Encapsulation of 10-Hydroxy Camptothecin in Supramolecular Hydrogel as an Injectable Drug Delivery System

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ABSTRACT: 10-Hydroxy camptothecin (HCPT) has been proven to be a cell cycle-specific chemotherapeutic agent, which is a necessary choice to inhibit tumor residue growth and prevent tumor metastasis after surgery. But it suffers from light decomposition, poor solubility, relatively low bioavailability, and some side effects, which are the major obstacles toward its clinical use. Integration of hydrophobic HCPT with hydrophilic hydrogel is a facile approach to change the disadvantageous situation of HCPT. In this study, a novel supramolecular hydrogelator with improved synthetic strategy was triggered by chemical hydrolysis, and then self-assembled to hydrogel. Taking advantage of the high-equilibrium solubility of HCPT in hydrogelator solution, this hydrogel was utilized to load HCPT via encapsulation as an effective carrier. HCPT hydrogels were characterized by several techniques including transmission electronic microscopy, rheology, and UV spectroscopy. *In vitro* release experiment indicated HCPT hydrogel could maintain long term and sustained release of HCPT at high accumulated rate. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay showed that HCPT hydrogel had an optimized anticancer efficacy. Besides, with prominent physical properties of carrier, HCPT hydrogel possessed satisfactory stability, syringeability, and recoverability, demonstrating itself as a potential localized injectable drug delivery system. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2266–2275, 2015

Keywords: 10-hydroxy camptothecin; supramolecular hydrogel; chemical hydrolysis; injectable drug delivery; Drug delivery systems; Hydrogels; Cancer chemotherapy; Encapsulation; Peptides

INTRODUCTION

Hydrogels, with highly hydrated, porous nature, and alterable chemical or physical properties, have been applied in fields such as medical implants, tissue engineering, and drug delivery.[1–6] For delivery applications, hydrogels have been used to incorporate drug molecules into the gel matrix to create reservoirs that deliver bioactive agents.[7] Although a majority of hydrogels are polymeric ones whose networks consist of covalently cross-linked natural or synthetic polymers, supramolecular hydrogels, whose networks consist of nanofibers formed through the self-assembly of small molecules (i.e., hydrogelators), have received remarkable scientific interests as promising biomaterials in the last decade.[2,8–11] Excellent drug delivery systems have been successfully developed based on hydrogelators of therapeutic agents.[12–15] By introducing a pyrene group, Xing et al.[16] modified vancomycin and turned it into a vancomycin–pyrene hydrogel, serendipitously enhancing nearly three orders of magnitude potency. One of these therapeutic agents commercially called "Lanreotide" has been approved by US FDA for clinical use.[17,18] First example of a codelivery hydrogel system of two complementary anticancer drugs,[19] for chemotherapy was successfully completed by chemical covalent binding, which might be administrated in the cavity after surgical tumor removal for the long-term release of anticancer drugs to improve the efficiency, reduce side effects, and overcome drug resistance during chemotherapy. Recently, Ou et al.[20] provided an additional excellent choice of aromatic capping group, for example, PTZ-acetic acid, to generate short peptide-based supramolecular hydrogelators with super gelation ability. This achievement brought more opportunities for the development of supramolecular hydrogels.

Cancer chemotherapy is essential and to date remains the best way to eliminate tumor residues and prevent tumor metastasis after surgery, yet widely used anticancer drugs such as Taxol and cis-platinum-based drugs often have limited water solubility and result in side effects.[19] In order to minimize the side effects, some research groups have successfully developed a few supramolecular hydrogels combining chemotherapy with target. A folic acid–Taxol conjugated molecular hydrogel[21] was formed by the reduction of disulfide bond by glutathione. Compared with intravenous injection of clinically used Taxol® with four times the dosage, this hydrogel could inhibit tumor growth more efficiently by a single dose of intratumor administration, which suggested the big potential of this novel gelation system of Taxol for cancer therapy. In one of our previous articles,[22] a novel synergistic dual-targeting molecular self-assembly hydrogel of Taxol was developed by conjugation with estrone and RGD peptide, which transported drugs, specifically targeted tumors, and penetrated breast cancer cells. Confocal imaging

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in vitro and real-time visualization *in vivo* were carried out to evidence enhanced targeted delivery and anticancer effect of Taxol.

Except for chemical conjugation, some groups obtained promising drug delivery systems by simple noncovalent interaction as well. Soukasene et al.[23] used self-assembling peptide amphiphile nanofibers to encapsulate camptothecin using solvent evaporation technique. *In vitro* and *in vivo* tests showed the ability of these amphiphilic nanofibers to inhibit tumor growth, demonstrating the potential of these systems to deliver hydrophobic drugs.[24] Peptide-based hydrogel nanoparticles also represent a promising alternative to current drug delivery approaches. Ischakov et al.[25] designed and developed a new class of self-assembled aromatic peptide-based HNPs consisting of Fmoc-FF core, and vitamin E-TPGS monolayer as an outer shell. Encapsulation of doxorubicin and 5-flourouracil within the HNPs matrix showed release kinetics of the drugs depending on their chemical structure, molecular weight, and hydrophobicity. Results clearly indicated that these HNPs have potential to be used as encapsulator and delivery system. In spite of some significant limitations about supramolecular hydrogels, the most important being that injectable liquids can be cross-linked into hydrogels after injection and then facilitate local delivery of drug molecules. For instance, curcumin hydrogel[26] is prepared *in situ* where curcumin encapsulation within the hydrogel network is accomplished concurrently with peptide self-assembly and demonstrates its effectiveness as an injectable agent for localized curcumin delivery. An injectable delivery system that can sustainedly release drugs may improve the efficiency and reduce their side effects on other organs, which ameliorate the pains of patients more directly.

Hydrophilicity, biocompatibility, and physiologically benign processing conditions are also beneficial properties that make peptide hydrogels attractive candidates as injectable delivery vehicles for therapeutic agents.[27] So hydrogel is absolutely a promising choice to transform hydrophobic drugs into hydrophilic agents. 10-Hydroxy camptothecin (HCPT), a cell cycle-specific agent that can inhibit DNA replication and arrest the cells at S phase, has proven potential for cancer chemotherapy. Similar to other hydrophobic compounds, it suffers from light decomposition, poor aqueous solubility, low bioavailability, and side effects, which are the major obstacles toward its medical deployment.

In order to change the situation, a supramolecular hydrogel was adopted to load HCPT via noncovalent interaction.

Building a conventional drug delivery system[28] usually requires a carrier that loads the therapeutic agents via either noncovalent interaction (physical loading) or covalent bonds (chemical loading).[18] Zhao et al.[29] have successfully developed a new type of supramolecular hydrogel that induced gelation in a new way of chemical hydrolysis by replacing a hydroxyl group with a carboxylate group on the molecular structure of hydrogelator. The controlled hydrolysis of a carboxylic ester bond by a base or an enzyme can act as a simple trigger to initiate self-assembly and form hydrogels, even if the molecule itself is unable to form hydrogels by commonly used methods, such as directly changing pH,[30–34] temperature,[35,36] or ionic strength.[37] Besides, this hydrogel is stable over a wide pH range and insensitive to ionic strength.

In this paper, the synthetic method of supramolecular hydrogel precursor (NapFES) was thoroughly renewed by combining with solid phase synthesis, which raised the yield. As shown in Figure 1, the precursor was turned into hydrogelator (NapFE) by hydrolysis. The hydrogel, derived from self-assembly of hydrogelator, was used to encapsulate HCPT. We believe that the integration of hydrogel with HCPT can not only form a new formulation with better solubility and stability, it also takes advantage of the excellent performance of hydrogel to establish a simple yet robust drug delivery system, which can maintain a relatively stable and sustained-release HCPT in an aqueous, physiological medium with a fairly well-accumulated release rate.

The purpose of this article is to demonstrate the suitability of HCPT hydrogel as an injectable drug delivery system. The upcoming sections emphatically certified its suitability from the aspects of drug loading, mechanical characteristics, *in vitro* drug release, and anticancer efficacy.

MATERIALS AND METHODS

All the Fmoc-amino acid and 2-chlorotrityl chloride resin were obtained from GL Biochem (Shanghai, China). HCPT was obtained commercially from Dalian Meilun Biotech Company, Ltd. (Dalian, China) 1-Hydroxybenzotriazole, N,N-diisopropylethylamine, O-benzotriazole-N,N,N′,N′-tetramethyl -uroniumhexafluoro-phosphate, trifluoroacetic acid, and 4-dimethylamino-pyridine were purchased from Aladdin Reagent Corporation (Shanghai, China). 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was obtained from Biosharp Company (Hefei, China). The human breast cancer stem cells MCF-7 were purchased from American Type Culture Collection (ATCC, China's district general agent, Beijing, China).

Preparation of Precursor Peptide

In this part, we adopted solid phase peptide synthesis method (SPPS), which is easier and more straightforward than solution phase synthesis.[29] Scheme 1 shows the synthetic route and the structure of each small molecule.

First, Fmoc–glycinol and succinic anhydride were used to synthesize the Fmoc–ethanolamine–succinic anhydride (**1**). Then, **1** was used to couple with Fmoc–phenylalanine and 2-naphthylacetic acid by SPPS method. The final peptide (**3**) was obtained easily. Such a simple synthetic pathway ensures large-scale preparation of the compounds for practical use.

As illustrated in Figure 1, upon the action of sodium bicarbonate, precursor **3** hydrolyzes to the hydrogelator (**2**), which self-assembles into nanofibers and affords a supramolecular hydrogel.

Hydrolysis of 3 and the Formation of Hydrogel of 2

Two milligram of **3** was added to 1 mL weak basic pure water (pH 7.5). Gradual addition of base (0.1 M Na_2CO_3) caused the hydrolysis of **3** and induced self-assembly. The solution should be oscillated sufficiently after each addition. Till there was no more solid and the pH of solution was measured as 8, the total volume of base was 80 μL. A clear solution of **2** at the concentration of 0.2% (w/v) self-assembled into a solid and translucent hydrogel of **2** in 12 h without a noticeable pH change. The same protocol was used to prepare 0.3% (w/v) and 0.4% (w/v) hydrogels.

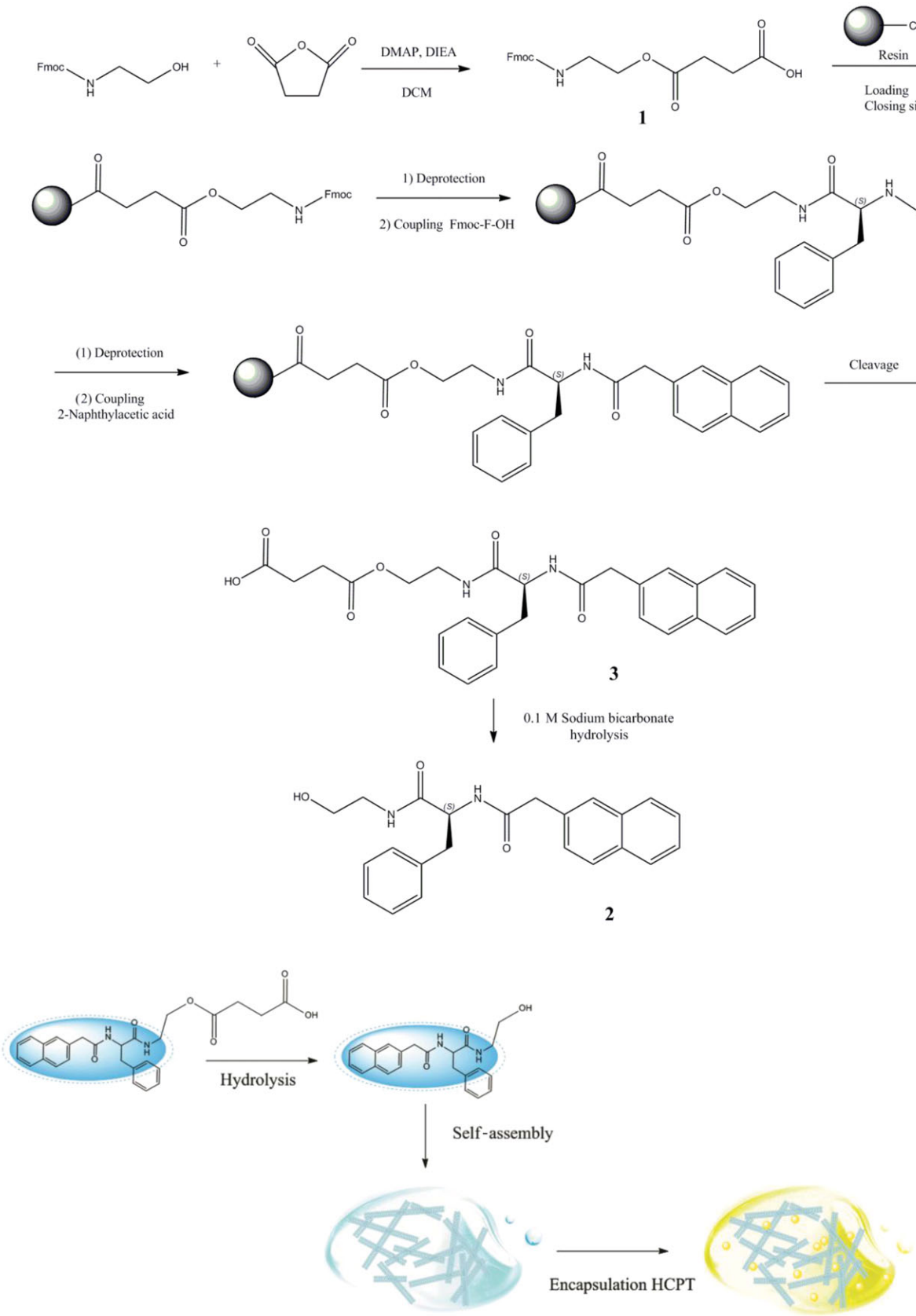

Figure 1. The synthesis route of **3** and schematic of the formation mechanism of HCPT hydrogel.

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Preparation of HCPT Hydrogel

Although HCPT is poorly soluble in organic solvents, it is soluble in weak basic solutions. And the pH of the solution of **2** is just measured as around 8.0. Thus, we took advantage of the weak basic solution of **2** to load HCPT. HCPT (0.2 mg) was dissolved and evenly dispersed to 0.5 mL solution of **2**. Then, self-assembly took place and HCPT hydrogel was formed gradually.

Transmission Electron Microscopy of HCPT Hydrogels

Several drops of diluted hydrogel of **2**, heat-treated hydrogel of **2**, HCPT hydrogel at 0.3% (w/v) and 0.8% (w/v) were applied to a carbon-coated grid and excess water was blotted away with filter paper. The grids were left to dry in ambient condition for 1 h. Transmission electronic microscopy (TEM) was performed with JEOL JEM-2100f TEM (Japan) operating at 220 kV and used to characterize the self-assembled structures in hydrogels.

Rheology

Rheological measurement with the mode of dynamic time sweep, dynamic frequency sweep, and dynamic strain sweep provides useful information on gelation properties of HCPT hydrogel. Rheology experiments were conducted on a Thermo RheoStress 600 instrument using a set of 60 mm diameter parallel plates with the thickness of 0.3 mm at 37°C. Aged HCPT hydrogel (0.8%, w/v) was prepared as described previously. After aged HCPT hydrogel was subjected to 1000 s^{-1} shear for 30 s and immediately transferred to the rheometer, the recovery of the storage (G') and loss (G'') modulus as a function of time (6.28 rad/s frequency, 1% strain) was subsequently monitored for 240 min. Then, dynamic frequency sweep and dynamic strain sweep were performed in turn. Dynamic frequency sweep experiment was performed to establish the frequency response of the samples in the region of 0.1–100 rad/s at the strain of 1%. Dynamic strain sweep experiment was performed on samples to establish the linear viscoelastic region at the frequency of 6.28 rad/s and the strain of 0.1%–100%.

UV Spectroscopy

Ultraviolet–visible spectra of HCPT, supernatants of hydrogel of **2** and HCPT hydrogel at concentration of about 10^{-5} mol/L were recorded using Agilent Technologies UV1800 spectrophotometer (Shanghai, China). In all experiments, solutions were taken in quartz cuvette of 0.1 cm path length.

Equilibrium Solubility of HCPT in Solution of 2

Excessive amount of HCPT was added to fresh solutions of **2** at the concentration of 0.2% (w/v), 0.3% (w/v), and 0.4% (w/v), respectively. After ultrasonic sonication for 6 h to maximize the solubility of HCPT and centrifugation at 10^4 r/min for 15 min, the saturated supernatants were taken out and diluted appropriately by phosphate-buffered solution (PBS) solution at pH 7.4. The absorbance of diluted supernatants was determined and then used to calculate the solubility of HCPT in the solution of **2** according to the standard curve of HCPT. The experiment was conducted in three parallel experiments and the results averaged.

In Vitro Release Profile of HCPT Hydrogels

In vitro release experiment was studied under physiological temperature (37°C). HCPT (0.5 mL) hydrogels at an increasing peptide concentration, that is, 0.2% (w/v), 0.3% (w/v), and 0.4% (w/v), all at fixed 400 μg/mL drug concentration, were prepared to quantify released HCPT into PBS supernatants. First, 0.2 mL of fresh PBS solution (100 mM, pH 7.4) was added onto the top of each hydrogel. At each desired time point, the PBS supernatant was totally taken out for measurement of accumulating release amount of HCPT from hydrogels; then, an equal volume of fresh PBS buffer was added. The experiment was conducted in three parallel experiments.

Cell Culture and HCPT Hydrogels in Contact with MCF-7 Cell

Michigan Cancer Foundation-7 cells line, MCF-7 cells, were cultured in RPMI-1640 supplemented with 10% fetal bovine serum and penicillin/streptomycin (complete cell growth medium) in a humidified, 5% CO_2 atmosphere at 37°C.

Five-hundred microliter of 0.1% (w/v), 0.2% (w/v), 0.4% (w/v), and 0.8% (w/v) HCPT hydrogels was prepared with 0.2 mg and a control group without HCPT. MCF-7 cells were planted in the 24-well plate (1500 cells/well) and incubated at 37°C overnight in cell culture medium. One-hundred microliter of each sample was added into 24-well plate and incubated for another 4 h. And then, light microscope images were taken by MOTIC AE31 inverted biological microscope (CCIS Ph 10×. Xiamen, China).

Anticancer Efficacy Measurement

A total medium volume of 100 μL MCF-7 cells were seeded in a 96-well plate with the density of 1500 cells per well. Twenty-four hour after seeding, the solutions with six geometric concentrations of three different compounds (HCPT, hydrogel of **2**, and HCPT hydrogel) in 100 μL of medium were added to each well (three wells for each concentration). Cells in wells with the treatment of the blank PBS solution were used as the control. After an extra 48 h culture time, the MTT assays were performed. Twenty microliter of MTT solution (5 mg/mL) was added for each well, and then incubated for 4 h in 37°C. All the spent solution was pipetted out; formazon crystals at the bottom of each well were dissolved in 150 μL dimethyl sulfoxide (DMSO). After oscillation for 15 min at room temperature, absorbance at wavelength of 490 nm was tested using a microplate reader (iMark TM, BIO-RAD, Hercules, CA, USA).[19] The experiment was repeated for three times.

RESULTS AND DISCUSSION

Hydrolysis of 3 and the Formation of Hydrogel of 2

Self-assembly of the peptide is initiated with the generation of **2**. The loss of succinic acid from **3** afforded **2** in almost quantitative yield.[29] Critical gelation concentration can be reached at 0.1% (w/v). The time of different peptide concentrations needed for gelation was different: falling within 10 min and approximately 72 h for 1.6% (w/v) and 0.1% (w/v). Higher peptide concentration resulted in shorter gelation time. Rigidity of hydrogel is also proportional to peptide concentration. Increase in peptide concentration may further strengthen the network of hydrogel. Interestingly, we found that heat to a boil could speed up gelation. Once boiled, the formation of hydrogel at 0.3% (w/v) would be observed in about 20 min.

Hydrogel of **2** is able to remain stable for more than 1 month at 4°C, which proved that hydrogel of **2** is a stable platform for loading drugs. Besides, this hydrogel displays shear-thinning

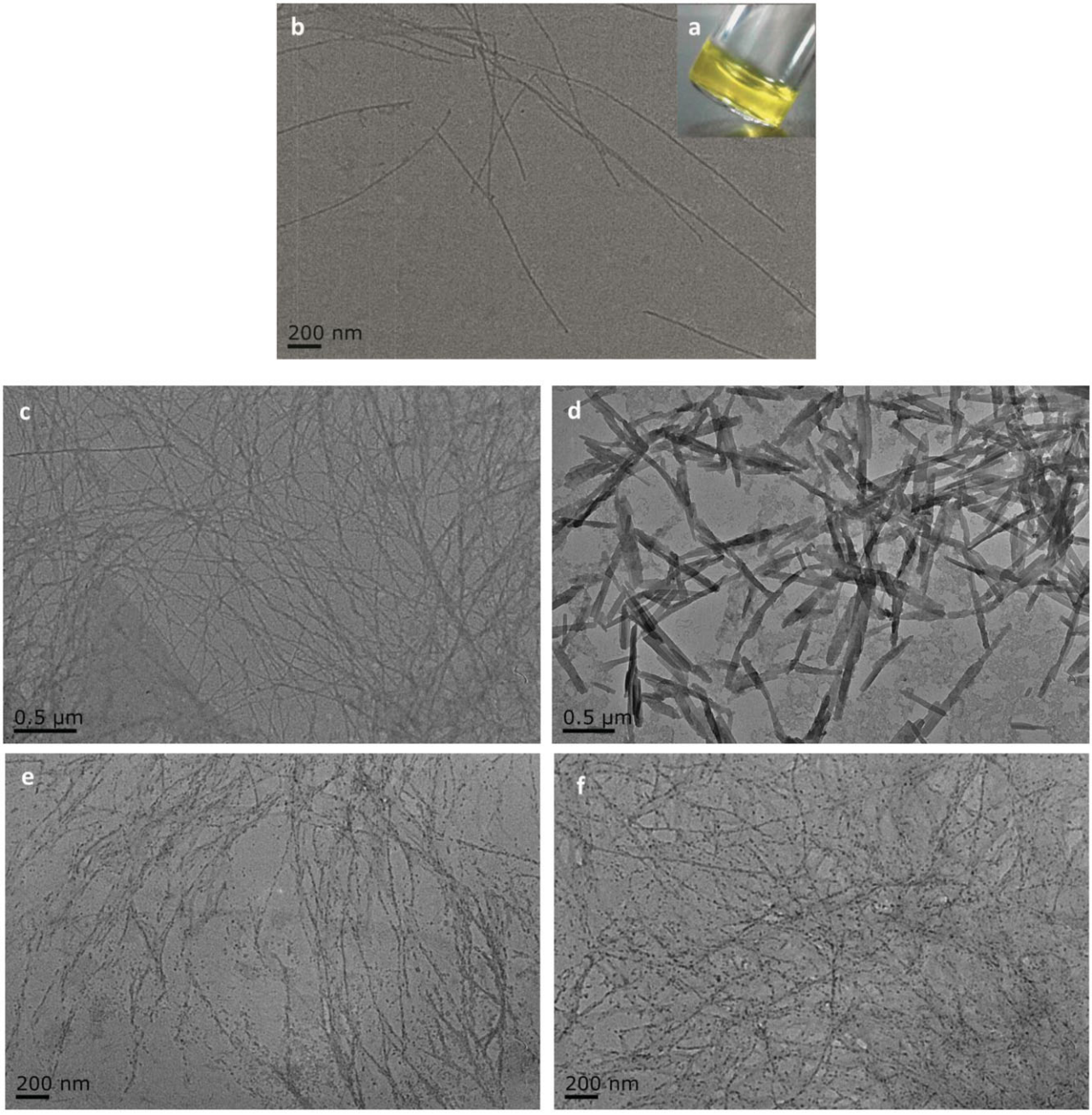

Figure 2. (a) Optical image of HCPT hydrogel. TEM images of (b) hydrogel fibrils; (c) hydrogel of **2** at 0.8% (w/v); (d) heat-treated hydrogel of **2**; HCPT hydrogel at 0.3% (w/v) (e) and 0.8% (w/v) (f).

and recovery properties showing its ability to flow with low viscosity under shear and soon recover back to a solid hydrogel on cessation of shear. Shear-thinning and recovery properties enable injection, without syringe clogging, to a specific site and provide site specificity because of solid-like properties of hydrogel after injection.[38]

Preparation of the HCPT Hydrogel

A clear, stable, bright yellow HCPT hydrogel with 400 μg/mL drug concentration was obtained after standing 1 day, as shown in Figure 2a. Its drug concentration is comparable to Hydroxycamptotbecine Injection® on the market, in which 4–6 mg HCPT was dissolved in 20 mL 0.9% NaCl solution. The pH of HCPT hydrogel was measured as approximately 7.5. We took advantage of the weak basic characteristics of solution of **2** to efficiently dissolve and encapsulate HCPT, directly avoiding the introduction of toxic substance, such as DMSO, and reaching a preferable effect.

Connecting link in HCPT hydrogel between the drug and carrier is achieved through noncovalent cross-linking. Structure of both HCPT and **2** has benzene ring and hydroxide radical, thus π–π stacking and hydrogen bonding cannot be ignored. Because of the hydroxide radical and lactonic ring in chemical structure, HCPT has two forms in aqueous solutions: E-lactone form and carboxylic salt form. *In vivo* at physiological

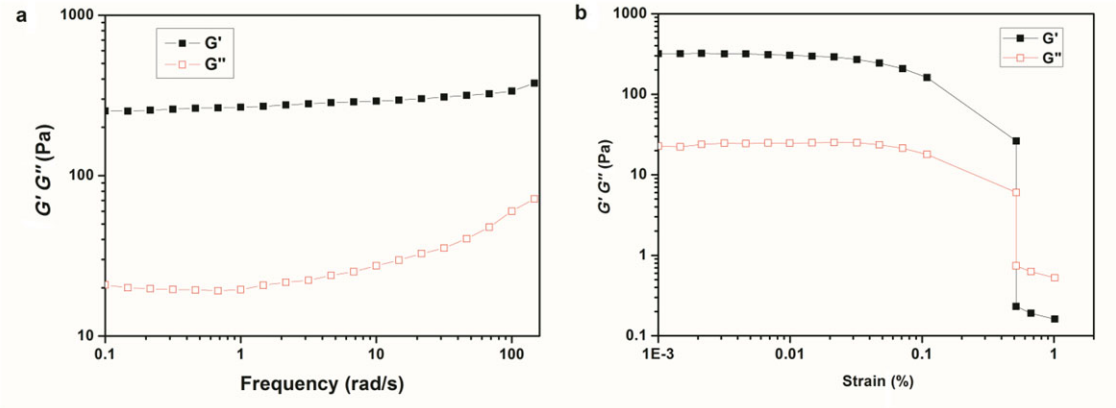

Figure 3. (a) Dynamic frequency sweep (0.1–100 rad/s frequency, 1% strain) and (b) dynamic strain sweep (0.1%–100% strain, 6.28 rad/s frequency) of 0.8% (w/v) HCPT hydrogel.

pH, HCPT remains a balance of E-lactone form and carboxylic salt form. Along with the increasing strength of alkaline solution, E-lactone form could transform to carboxylic salt form, so there were likely charge interactions between HCPT and peptide.

Transmission Electron Microscopy of HCPT Hydrogels and Nanofiber Morphology

Transmission electronic microscopy image provides useful information on morphology of self-assembled structures in the hydrogel. As shown in Figure 2b, nanofibers were fine filaments with a width of about 10 nm and length of several microns. These confirmed the self-assembly of nanofibers. Figure 2c showed the nanofibers of hydrogel of **2** at 0.8% (w/v). Those nanofibers tightly intertwined together to form three-dimensional networks to support the hydrogel formation.[39] Desired stiffness of hydrogel can explain how fiber entanglements and fiber branching[40] account for the mechanical rigidity of the hydrogel.[26] Figure 2d is the TEM image of heat-treated hydrogel of **2**. We observed uniform rod-like nanofibers with a width of about 100 nm and length of less than 1 μm stacked with each other, which are quite different from those in unheated hydrogel. It seems that heating process could speed the hydrolysis of **3** and aggregation of **2**, thus forming nanofibers in different shapes. TEM results of HCPT hydrogel at 0.3% (w/v) and 0.8% (w/v) are shown in Figures 2e and 2f, respectively. Nanofibers of 0.8% (w/v) bear similarity to that of 0.3% (w/v), but it has denser networks. Black dotted HCPT was stuck to nanofibers or trapped in networks, which distinctly provided certification for drug encapsulation. Obviously, the quantity of HCPT in 0.8% (w/v) hydrogel is more than that in 0.3% (w/v) hydrogel, which proved that the equilibrium solubility of HCPT may increase with the concentration of solution of **2.**

Moreover, TEM was used to investigate whether the fibrillar nanostructure was affected by the presence of HCPT. This result showed that the presence of HCPT does not affect self-assembly of **2**.[26] Except for the color from HCPT, HCPT hydrogel has the same appearance as neat hydrogel with no discernable changes.

Rheology

As shown in dynamic time sweep (Supplementary Fig. S3), HCPT hydrogel at concentrations 0.8% (w/v) spontaneously recovered within 4 h at 37°C, indicated by G' values dominating G'' values. The observed modulus directly after the shear-thinning treatment was 19.3 and 5.4 Pa, respectively, and 304 and 26.2 Pa 240 min after the shear-thinning treatment. The growing trend of G' is much faster than G'' over time, which is the main characteristics of hydrogel elastic behavior, indicating that peptides in the hydrogel system have successfully formed fibrous network structure by various intermolecular forces. Dynamic frequency (0.1–100 rad/s frequency, 1% strain) sweep is shown in Figure 3a. G' values exhibited weak frequency dependence at the range of 0.1–100 rad/s, suggesting the presence of high elastic matrixes in hydrogels.[26] The result of dynamic strain (0.1%–100% strain, 6.28 rad/s frequency) sweep was Figure 3b. Once applying strain beyond the critical strain (about 0.5%), G' and G'' obviously appear declining, thus HCPT hydrogel system was damaged. As long as the strain or shear force was removed, HCPT hydrogel system can recover soon. Rheological experiments not only confirmed the formation of hydrogel, but also demonstrated that the HCPT hydrogel indeed has recoverability. The shear-thinning and recovery capability of HCPT hydrogel makes it quite suitable for injecting, laying the foundation as a potential candidate for injection.

UV Spectroscopy

Concentrations of HCPT in PBS supernatants of samples were determined by UV. In Figure 4, the absorbance spectra of HCPT showed ultraviolet absorption peak at 267 and 384 nm. Because of the benzene structure of **2**, absorption peak of pure hydrogel of **2** was at about 280 nm, which would apparently affect the HCPT absorption peak at 267 nm. But, there was no absorption over 300 nm range for hydrogel of **2**. So the ultraviolet absorption at 384 nm was determined to quantify released HCPT.

Equilibrium Solubility of HCPT in Solution of 2

According to the standard curve of HCPT, the equilibrium solubility of HCPT in solution of **2** at concentrations of 0.2% (w/v), 0.3% (w/v), and 0.4% (w/v) is shown in Table 1. The equilibrium solubility of HCPT in PBS solution at pH 7.4 is determined as 9.08 μg/mL.[41] Compared with that in PBS solution, the solubility of HCPT in solution of **2** increased at least 40

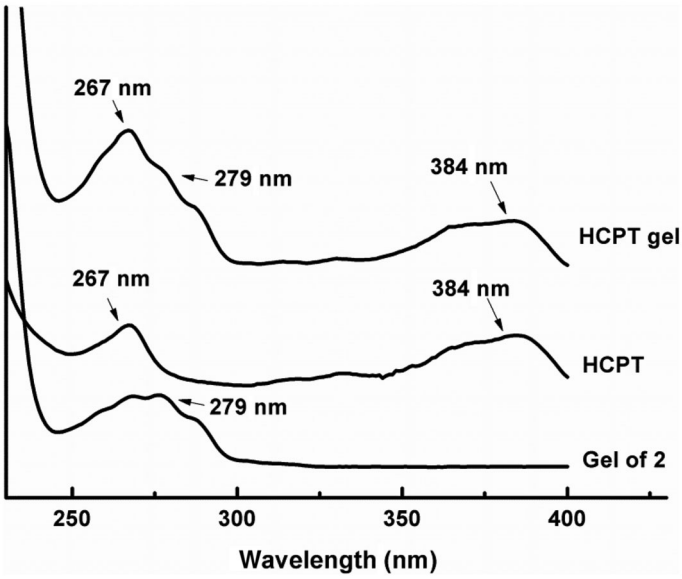

Figure 4. The ultraviolet absorption spectra of HCPT, pure hydrogel of **2**, and HCPT hydrogel at the concentration of about 10^{-5} mol/L.

Table 1. The Equilibrium Solubility of HCPT in Solution of **2** at Concentration of 0.2% (w/v), 0.3% (w/v), and 0.4% (w/v)

Concentration of Solution of 2	Equilibrium Solubility (μg/mL)
0.2% (w/v)	416
0.3% (w/v)	743
0.4% (w/v)	863

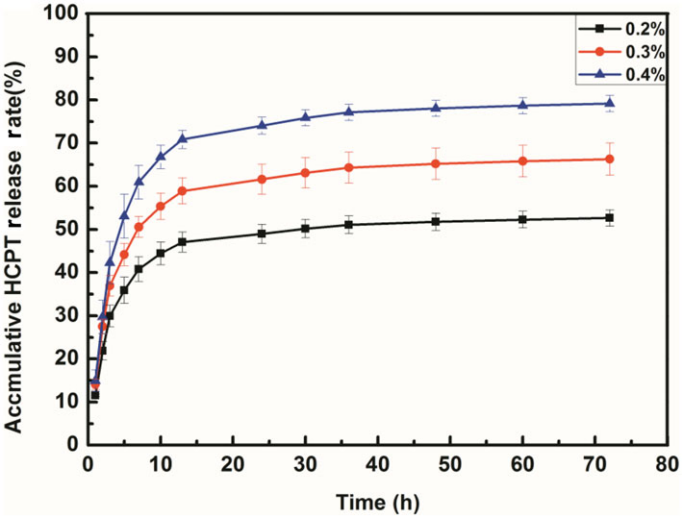

Figure 5. 10-Hydroxy camptothecin accumulative release profiles of 0.2% (w/v) (■), 0.3% (w/v) (●), and 0.4% w/v (▲) HCPT hydrogel prepared at fixed drug concentration of 400 μg/mL.

times, which fully demonstrated that a hydrophilic preparation of HCPT have been created. It can be easily concluded that the equilibrium solubility increases with the concentration of solution of **2**, which leads to the increase of maximum drug loading in unit volume. The optimization in maximum drug loading showed that hydrogel of **2** is suitable for loading drug. Under the same drug dose, drug loading in unit volume of hydrogel increased; the volume administrated can be partially reduced eventually, which is more sustainable for patients. Furthermore, the increase in equilibrium solubility implied that the bioavailability of HCPT could be promoted to some extent.

In Vitro Release Profile of HCPT Hydrogels

Figure 5 shows the release profiles and accumulative percentages of HCPT released from their corresponding hydrogels. Three curves with different peptide concentrations exhibit similar release trend: relatively higher rates during the first 12 h, followed by lower rates after 60 h. The highest release rate of 1 h was 18%, meeting the required under 40% for the sustained- and controlled-release preparations.[39] For 72 h, approximately 52.6%, 66.3%, and 79.2% of HCPT were released from HCPT hydrogels at 37°C. Comparison of three accumulative release rates state clearly that higher peptide concentration has higher drug release under the prerequisite of the same drug loading. This result may be associated with the increase of equilibrium solubility and hydrophilicity of HCPT as peptide concentration increased. These preliminary results demonstrated the long term and sustained release of HCPT at high-accumulated release rate. In addition, either before or after injection, HCPT hydrogels do not swell when exposed to additional solution or bodily fluids. Rather, new solutes and aqueous fluids can begin to diffuse into and within the stable hydrogels as defined by the porosity of the self-assembled peptide network.[26] It means that HCPT hydrogel is likely to work at a steady state *in vivo* as an injectable therapeutic agent.

HCPT Hydrogels in Contact with MCF-7 Cell

Through the microscope (Figure 6), MCF-7 cells showed morphological features of cell death (cell rounding, cell shrinkage, detachment, or floatation) when incubated with HCPT hydrogels, whereas there were few obvious signs of cytotoxicity in cells cultured with hydrogels lacking HCPT. Cell death observed clearly indicated the release of HCPT from HCPT hydrogel network. Comparison of cells cultured with a fixed concentration of HCPT at an increasing peptide concentration, that is, 0.1% (w/v), 0.2% (w/v), 0.4% (w/v), and 0.8% (w/v), showed signs of increased cell death. This increasing trend of cell death is caused by the higher drug release of higher peptide concentration. This result is coincident with the previous *in vitro* release profile of HCPT hydrogel.

Anticancer Efficacy Measurement

The activity of three compounds was evaluated by treating MCF-7 cells at a serial of concentrations. Although MCF-7 cells grew in hydrogel of **2** freely, HCPT hydrogels inhibited their growth (Fig. 7). By calculating cell inhibition rate, the hydrogel of **2** was nontoxic at gelation concentration, whereas pure HCPT and HCPT hydrogel possessed IC_{50} values of 52.3 ± 2.9 and 23.5 ± 3.5 μg/mL. This result is in agreement with the cell state observed in cell imaging. Unlike some other anticancer drug hydrogels,[8,19] the anticancer efficacy of HCPT was indeed increased. The most likely reason is that the solubility and hydrophilicity of HCPT improved through encapsulation by hydrogel. So hydrophilic HCPT hydrogel is much easier than hydrophobic HCPT in entering the cells to exert its cytotoxic effect and cause fatal damage

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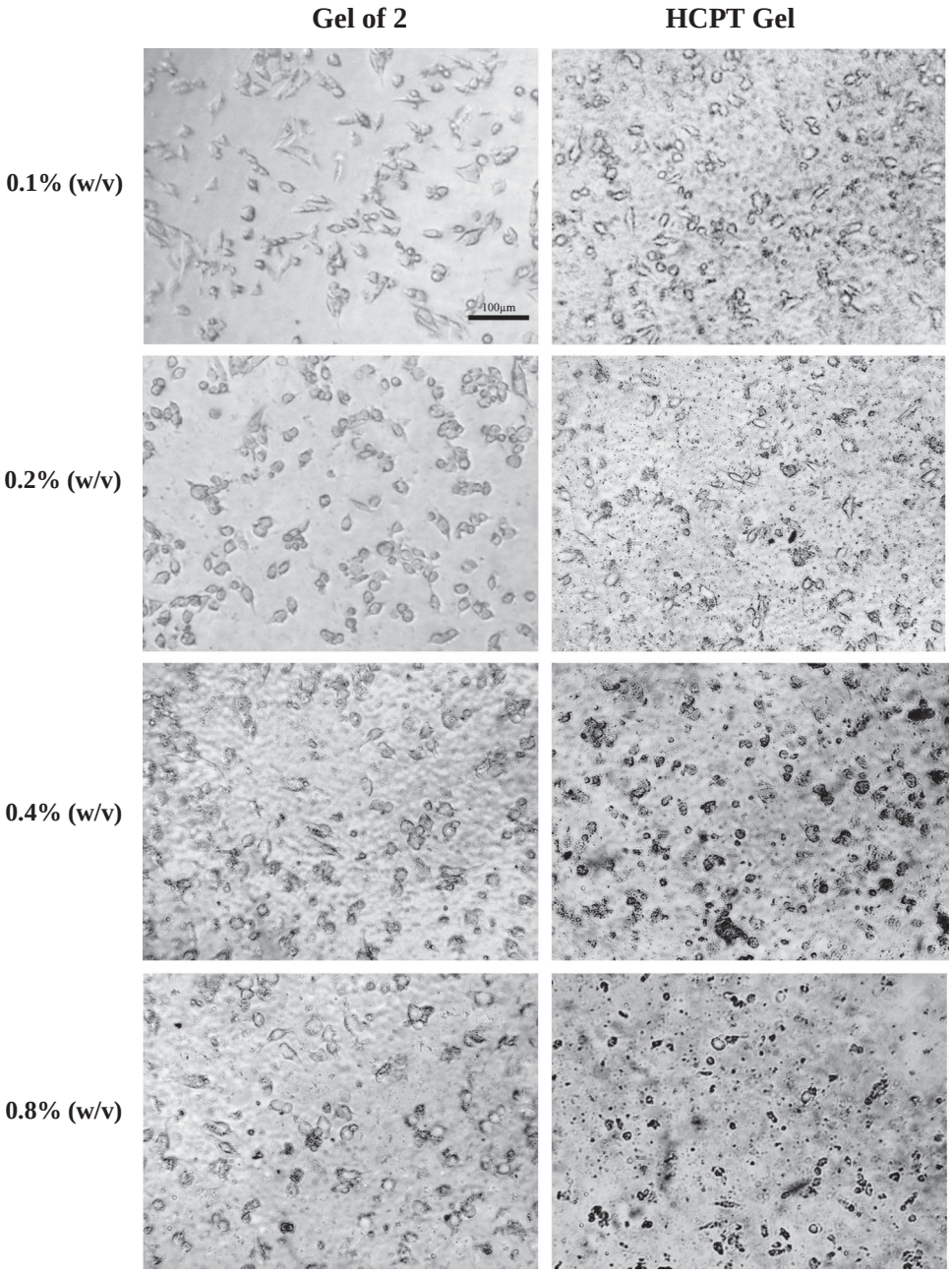

Figure 6. Images of MCF-7 cells affected by 0.1% (w/v), 0.2% (w/v), 0.4% (w/v), and 0.8% (w/v) hydrogel of **2** (left) and HCPT hydrogel (right) at fixed 400 μg/mL drug concentration. Scale bar represents 100 μm.

to cells. Although HCPT hydrogel showed promising potency, its toxicity toward normal mammalian cells remains to be evaluated.

CONCLUSION

10-Hydroxy camptothecin hydrogel was successfully developed as an injectable and hydrophilic anticancer formulation, which radically turned several drawbacks of HCPT. Excellent physical properties of carrier, such as stability, shear-thinning, and recovery properties, enable injection to a special location. High-equilibrium solubility of HCPT in hydrogelator solution brought HCPT hydrogel a superior drug loading, which indeed improved anticancer efficacy simultaneously. Cytotoxicity test indicated that the hydrogel without drug is nontoxic, ensuring the safety of carrier. Long term and sustained release of HCPT enhanced the potential effectiveness of HCPT hydrogel as an injectable agent for local delivery. We believe that the integration of hydrophilic hydrogel with hydrophobic drugs is a feasible approach to formulate hydrophobic drugs into an aqueous form for improving their clinical applicability. Further studies will focus on the *in vivo* stability of HCPT hydrogel, with the hope that its release rate could withstand the complex and volatile physiological environment.

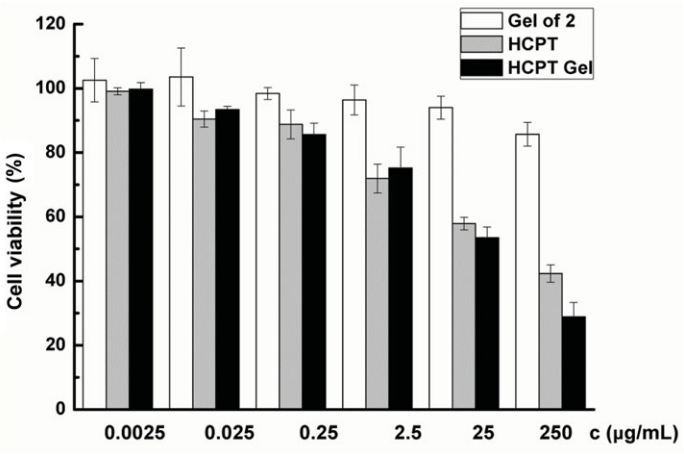

Figure 7. Cell viability of hydrogel of **2**, HCPT, and HCPT hydrogel from low to high concentration.

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