formation of a molecular hydrogel (DexK(Taxol)E-gel in Fig. 1A) by adding 4 equiv. of GSH to a phosphate buffer solution (PBS, pH = 7.4) containing 1.0 wt% of Dex-K(Taxol)E-ss-EE within 10 minutes. Similar to gels formed from solutions of Dex-FFFK(Taxol)E-ss-EE, the gels from Dex-K(Taxol)E-ss-EE were not very stable either and only stable for 3 days at 37 °C. We then removed the part of Dex from Dex-K(Taxol)E-ss-EE to make Ac-K(Taxol)E-ss-EE (Scheme 1). Surprisingly, Ac-K(Taxol)E-ss-EE could also form hydrogels (AcK(Taxol)E-gel in Fig. 1B) by GSH. These results indicated that both Dex and the tripeptide of FFF were not requisites for building molecular hydrogelators of Taxol.

There are many gelators of lysine (K) derivatives with hydrophobic molecules attached on the ε-position amine group of K.[13] In order to study the influence of the position of Taxol on the two amine groups of K, we prepared the compound of Taxol-K(Ac)E-ss-EE (Scheme 1). It could also form gels (Taxol-K(Ac)E-gel in Fig. 1C) by GSH. We then removed K(Ac) from Taxol-K(Ac)E-ss-EE to make Taxol-E-ss-EE. It was interesting that Taxol-E-ss-EE could also gel aqueous solutions after being converted to Taxol-E-s (Taxol-E-gel in Fig. 1D). We then changed E to other amino acids with different charges such as arginine (R) with a positive side chain and serine (S) with a neutral side chain. Both solutions of Taxol-R/S-ss-EE could also be converted to gels by adding GSH (Taxol-R/S-gel in Fig. 1E and 1F). These results indicated that conjugates of Taxol and even a single amino acid were efficient molecular hydrogelators. We found that the Taxol-E-gel was stable at room temperature and 37 °C for at least 6 months. However, other five gels were only stable for less than 7 days and would change to precipitates



Scheme 1 Chemical structures of precursors of molecular hydrogelators.

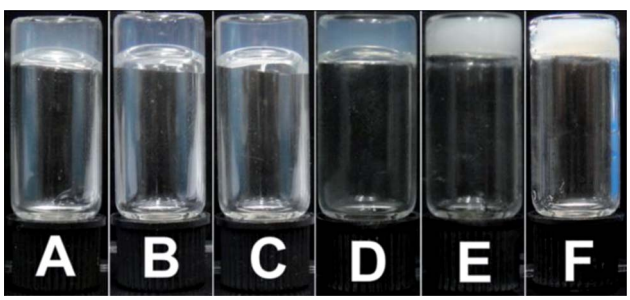


Fig. 1 Optical images of hydrogels formed by treating PBS solutions containing 1.0 wt% of different precursors with 4 equiv. of GSH. (A) Dex-K(Taxol)E-gel (B) AcK(Taxol)E-gel, (C) Taxol-K(Ac)E-gel, D) Taxol-E-gel, (E) Taxol-R-gel, and (F) Taxol-S-gel.

in the end. Except the Taxol-S-gel, the other five as-formed hydrogels were injectable. As shown in Fig. S-15 and S-16,† three hydrogels (AcK(Taxol)E-gel, Taxol-K(Ac)E-gel, and Taxol-E-gel) could re-form within 5 minutes after injection through a 1.0 mL syringe (Becton Dickinson Consumer Products). Both Dex-K(Taxol)E-gel and Taxol-R-gel could not re-form until after at least 12 h. The Taxol-S-gel changed to a partial gel with precipitation after injection. The phenomena suggested that the as-formed hydrogels, except Taxol-S-gel, were injectable, which is beneficial for their future applications. We then characterized these hydrogels by different techniques including rheology and TEM.

3.2 Rheology

We studied the viscoelasticity of all hydrogels, which reflected the mechanical property of them. Dynamic time sweeps could provide useful information about the process from the flow state to gel state and indicate the gelling point when the storage modulus (G') was bigger than the loss modulus (G''). According to Fig. S-13,† six hydrogels could form within 30 min at room temperature after 4 equiv. of GSH had been added to reduce the disulfide bond and convert precursors to their corresponding gelators. Dynamic frequency sweeps (Fig. 2) were then used to compare the mechanical properties of the resulting gels formed 2 hours after the addition of GSH, all hydrogels exhibited similar G' values of around 1000 Pa, except the Taxol-S-gel whose G' value was above 10 000 Pa. Both G' and G'' values of all gels showed weak frequency dependencies at the region of 0.1 to 100 rad s^{-1}, suggesting elastic networks in the hydrogels.

3.3 TEM images of hydrogels

Negative-stained transmission electron microscopy (TEM) images of hydrogels were recorded to study the self-assembled structures in hydrogels. As shown in Fig. 3, we observed networks of nanofibers in all hydrogels. However, the density and morphology of fibers were different. Dex-K(Taxol)E-gel (Fig. 3A), Taxol-R-gel (Fig. 3E), and Taxol-S-gel (Fig. 3F) exhibited dense networks of fibers with the width of around 10–30 nm, suggesting heavy aggregations of self-assembled structures in these gels. Compared with these three gels, both AcK(Taxol)-E-gel (Fig. 3B) and Taxol-K(Ac)-E-gel (Fig. 3C) also showed dense networks of fibers of

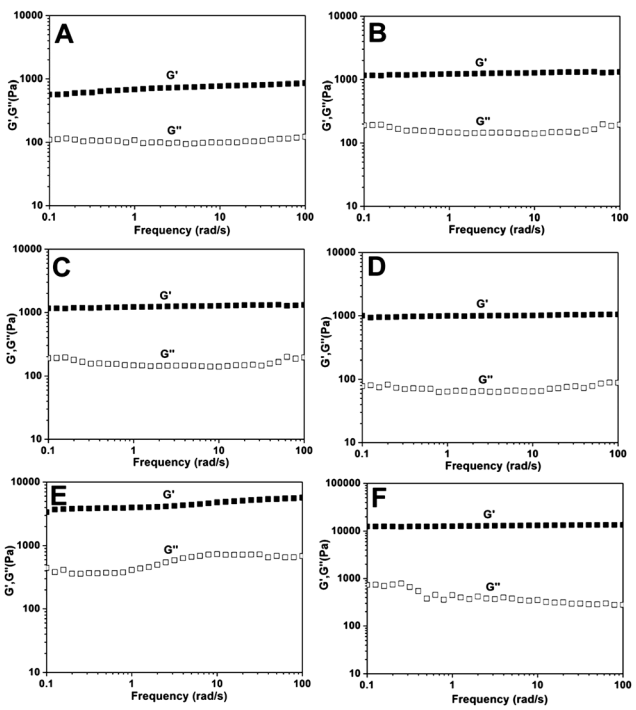


Fig. 2 Rheological measurements in the dynamic frequency sweep mode for all the gels formed 2 h after the addition of 4 equiv. of GSH at a strain of 1.0% (initial precursor concentration = 1.0 wt%): (A) Dex-K(Taxol)E-gel, (B) AcK(Taxol)E-gel, (C) Taxol-K(Ac)E-gel, (D) Taxol-E-gel, (E) Taxol-R-gel, and (F) Taxol-S-gel.

about 10–20 nm in width but the fibers were more evenly distributed. Taxol-E-gel exhibited a totally different morphology of nanofibers to other five gels in where we observed well-distributed uniform nanofibers of about 20 nm. The aggregation degree was followed by the order of Dex-K(Taxol)E-gel ~ Taxol-R-gel ~ Taxol-S-gel > AcK(Taxol)E-gel ~ Taxol-K(Ac)E-gel > Taxol-E-gel, which interpreted the stability of these gels that Dex-K(Taxol)E-gel, Taxol-R-gel, and Taxol-S-gel were only stable for three days, AcK(Taxol)E-gel and Taxol-K(Ac)E-gel were stable for 5–7 days, and Taxol-E-gel was stable for more than 6 months.

3.4 Release profile of Taxol from gels

Fig. 4 (top) showed the release profiles of Taxol from different hydrogels at physiological temperature condition (37 °C). All hydrogels released original Taxol molecule but not Taxol derivatives by the ester bond hydrolysis. Dex-K(Taxol)E-gel released Taxol at a constant rate of about 0.48 μg mL^{-1} per hour from 2 to 24 hours. AcK(Taxol)E-gel exhibited a two stage release profile—it released Taxol at a rate of ~6.0 μg mL^{-1} per hour in the first 12 hours, followed by a rate of ~2.6 μg mL^{-1} per hour in the next 12 hours. Similar to AcK(Taxol)E-gel, Taxol-K(Ac)E-gel also showed a two stage release profile but the rates were different—1.6 and 1.13 μg mL^{-1} per hour in the first 8 hours and the following 16 hours, respectively. Both Taxol-R-gel and Taxol-S-gel showed burst release profiles in the first 12 hours and possessed much slower release speeds in the following 12 hours. Interestingly, Taxol-E-gel exhibited a totally different release profile to the other five gels. There were no burst releases of

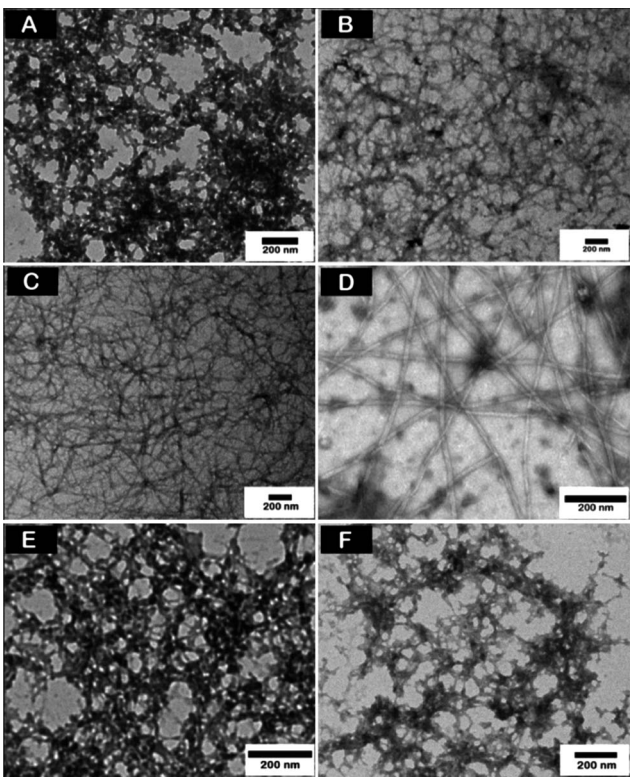


Fig. 3 Negative-stained TEM images of the gels: (A) Dex-K(Taxol)E-gel, (B) AcK(Taxol)E-gel, (C) Taxol-K(Ac)E-gel, (D) Taxol-E-gel, (E) Taxol-R-gel, and (F) Taxol-S-gel.

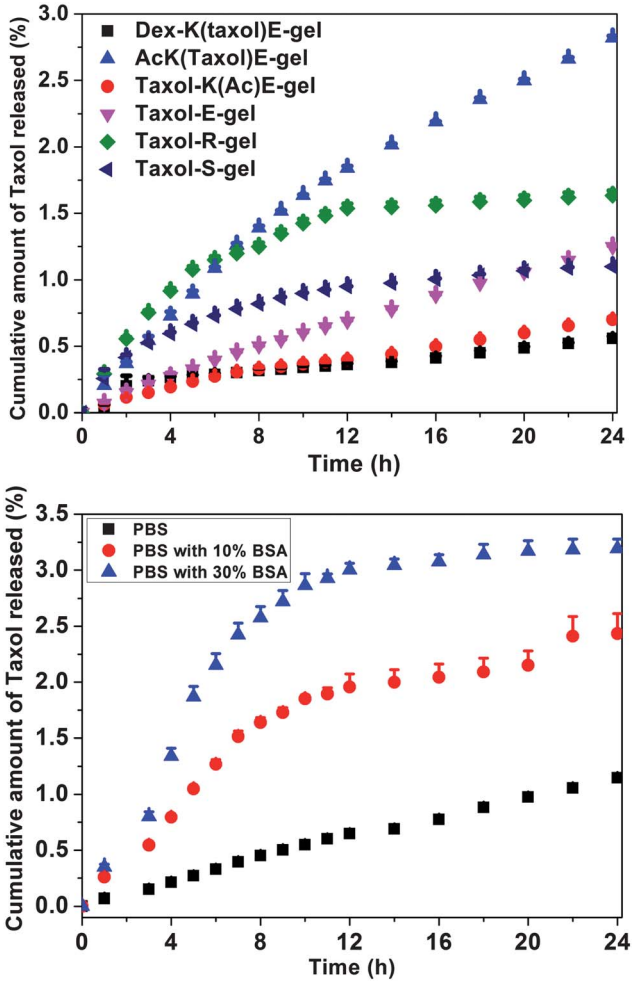


Fig. 4 (top) Accumulative release profile of Taxol from different kinds of hydrogels at 37 °C in 100 mM PBS buffers (pH = 7.4) and (bottom) accumulative release profile of Taxol from Taxol-E-gel at 37 °C in PBS and PBS containing different concentration of BSA.

Taxol from the Taxol-E-gel, and the Taxol-E-gel released Taxol at a constant rate of about 2.3 μg mL^{-1} per hour throughout the entire measurement period of 24 hours. We could not conclude a relationship between the release profiles of Taxol from gels and other characterizations of the gels. We believed that the constant release profile of Taxol-E-gel could be beneficial to its future practical applications.

The presence of surfactant or serum would affect the release profile of drug molecules from hydrogels because surfactant or serum could serve as a solubilising agent to increase the solubility of the hydrophobic drug molecules in aqueous solution.[14] We also obtained the release profile of Taxol from Taxol-E-gel with bovine serum albumin (BSA) as the solubilising agent. As shown in Fig. 4 (bottom), the addition of BSA enhanced the release and changed the release profile of Taxol from the gel. Taxol-E-gel exhibited a constant release in the 24 hour experimental period in the absence of BSA (Fig. 4 (top)), while it showed a two stage release profile in the presence of BSA (Fig. 4 (bottom)). For example, Taxol-E-gel released Taxol at a rate of ~8.15 μg mL^{-1} per hour in the first 10 hours, following by a rate of ~1.72 μg mL^{-1} per hour in the next 14 hours when PBS containing 10% of BSA was used.

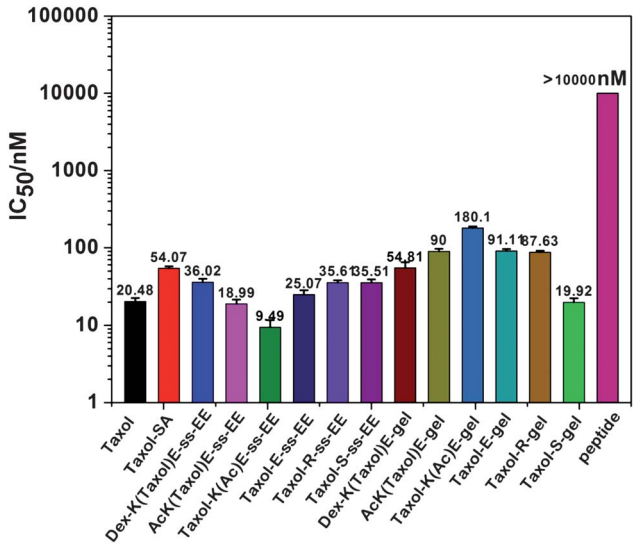


Fig. 5 Cytotoxicity (*y*-axis in log10 scale) of Taxol, Taxol-SA, precursors of gelators, gelators, and peptides without Taxol against HepG2 cells.

3.5 IC_{50} values against HepG2 cells

We also obtained the IC_{50} value of the precursors of gelators and gelators against HepG2 cells (Fig. 5). After 72 hours of incubation with the cells, Taxol and Taxol-SA exhibited an IC_{50} value of 20.48 ± 2.12 and 54.07 ± 3.49 nM, respectively. While, peptides without Taxol showed no obvious toxicities to the cells at a concentration of 10 μM (Fig. S-18†). Precursors of gelators exhibited IC_{50} values of 36.02 ± 3.88, 18.99 ± 2.72, 9.49 ± 2.24, 25.07 ± 3.46, 35.61 ± 2.52, and 35.51 ± 3.83 nM and gelators possessed IC_{50} values of 54.81 ± 10.32, 90.0 ± 7.21, 180.1 ± 8.68, 91.11 ± 5.11, 87.63 ± 4.17, and 19.92 ± 2.61 nM for Dex-K(Taxol)E-gel, AcK(Taxol)E-gel, Taxol-K(Ac)E-gel, Taxol-E-gel, Taxol-R-gel, and Taxol-S-gel, respectively. The IC_{50} values of most of Taxol derivatives were within the range of 10 to 100 nM, indicating that the modification of Taxol had not reduced the activity of Taxol dramatically.

4. Conclusions

In summary, we have developed a series of hydrogelators based on Taxol and short peptides/amino acids with simple synthetic strategies and high yields (more than 75%). The gels showed high weight percentages of Taxol in the self-assembled structures (about 50.0%) and most of them were injectable. These gels exhibited different stabilities and release profiles of Taxol due to the different chemical structures of gelators. Though we were unable to conclude a relationship between the chemical structures and the release profiles/stabilities of the hydrogels, we have found the gel of Taxol-E-gel, with an extraordinary stability, could release original Taxol at a constant rate without any burst releases. This was a detailed study on the gelation ability of Taxol conjugates with short peptides and amino acids, which could provide useful information for the development of Taxol-hydrogels for practical chemotherapy applications.

Acknowledgements

This work is supported by NSFC (21172023), A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institution, and Tianjin MSTC (11JCZDJC17200).

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