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Evaluation of micelles incorporated into thermosensitive hydrogels for intratumoral delivery and controlled release of docetaxel: A dual approach for *in situ* treatment of tumors

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ABSTRACT

The *in situ* gelling hybrid hydrogel system has been reported to effectively concentrate chemotherapeutic drugs at the tumor site and sustain their release for a long period. DTX-micelles (docetaxel-loaded mixed micelles) are able to increase the solubility of DTX in water, and then a high drug loading rate of hydrogels can be achieved by encapsulating the docetaxel-loaded mixed micelles into the hydrogels. The thermosensitive nature of DTX-MM-hydrogels (thermosensitive hydrogels incorporated with docetaxel-loaded mixed micelles) can accelerate the formation of a depot of this drug-loaded system at the site of administration. Therefore, the hydrogels provide a much slower release compared with DTX-micelles and DTX-injection. An *in vivo* retention study has demonstrated that the DTX-MM-hydrogels can prolong the drug retention time and *in vivo* trials have shown that the DTX-MM-hydrogels have a higher antitumor efficacy and systemic safety. In conclusion, the DTX-MM-hydrogels prepared in this study have considerable potential as a drug delivery system, with higher tumor inhibition effects and are less toxic to normal tissues.

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1. Introduction

Systemic administration of chemotherapeutic drugs for the treatment of cancers always results in unfavorable and toxic adverse effects, such as cardiotoxicity, hair loss, and weight loss [1–4], due to their high plasma concentrations and only a fraction of the entire dose administered arrives the tumor via the systemic circulation. Tumor responsiveness to chemotherapeutic agents can be increased by various methods, such

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as the production of higher concentrations and more prolonged drug exposure [5]. However, several anticancer drugs are rapidly cleared from plasma and so these two methods have little effect. To overcome these disadvantages, drug delivery depot technologies have been developed with the aim of providing a sustained and controlled drug delivery within the targeted tumor site and allowing the drug to avoid normal organs [6,7]. The treatment effects of a topical formulation depend on the type of vehicles and the physicochemical properties of the active substances [8]. Several regional forms of administration of sustained drug delivery systems, such as *in situ* gels, wafers, particles, rods, and films combined with targeting intratumoral (i.t.) injection, can deliver chemotherapeutic agents to their target sites, resulting in the maximum anticancer effects and minimum side effects [9,10]. Furthermore, the drug can reach the tumor regardless of its vascular status [11,12].

Injectable hydrogels can be divided into physical and chemical gels with regard to their type of cross-linking used for the formation of the three dimensional depot, reversible noncovalent and covalent interactions, respectively [13–16]. The physical gels are useful for inducing sol−gel transitions by changing the temperature, the solvent or the pH value, since noncovalent interactions are sensitive to the surrounding environment [17–21]. The systems of thermosensitive injectable hydrogels have attracted attention because of their physical targeting which has additional advantages over passive or other actively targeted therapies [11,12]. Compared with preformed implants, injectable biodegradable *in-situ* forming depots are less invasive and cause patients less pain which make them attractive systems for topical administration of anticancer drugs [9,22].

After topical administration, *in situ* gel systems can undergo sol–gel transition under physiological conditions and change into gels at the administration sites as drug reservoirs [9,21]. After intratumoral administration, thermal-reversible hydrogels can form a "depot" at the administration sites, and the drug can then be released into the tumor and surrounding tissues slowly and continuously [12]. Thus, the prolonged exposure of the tumor to the drug could help reinforce its therapeutic efficacy.

Poloxamers (Pluronic®) with concentrations between 16% and 30% (w/v) possess the special characteristics of reverse-gels [23]. Poloxamer 407 (F127) and Poloxamer 188 (P188) are used extensively due to their commercial availability and nontoxicity and they have been approved by the US FDA. For the reasons mentioned above, they have been intensively investigated with regard to different routes of application [6,11].

The network of hydrogels is hydrophilic in nature. Therefore, the amount and homogeneity of hydrophobic drug loaded (DL) into hydrogels may be limited. In addition, due to the high water content and large pore sizes of hydrogels, drugs loaded directly into hydrogels are often released relatively rapidly [24].

In this study, it was difficult to load lipophilic drug into the hydrogels due to their incompatibility. Hence, a two-step delivery system consisting of micelles and hydrogels was designed to solve the poor drug loading associated with hydrogels. Docetaxel was chosen as the hydrophobic drug, and it could be incorporated into micelles composed of F127 and Solutol®HS15 [25]. As a result, the solubility of docetaxel in water was increased, and then the DTX-micelles were incorporated into the networks of hydrogels to achieve a high drug loading rate and prolong the release of DTX from the DTX-micelles. Therefore, it could be concluded that this combination of dual delivery systems of drug loaded micelles entrapped in hydrogels is an attractive approach for hydrophobic drugs in cancer therapy given by *in situ* treatment, providing both increased efficacy and reduced side effects [11,26].

2. Materials and methods

2.1. Materials

Docetaxel (DTX) was obtained from NanjingJingzhu Biotechnology Co., Ltd. (Nanjing, China). F127 and P188 were supplied by BASF Ltd. (Beijing China). Solutol®HS15 was obtained from BASF SE Ludwigshafen, Germany, and mehanol and acetonitrile were obtained from Tianjin Concord Technology Co., Ltd. (Tianjin, China). Dehydrated ethanol was obtained from Tianjin Damao Technology Co., Ltd. (Tianjin, China). Dir (Dir is the abbreviation of Dir iodide [1,1-dioctadecyl-3,3,3,3-tetramethylindotricarbocyanine iodide], which is a lipophilic fluorescent dye) was obtained from Beijing Fluorescence Biothecology Co., Ltd. (Beijing, China).

The HT-29 cell lines (human colon cancer cells) were gifts from the pharmacology laboratory of Yanhua Mou. Male Kunming mice (18–22 g) and Balb/c nude mice (18–20 g) were provided by the Laboratory Animal Centre of Shenyang Pharmaceutical University (Shenyang, China).

All the animal experimental protocols conformed to the Animal Management Rules of the Ministry of Health of the People's Republic of China (Document no.55, 2001). All the experiments were performed satisfying the principles of laboratory animal care of Shenyang Pharmaceutical University and approved by the Experimental Animal Use and Care Committee, Shenyang Pharmaceutical University. All the animals were clinically healthy during the experimental period.

2.2. Preparation of DTX-injection, DTX-micelles, DTX-MM-hydrogels, blank hydrogels, Dir-Micelles, and Dir-MM-hydrogels

2.2.1. The preparation of DTX-micelles

Docetaxel-loaded mixed micelles were prepared using the solvent evaporation method [25]. Solutol®HS15 and Pluronic F127 in different ratios and concentrations were co-dissolved in ethanol by gentle agitation at 40 °C until a clear solution was obtained. Then, a certain amount of docetaxel dissolved in 10 ml ethanol was pre-warmed to 40 °C and slowly added dropwise to the above solution. After agitation for a few min, the mixed solution was evaporated under a vacuum of −0.1 MPa and at a temperature of 40 °C until a transparent thin film was formed and the ethanol was then evaporated to dryness. Deionized water (5 ml) was then injected into the flask and shaken until a clear solution formed. The solution was then passed through a 0.45 μm micro-porous membrane filter and the obtained filtrate consisted of mixed micelles.

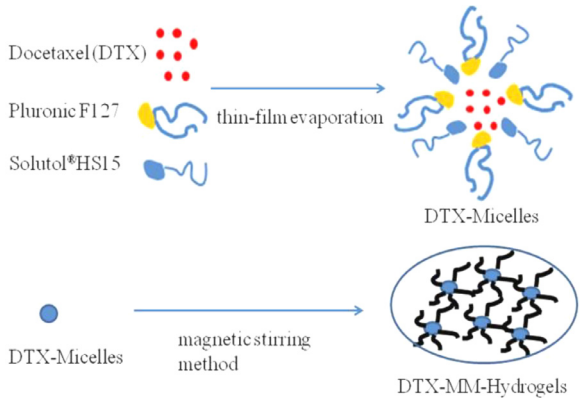

Fig. 1 – The drug loading process of micelles incorporated into hydrogels.

2.2.2. *The preparation of blank hydrogels*

A selected amount of F127 and P188 was added to 10 ml deionized water and the mixture was stirred magnetically until all the F127 and P188 granules were thoroughly dispersed in the solution. Then, the mixed solution was kept at 4 °C for 24 h until a clear solution was obtained with final concentrations of F127 and P188 of 20% and 6% (w/v), respectively.

2.2.3. *The preparation of DTX-MM-hydrogels*

Suitable amounts of F127 and P188 granules of the blank hydrogels were added to a 10 ml solution of docetaxel-loaded mixed micelles, and the method was the same as that used for preparing blank hydrogels. The brief process of preparation was shown in Fig. 1.

2.2.4. *The preparation of DTX-injection, Dir-Micelles and Dir-MM-hydrogels*

As control, a DTX formulation, DTX-injection, was prepared as follows [27]. In brief, 20 mg docetaxel was dissolved in 0.5 ml Tween-80, and then a mixed solution of 1.5 ml ethanol-water (13:87, v/v) was added to the above solution. This drug solution was then shaken until the drug was dispersed in the solvent uniformly and then it diluted with normal saline.

The method for preparing Dir-Micelles (Dir-loaded mixed micelles) and Dir-MM-hydrogels (hydrogels incorporated with Dir-loaded mixed micelles) was the same as that used for preparing DTX-micelles and DTX-MM-hydrogels.

2.3. *In vitro characterization*

2.3.1. *Particle size, zeta potential of DTX-micelles and the dilution of DTX-MM-hydrogels and morphology of DTX-micelles*

The particle size and zeta potential of DTX-loaded mixed micelles were determined by dynamic light scattering using a Malvern system (Nano ZS, Malvern Co., UK). Samples were equilibrated at 25 °C for 30 s prior to analysis. The results were expressed as the average of three measurements. Then, the polydispersity index (PDI) was measured to evaluate the particle size distribution of docetaxel-loaded mixed micelles. The morphology of micelles was examined by transmission electron microscopy (TEM, H-600, Hitachi, Japan). A drop of docetaxel-loaded mixed micelles was placed on a carbon-coated copper grid for 30 s and then dried with filter paper. The sample was then stained with 0.5% phosphotungstic acid (10 μl) for 30 s and dried in air. The sample was examined by TEM.

2.3.2. *Determination of drug content in DTX-micelles and DTX-MM-hydrogels*

In this study, the amount of loaded docetaxel in the mixed micelles was determined by HPLC using a suitable column (250 mm × 4.6 mm, 5 μm, Thermo BDS-C_{18}). The mobile phase was acetonitrile: water (65: 35, v/v) and the mixed micelles were passed through a 0.45 μm micro-porous membrane filter to remove any unencapsualted drug. Because of its poor solubility in water (experimentally, measured < 10 μg/ml), the unencapsulated drug would aggregate. The aggregations were usually visible and could be removed by passage through a 0.45 μm membrane filter. The docetaxel concentration in water was negligible compared to its concentration in micelles. Therefore, during the determination of the entrapment efficiency and drug loading, this method could be used to remove the unencapsulated drug [25,28,29]. Then, 0.2 ml of the filtrate was diluted to 2 ml with acetonitrile and the micelles were destroyed by this method. The mixed solution was passed through a 0.22 μm micro-porous membrane filter and then analyzed by HPLC. The drug loading (DL%) and entrapment efficiency (EE%) were calculated by the following equations.

$$DL\% = W_1/(W_1+W_2)\times 100\%$$

$$EE\% = W_1/W_3\times 100\%$$

where W_1 is the weight of drug encapsulated in the micelles, W_2 is the weight of total excipients and W_3 is the weight of drug in the micelles solution.

2.3.3. *The determination of gel formation temperature and the gelation time (GT) determination*

The gel formation temperature (GFT) was determined using the test tube inversion method [11]. Briefly, each formulation of 2 ml was sealed in a 10 ml test tube and heated slowly from 10 °C to 50 °C at an increment of 2 °C/step. The sol–gel transition temperature was determined as that when the liquid was nonflowing after the tube was inverted for 30 s.

We had conducted the GT determination studies referred to the articles [1,11,30]. The gelation time (GT) of the samples was similarly determined at 37 °C water bath. Time measurements were initiated when the DTX-MM-hydrogels solution was placed into the water bath. The time at which the samples stopped movement was recorded as the GT.

2.3.4. *In vitro degradation studies*

One milliliter of hydrogel was placed on the bottom of a 10 ml tube and the solution underwent a sol–gel transition immediately once 5 ml PBS (pH 6.8) was added to the tube. All the experiments were conducted at 37 °C and changes in the hydrogel layer were examined at predetermined time points [11].

2.3.5. *The rheological measurement of DTX-MM-hydrogels*

Rheological measurements were carried out using an AR2000 rheometer (TA, Co Ltd., New Castle, DE). For the whole determination, a parallel-plate 20 mm in diameter was used and the gap between the two plates was 1 mm. The tests were performed as follows: The lower plate was initially maintained at 37 °C and the sample was placed on the plate. Oscillation strain sweeps were carried out with a constant frequency of 1 Hz over a strain pressure range of 0.05–10% and oscillation frequency sweeps were carried out with a constant strain of 0.5% over a frequency range of 0.1–100 Hz. The elastic modulus (G′) and viscous modulus (G″) were obtained after the three repeated measurements of each sample [31].

2.3.6. *Studies of storage stability of DTX-MM-hydrogels*

The dehydrated hydrogels were freeze-dried under vacuum for 36 h and then stored in a desiccator to protect them from water in the air. At predetermined time points, the morphology of the dehydrated hydrogels was observed by scanning electron microscopy (SEM), after the hydrogels were coated with gold, using a scanning electron microscope (SEM, S-3400, Hitachi, Japan).

2.3.7. *In vitro drug release studies*

The DTX release profiles of the DTX-micelles and DTX-MM-hydrogels were determined by a dialysis method [12]. Briefly, 1 ml of free DTX-injection, DTX-micelles, and DTX-MM-hydrogels were placed in the dialysis bag (MWCO = 14,000 Da), and then the samples were immersed in 75 ml phosphate buffer (pH 6.8) which was prewarmed and allowed to stand in a water bath at 37 °C with uninterrupted shaking at 100 r/min. Then 2 ml supernatant was collected and filtered for HPLC analysis and the supernatant was replaced with an equal volume of fresh medium at predetermined times. All the experiments met the sink conditions. Three independent experiments were carried out, and the average values were used for the data presentation. The cumulative release was calculated using the following equation:

$$A(\%)=\left(\Sigma_1^{n-1}C_i \cdot V+V_0 \cdot C_n\right)/M_0\times 100\%$$

where A represents the cumulative release (%), V is the sampling volume (2 ml), V_0 is the total volume (75 ml), C is the drug concentration (μg/ml), and M_0 is the total mass of DTX.

2.3.8. *In vivo distribution studies*

An *in vivo* fluorescence imaging system was used to observe the distribution over the whole body. In order to monitor the localized retention capacity of DTX-MM-hydrogels and DTX-micelles, fluorescence spectroscopy (Dir, a fluorochrome, λ_{ex}748 nm, λ_{em}780 nm) was used. Six KM mice were randomly divided into 3 groups and given Dir solution, Dir-Micelles and Dir-MM-Hydrogels subcutaneously. At the predetermined time points, the KM mice were subjected to fluorescence spectroscopy after ether anesthesia at 1, 6, 12, 24, 48 and 72 h after administration.

2.3.9. *In vivo pharmacodynamic studies*

The *in vivo* antitumor efficacy was investigated in HT-29 tumor-bearing Balb/C nude mice following intratumoral administration. The mice (18–20 g) were subcutaneously injected with 0.2 ml cell suspension containing 10^7 tumor cells per milliliter in the armpit of the left anterior limb. The mice were weighed and divided into four groups randomly when the tumor volume reached 100 mm^3. Docetaxel-Injection, DTX-micelles, DTX-MM-hydrogels (10 mg/kg) and blank hydrogels were administered by the intratumoral route. The tumor volume and body weight of the mice were monitored as a function of time and the results were recorded every other day. The tumor volume was calculated by the equation: $V = (\text{length} \times (\text{width})^2)/2$. Mice were sacrificed on day 12 and tumors were excised and weighed. The evaluation methods of relative tumor volume (RTV), tumor volume doubling time (DT) and body weight in [32,33] were adopted to assess the treatment effects and safety. The RTV% was calculated by the equation:

$$RTV\%=V_t/V_0\times 100\%$$

where V_t was the tumor volume at predetermined time points, and V_0 was the initial tumor volume. The DT was calculated by the equation:

$$DT(d)=T\times\log 2/(\log V_F-\log V_I)$$

where V_F was the final tumor volume, V_I was the initial tumor volume and T was the time difference between the initial and final day.

Tumor volume and body weight curves were plotted using the average tumor size and mean body weight in each experimental group at predetermined time points.

2.4. *Statistical analysis*

All data are expressed as the mean value ± SD. Student's t-test was performed using SPSS software. A P value less than 0.05 was considered to be statistically significant.

3. Results and discussion

3.1. *Particle size and morphology of DTX-micelles, drug content in DTX-micelles and DTX-MM-hydrogels*

Routinely, the mean diameter of the DTX-micelles and the dilution of DTX-MM-hydrogels was about 20 nm (Fig. 2A) and the size distribution was relatively narrow with a PDI of 0.170. The results obtained showed that the hydrogel formation had no influence on the size of the micelles. The zeta potential of the DTX-micelles was neutral (Fig. 2A), and the dilution of the DTX-MM-hydrogels was −9.14 mV. The morphology of the DTX-micelles is shown in Fig. 2B. The results obtained showed that Solutol®HS15 and F127 could form self-assembled micelles with a spherical shape [5].

The determination results of drug content in DTX-micelles and DTX-MM-hydrogels showed that these DTX-micelles and DTX-MM-hydrogels contributed to a high solubility (1.0 mg/ml in water) of docetaxel. The EE% and DL% of the mixed micelles reached 86.6% and 1.27%, respectively.

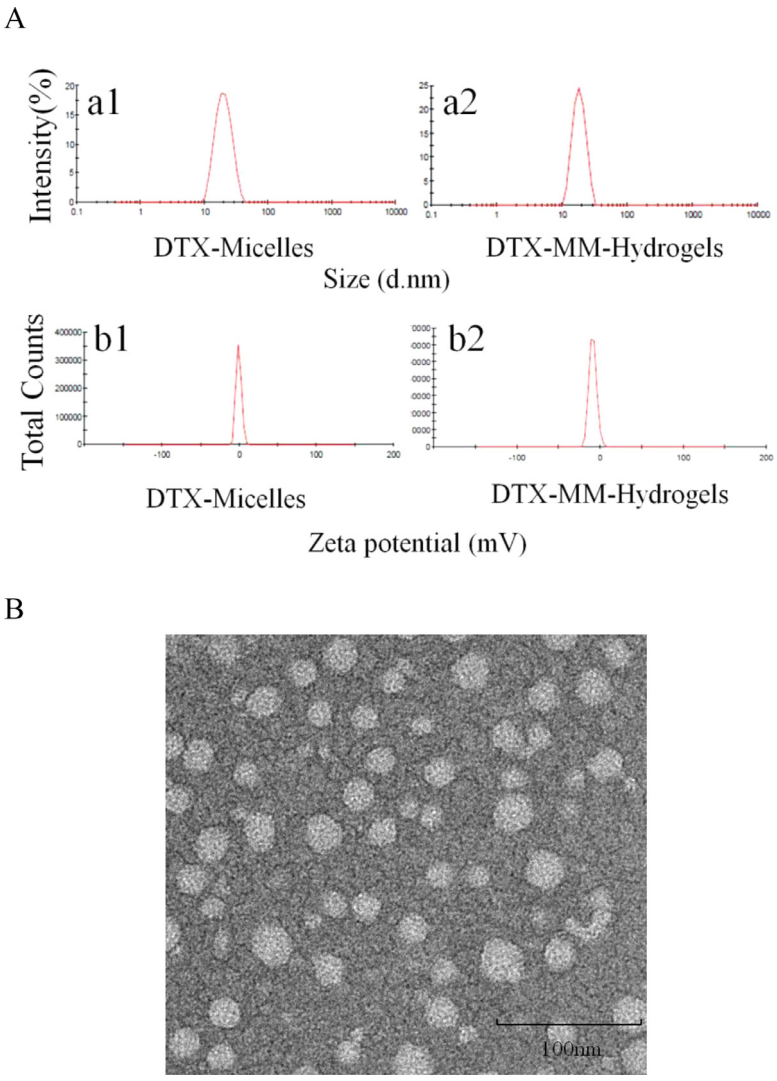

Fig. 2 – (A) Particle size and zeta potential of DTX-micelles and DTX-MM-hydrogels; (B) and the TEM of DTX-micelles.

3.2. *Determination of the gel formation temperature and the gelation time (GT) determination*

The gelation temperature of the blank and DTX-MM-hydrogels both varied with the percentages of F127 and P188, and the most appropriate percentages of F127 and P188 were 20% and 6% (w/v), respectively. At those concentrations, they exhibited reversible sol–gel transition properties. This means that they were in a free-flowing solution state at 4 °C and a gelation state at 37 °C (Fig. 3A).

The GT determination results showed that they could form hydrogels at 37 °C within 30 s. These characteristics ensure the formulations could form hydrogels at the administration sites rapidly. This results were comparable with that in the articles [1,11,30]. The results in the abovementioned articles have showed that this thermosensitive hydrogels could change into the solid state. Hence the hydrogels could gather at the local sites with the least DTX-MM-hydrogels diffusing into the circulation system.

3.3. *In vitro degradation studies*

Samples of the formulations (2 ml) were added to the bottom of the tubes, and then placed in a water bath at 37 °C. Phosphate buffer (pH 6.8, 5 ml) was added to the tubes and then the solutions immediately turned into stationary hydrogels. A clear layer could be observed between the PBS solution and the hydrogels, and then the layer became unclear as time passed. By day 4, the boundary between the medium and hydrogels had disappeared. The results obtained are shown in Fig. 3B and suggest that this thermosensitive formulation can form hydrogels at body temperature *in vitro* and is biodegradable.

3.4. *The rheological profiles of DTX-MM-hydrogels*

As shown in Fig. 4A, the linear viscoelastic region (LVR) [12] of the DTX-MM-hydrogels spread over the range of 0.1%−10% Pa of strain stress, which showed that the structure of the DTX-MM-hydrogels remained stable over the range 0.1%−10% Pa. Therefore, G' and G'' were determined as a function of the oscillation frequency under a constant strain stress of 0.5% Pa.

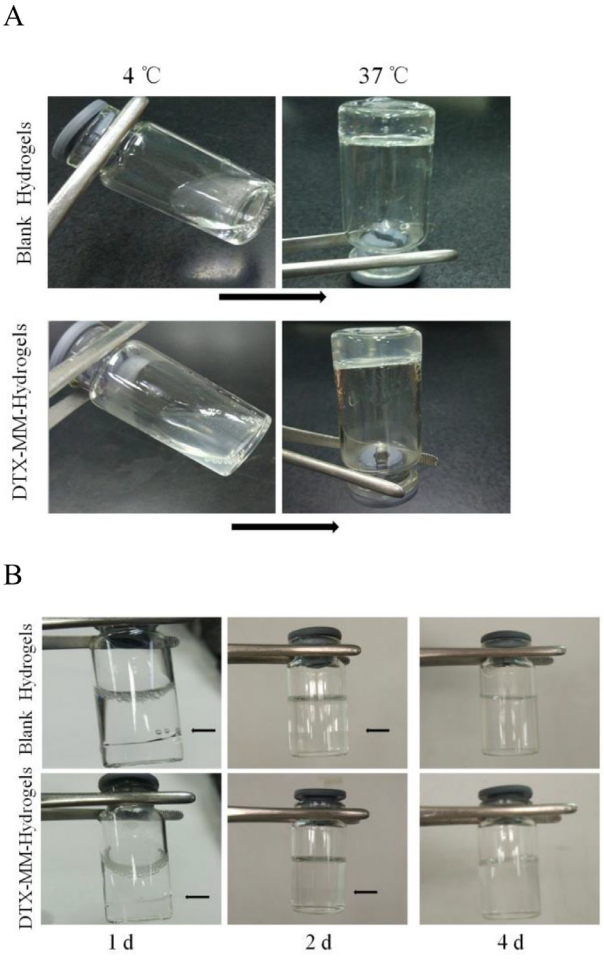

Fig. 3 – (A)The sol–gel transition of blank hydrogels and DTX-MM-hydrogels; (B) the degradation behavior of DTX-MM-hydrogels.

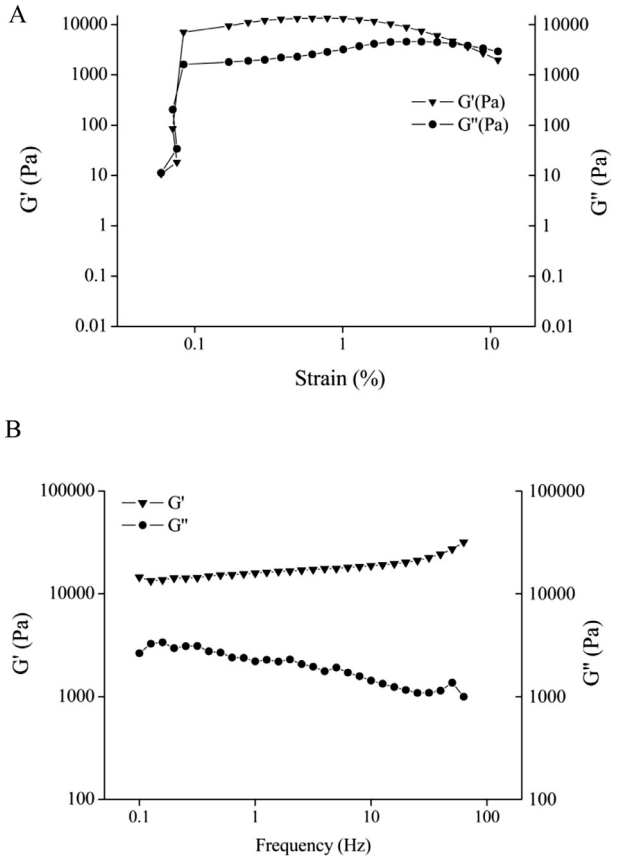

Fig. 4 – Evaluation of rheological behavior. (A) Diagram of strain *versus* G′ and G″ values of DTX-MM-hydrogels. The oscillation frequency was 1 Hz; (B) G′ and G″ as a function of the oscillation frequency at 37 °C. The strain stress was 0.5% Pa.
G′: elastic modulus; G″: viscous modulus.

G′ and G″ as a function of the oscillation frequency at 37 °C are shown in Fig. 4B. Fig. 4B shows that the elastic modulus (G′) was higher than the viscous modulus (G″) as the oscillation frequency varied from 0.1 Hz to100 Hz when the temperature reached 37 °C. These results showed that the DTX-MM-hydrogels could change into a solid form at 37 °C which is close to body temperature. Based on these results, the rheological profiles of the DTX-MM-hydrogels make them suitable as thermosensitive hydrogels for *in situ* administration during antitumor studies [30].

3.5. *Studies of storage stability of DTX-MM-hydrogels*

The morphology of the dehydrated hydrogels at different storage times was observed by scanning electron microscopy (SEM). The results obtained are shown in Fig. 5 and confirm that the morphologies of the hydrogels were stable over a period of 3 months.

3.6. *In vitro drug release studies*

The docetaxel release profiles of the DTX-micelles and DTX-MM-hydrogels are shown in Fig. 6 and it is clear that the DTX-micelles and DTX-MM-hydrogels both produced controlled release of docetaxel. The DTX-injection showed the fastest release of docetaxel within 48 h, followed by DTX-micelles, and the slowest release was obtained by the DTX-MM-hydrogels. The cumulative release of docetaxel of the DTX-injection and DTX-micelles at 120 h were 84% and 69%, respectively, while DTX-MM-hydrogels produced the slowest release of docetaxel of 56%. In summary, this dual drug delivery system could allow the sustained release of docetaxel for a long period, leading to a better anticancer effect [30]. The release of the drug from the DTX-MM-hydrogels was mostly due to diffusion of the drug from the hydrogels [34,35]. In the case of DTX-MM-hydrogels, when the medium diffuses into the matrix, and then dissolves the drug and relaxes the polymeric chains, the release starts [36]. Hence, the hydrophilic nature of the inclusions and the stability of the hydrogels could both influence the release profiles. The DTX-micelles encapsulated into the DTX-MM-hydrogels are soluble in the release medium, and our studies showed that the DTX-MM-hydrogels could remain stable in the release medium for 2 d. Therefore, the difference between the DTX-micelles and DTX-MM-hydrogels was mainly due to erosion of the polymers. In addition, the pore

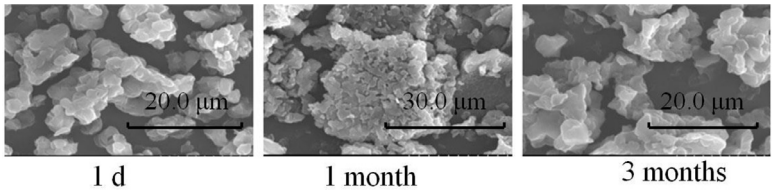

Fig. 5 – The SEM of DTX-MM-hydrogels at different storage time.

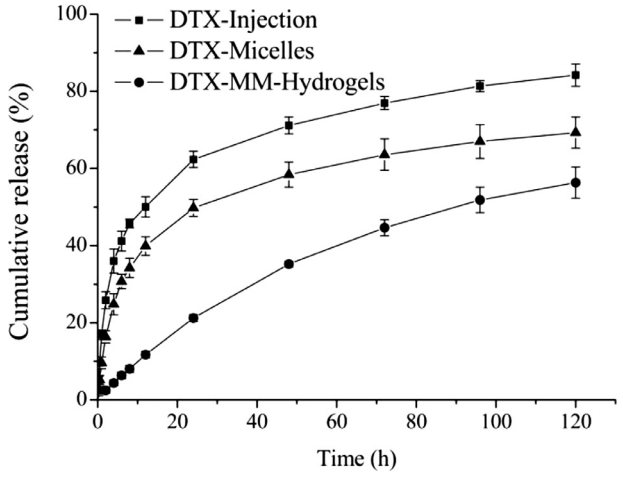

Fig. 6 – Docetaxel release curves of DTX-injection, DTX-micelles and DTX-MM-hydrogels *in vitro*. The experiments were performed in triplicate and the results were presented as the mean ±SD.

sizes within the hydrogels and the drug molecules or particle sizes of the micelles entrapped in the hydrogels also affect the release profiles [11]. At the late stage, the release behavior largely depended on the controlled release of the micelles. In the *in vitro degradation studies*, the boundary had disappeared after 4d, which indicated that the hydrogels began to break up, and the DTX-micelles diffused into the medium. However, the micelles could also sustain the release of docetaxel, thus, the structure of hydrogels and micelles both influenced the release of docetaxel. The amount of cumulative release was about 60% in the referenced articles [11,30]. Hence, the drug could keep a controlled release even if the hydrogels degrade in the medium and our results are comparable with the results in the articles [11,30,34,35].

3.7. *In vivo distribution studies*

As is shown in Fig. 7, the NIR signal in the three groups decreased with time. However, in the Dir-MM-hydrogels group, the signal was stronger at the same time and decayed much slower compared with the Dir-micelles and Dir-injection groups. This result showed that the retention of docetaxel at the local site of administration could be prolonged by the DTX-MM-hydrogels system. The state of the formulations at the site of administration and the release profiles could both affect the retention of docetaxel. Because of the thermo-sensitivity of the DTX-MM-hydrogels, they could become solid once administered, whereas the DTX-injection and DTX-micelles remained fluid. Based on these findings, the DT\X-MM-hydrogels formulations could remain at the local site for a longer time compared with other groups. In addition, the DTX-MM-hydrogels exhibited slower release profiles, and the drugs could continue to gather at the local sites. Therefore, the hydrogel formulations could produce a longer retention and higher concentration of drug at the site of administration.

3.8. *In vivo pharmacodynamics studies*

3.8.1. *The antitumor effects of DTX-injection, DTX-micelles and DTX-MM-hydrogels after intratumoral administration*

Fig. 8 shows the tumor volume as a function of time for four different formulations. The change curves showed that the DTX-MM-hydrogels could achieve a greater tumor inhibition effect compared with the control group. The changes in the tumor volume with the DTX-MM-hydrogels demonstrated the release profiles of the DTX-MM-hydrogels formulation. For example, the tumor volume showed a tiny rise in 2 d, and this may be due to the controlled release of the formulation. Also, the cumulative amount of drug in the DTX-MM-hydrogels was lower compared with the other groups. However, the other two formulations produced a high level of drug accumulation within 2 d and, therefore, they had a better tumor inhibition effect compared with the DTX-MM-hydrogels formulation within 2 d. As is shown in Table 1, the RTV at day 12 after initial therapy was 11.20 for blank hydrogels, 6.09 for the DTX-injection, 5.42 for DTX-micelles, and 2.38 for DTX-MM-hydrogels. And this shows that the DTX-MM-hydrogels formulation had marked antitumor effects compared with the blank hydrogels group. Also, as shown in Fig. 8B, there was a significant difference in the tumor volume doubling times between the hydrogels group and the other three groups. Compared with blank hydrogels, the tumor volume doubling time in DTX-MM-hydrogels group was significantly increased 2.7-fold. As a result, the DTX-MM-hydrogels reduced the rate of tumor proliferation. Therefore, after administration, a better antitumor efficacy was observed with the DTX-MM-hydrogels formulation over time and this remained stable for a relatively long time. The results obtained in the *in vivo* antitumor experiments were consistent with the *in vitro* release behavior.

The antitumor effect was in the following descending order: DTX-MM-hydrogels > DTX-micelles > DTX-injection and the above results demonstrate that the DTX-MM-hydrogels are a promising drug delivery system for the local administration of anticancer drugs.

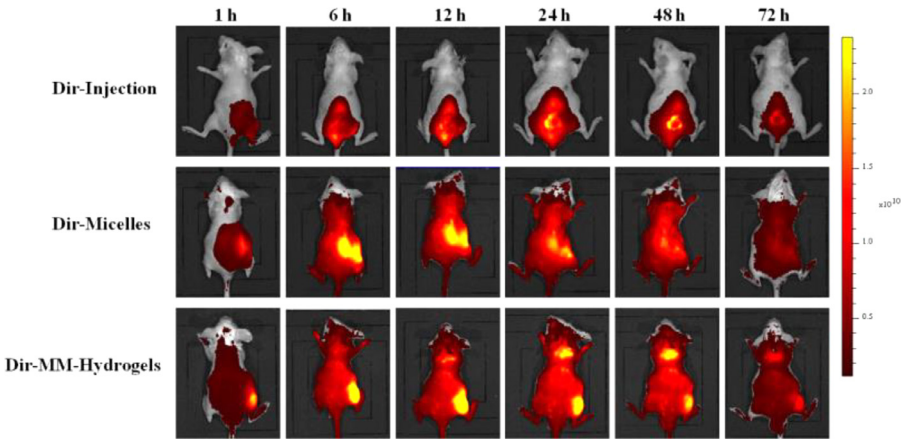

Fig. 7 – *In vivo* fluorescence images of the whole body distribution of the Dir in mice.

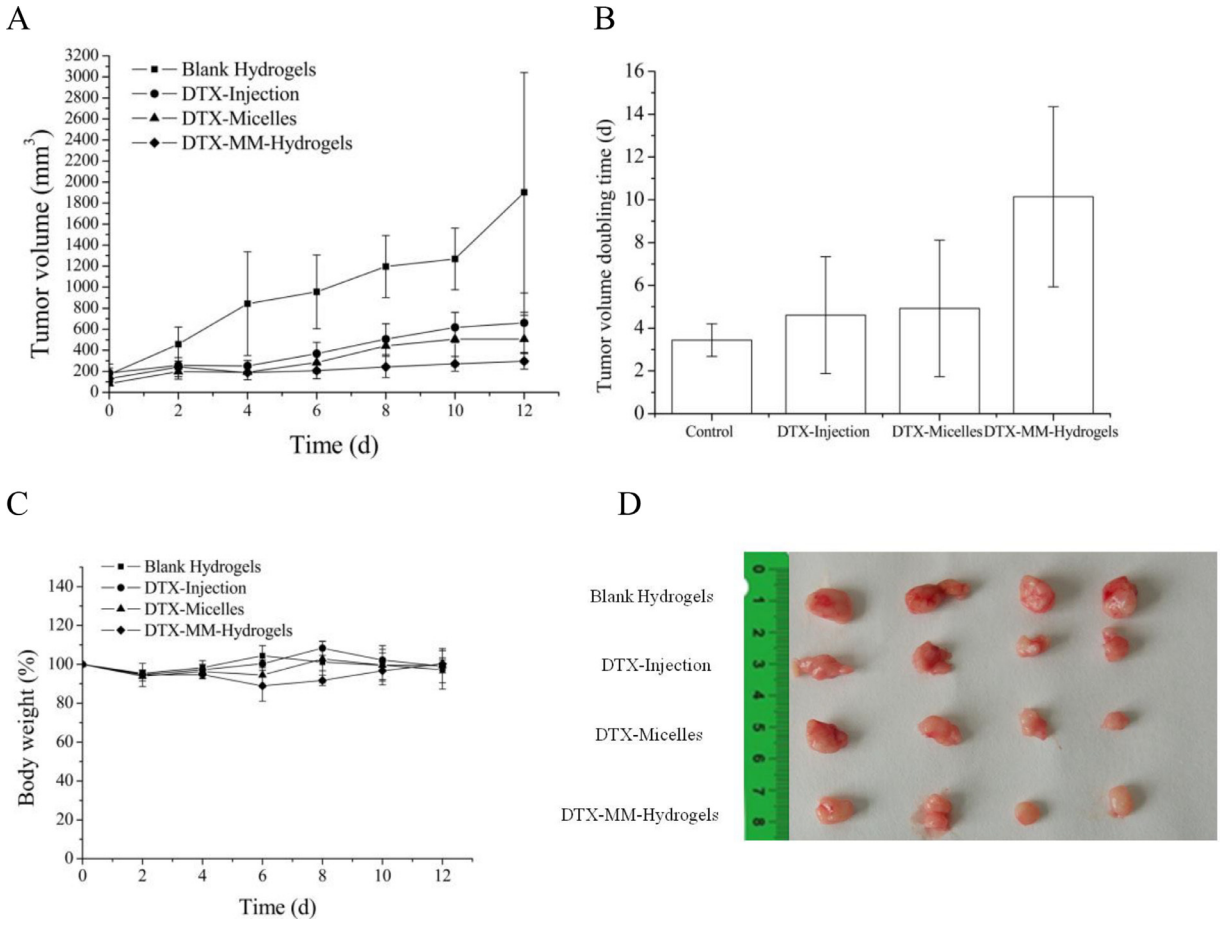

Fig. 8 – (A) Changes in tumor volumes of the *in vivo* antitumor activity against HT-29 tumor-bearing mice after a single administration of blank hydrogels, DTX-micelles, DTX-MM-hydrogels or DTX-injection at an equivalent dosage of 10 mg/kg. ($n = 4$, mean ± SD). (*$P < 0.05$, DTX-MM-hydrogels *versus* blank hydrogels at the same time-point; *$P < 0.05$, DTX-MM-hydrogels *versus* DTX-injection at the same time-point); (B) the tumor volume doubling times in each group. ($n = 4$, mean ± SD). ($P < 0.01$, DTX-MM-hydrogels *versus* blank hydrogels at the same time-point, *$P < 0.05$, DTX-MM-hydrogels *versus* DTX-injection at the same time-point, *$P < 0.05$, DTX-MM-hydrogels *versus* DTX-micelles at the same time-point); (C) body weight changes of each group after a single administration. ($n = 4$, mean ± SD). ($P > 0.05$, DTX-MM-hydrogels compared to blank hydrogels, DTX-injection and DTX-micelles). (D) Image of excised tumor masses at the time of sacrifice after 12 d post treatment.**

Table 1 – The relative tumor volumes in each group (RTV (%) = V_t/V_0 x 100).

group	Blank hydrogels		DTX-injection		DTX-micelles		DTX-MM-hydrogels	
Time	RTV	SD	RTV	SD	RTV	SD	RTV	SD
2 d	2.70	1.26	2.08	0.83	2.10	0.80	1.84	0.26
4 d	4.97	1.96	2.04	1.20	2.06	1.02	1.45	0.27
6 d	5.63	1.65	2.97	1.92	3.04	1.37	1.58	0.49
8 d	7.05	1.11	4.09	1.82	4.71	3.05	1.86	0.61
10 d	7.47	2.76	4.99	1.62	5.41	3.60	2.08	0.53
12 d	11.2	2.76	6.09	2.77	5.42	3.19	2.27	0.61

RTV(%) = V_t/V_0*100, where V_t was the tumor volume at predetermined time points, and V_0 was the initial tumor volume. *P > 0.05, DTX-injection *versus* blank hydrogels at the same time-point, *P > 0.05, DTX-micelles *versus* blank hydrogels, *P < 0.05, DTX-MM-hydrogels *versus* blank hydrogels ($n = 4$, mean ± SD).

3.8.2. *Changes in body weight*

Fig. 8 shows that there were no obvious differences among the four groups. In the DTX-MM-hydrogels treatment group, the mean body had fallen at the sixth day, however, in the following 6 d, the body weight increased with time and reached the same level as that in the other three groups. Based on the *in vitro* release profiles, the reason for this change may be that the drug reached a high level of accumulation at the tumor sites by the sixth day, however, this slight decrease in body weight could be reversed in the days that followed. In addition, the changes in the four groups were not statistically significant. All the evidence shows that the *in situ* intratumoral DTX-MM-hydrogels are a suitable drug delivery system for cancer therapy.

4. Conclusion

In this study, a drug delivery system of hydrogels incorporated with micelles was developed. The *in vitro* characterization showed that the network of hydrogels was irregular and lyophilized samples retained the same inner structure for 3 months. The *in vitro* release profiles and *in vivo* distribution both showed that the hydrogels can form a depot at body temperature and achieve a prolonged drug release at the site of administration. *In vivo* antitumor experiments showed the drug delivery system could produce better treatment effects and a higher tumor inhibition rate for a long time, up to 12d. Also, there were no obvious changes in body weight. All the evidence suggests that the materials are suitable for *in vivo* application. So it can be concluded that the DTX-MM-hydrogels are a promising drug delivery system for *in situ* administration in antitumor treatments.

Conflict of interest

We declare that we have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work entitled Evaluation of Micelles Included into Thermosensitive Hydrogels for Intratumoral Delivery and Controlled Release of Docetaxel: A Dual Approach for *In Situ* Treatment of Tumors.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajps.2018.05.004.

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