# Supplementary Materials

# Efficient inhibition of colorectal peritoneal carcinomatosis by drug loaded micelles in thermosensitive hydrogel composites

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## Supplementary methods

### 1. *In vitro* cell viability assay

The L929 cells were plated at a density of 1 $\times 10^4$ cells per well in 100 μL of medium in 96-well plates and grown for 48 hours. The cells were then exposed to a series of blank PCEC micelles or blank PCEC hydrogels at different concentrations for 48 hours respectively. Then, viability of cells was measured using the MTT method. Briefly, the mean percentage of cell survival relative to that of untreated cells was estimated from data of six individual experiments, and all data were expressed as the mean ± SD.

### 2. *In vitro* hemolytic test

The hemolytic study was performed on blank PCEC micelles and blank PCEC hydrogel *in vitro*. Briefly, 0.5 mL of blank PCEC micelles (20 mg/mL and 50 mg/mL) or blank PCEC hydrogel (20 mg/mL and 50 mg/mL) in normal saline (NS) was diluted into 2.5 mL by normal saline and added into 2.5 mL of rabbit erythrocyte suspension (2%) in normal saline at 37$^{o}$C. NS and distilled water were employed as negative and positive control, respectively. Three hours later, the erythrocyte suspensions were centrifuged, and then the supernatant of the erythrocyte suspension

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were collected. The collected supernatants were detected on a UV-Vis spectrophotometer at 545 nm to determine the hemolytic rate. The hemolytic rate was calculated according to the following equation:

$$\text{Hemolytic Rate} = \frac{\text{Test sample - Negative control}}{\text{Positive control - Negative control}} \times 100\% \quad (1)$$

All results were estimated from the data of three individual experiments, and all data were expressed as the mean ± S.D.

**3. Acute Toxicity Test**

Owing to great biodegradability and biocompatibility of PCEC copolymer and our preliminary experiment, no lethal dose or median lethal dose ($LD_{50}$) could be detected. Therefore, the toxicity of blank micelles/hydrogel DDDS was evaluated using Maximal Tolerance Dose (MTD) method here.

Twenty-four BALB/c mice of both sexes were equally divided into two groups (n=12, 6 male and 6 female mice). Each animal was intraperitoneally injected with 500 mg of blank micelles/hydrogel DDDS (20% wt). Therefore, the total micelles/hydrogel DDDS dose given each animal of subcutaneous injection group was up to 25g/Kg body weight (b.w.). The control group was treated in an identical manner except that a similar weigh of normal saline (NS) was used instead of the micelles/hydrogel DDDS.

All the animals were observed continuously for 14 days after administration including the general conditions (the activity, energy, hair, feces, behavior pattern, and other clinical signs etc), body weight, and mortality. The necropsy of dead animals was performed to observe the gross pathological changes. At 14th day, all animals were sacrificed. After dissection, gross histological examinations of the major organs were carried out.

**4. Histopathologic Study**

The samples were obtained from the following organs: heart, liver, spleen, lung, kidneys, intestinal tract and abdominal wall. All the samples were preserved in 10% buffered formaldehyde and were subsequently embedded in paraffin. Then, paraffin sections were stained with haematoxylin-eosin (HE) for histopathologic examination.

In order to investigate the major organs toxicity of the micelles/hydrogel DDDS, the histopathological changes of major organs were observed on light microscope.

**Supplementary Table 1** Hemolytic rates of PCEC micelles and hydrogel

| Concentration | PCEC micelles | | PCEC hydrogel | |
|---|---|---|---|---|
| (mg/mL) | 20 | 50 | 20 | 50 |
| Hemolytic Rate (%) | 0.28 ± 0.02 | 0.90 ± 0.04 | 0.36 ± 0.03 | 0.72 ± 0.04 |

# Supplementary Figure Legends

**Supplementary Figure 1** Cytotoxicity (A) and hemolytic test (B) of PCEC micelles and PCEC hydrogel at different concentration. a: distilled water as positive control; b: normal saline as negative control; c: 20mg/ml PCEC micelles; d: 50mg/ml PCEC micelles; e: 20mg/ml PCEC hydrogel; f: 50mg/ml PCEC hydrogel. Error bars represent the standard deviation (n=6).

**Supplementary Figure 2** Mice body weight of each group during the observation period.

**Supplementary Figure 3** Photograph of major organs after subcutaneous administration of blank micelles/hydrogel system (×400). Mice cardiac muscle, liver, spleen, lung, kidneys, intestinal tract, and abdominal wall photograph of control group (A, C, E, G, I, K, M) and blank micelles loaded hydrogel composite treated group (B, D, F, H, J, L, N) respectively.