Supporting Information

Thermoresponsive hydrogel for transcatheter arterial chemoembolization of hepatocellular carcinoma

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Materials and methods

Materials

Ethiodized poppyseed oil (Etpoil) was purchased from Hengrui Medicine Co. Ltd., Epirubicin hydrochloride (Epi) was acquired from Shandong New Times Pharmaceutical Co. Ltd (Shandong, China). Methylcellulose (MC) and xanthan gum were obtained from Sinopharm Chemical Reagent Co., Ltd. MTT and dimethyl sulfoxide (DMSO) were acquired from Sigma-Aldrich. Ultrapure water prepared by a Milli-Q system (18.2 MΩ·cm) was utilized in all experiments.

Construction of Epi/Etpoil@MC/XG

First, MC/XG hydrogel was synthesized as per the literature. MC/XG hydrogel were synthesized by adding 1.2 g MC to the 20 ml ultrapure water, respectively, at 4°C. After the MC aqueous solution was completely dissolved, 0.4 g XG was gently added and the mixture was stirred for 4 h at 4°C. Finally, the air bubbles were removed by centrifugation at 4,000 rpm/min for 10 min.

Secondly, 10 mg Epi was distributed in 10 ml Etpoil. Then, 0.5 ml Etpoil containing 1 mg ml^{-1} Epi were well mixed with 1 ml MC/XG hydrogel via three-way valve. The prepared Epi/Etpoil@MC/XG was stored at 4°C. Before the TACE treatment, the Epi/Etpoil@MC/XG was sterilized under UV light for 30 min.

Characterizations

Scanning electron microscope (SEM) image was captured in SU8220 (Hitachi). X-ray powder diffraction (XRD) patterns were taken on a D8 Advance X-ray polycrystalline diffractometer (Bruke). Circular dichroism (CD) spectra were recorded by MOS 450 (Bio-Logic Science Instruments). MTT assay was measured with a multi-functional microplate reader (Infinite M1000 Pro; Tecan). The Tensor 27 FT-IR spectrometer (Bruke) was used to collect fourier-transformed infrared spectroscopy (FT-IR) of Epi/Etpoil@MC/XG. X-ray images were scanned via Digital Radiography Systems (ARISTOS MX2, Siemens, GER, 52 kV/4.5 mAs). The fluorescence of Epirubicin was detected with a FluoroMax-4 (HORIBA). X-ray images were captured with a Digital Radiography System (ARISTOS MX2, Siemens, GER, 52 kV/4.5 mAs） The CT images were captured by SOMATOM Definition (Siemens) with the following settings: 56 mAs tube current, 120 kV tube voltage, 1 mm thickness, 0.5 mm overlap. The DSA images were captured with a DSA machine (Axiom Artis; Siemens).

Rheological measurements

The rheological properties of Epi/Etpoil@MC/XG were assessed using a Rheometer AR1000 (TA Instruments) under different temperatures with a 20 mm parallel-plate configuration. The changes in storage (G') and loss (G'') modulus were measured during the frequency cycles and strain cycles at room temperature (26°C) and body temperature (37°C). Frequency steps cycled between 1 and 30 rad/s with 1% strain at 26°C and 37°C was performed. Strain steps cycled between 0.1-1.0% with 10 rad/s frequency at 26°C and 37°C was performed. The thixotropic property of

Epi/Etpoil@MC/XG was measured by recording G' and G'' under alternating transformation of 1% strain and 200% strain.

MTT assay

HepG-2 cells and HUVEC (1×10^5 cells/well) were incubated with Dulbecco's Modified Eagle's Medium (DMEM) in 96 well plates at 37°C in a humidified atmosphere of 5 % CO_2 for 24 h. After removing the medium and washing with phosphate buffered solution (10 mM, pH=7.4, with 137 mM NaCl and 2.7 mM KCl), various concentrations of Epi/Lipdol@MC/XG were acquired by adding different dilutions of prepared hydrogels (control, 1/1,000, 1/800, 1/500, 1/100, 1/50 and 1/10) and DMEM (the total volume was 100 μl) to each well for 48 h, respectively. As the total volume was 100 μL, the dilution of 1/1,000, 1/800, 1/500, 1/100, 1/50 and 1/10 were corresponding to 0.100, 0.125, 0.2, 1, 2, 10 μL of prepared Epi/Lipdol@MC/XG hydrogel. Subsequently, 10 μl MTT solutions (5 $mg\cdot ml^{-1}$) and 90 μl DMEM were added into each well and cultured for another 4 h. Finally, DMSO (150 μl per well) was added to each well after removing the supernatant. The OD value was read at 490 nm to calculate cell viability.

In vitro drug release

The drug release performance of the 6 groups with different contents were compared. The group of free Epi was prepared by adding 0.5 mg Epi to 1.5 ml ultrapure water. The group Epi/Etpoil was emulsified 0.5 mg Epi with 1.0 ml H_2O and 0.5 ml

Etpoil. The groups Epi/Etpoil@MC and Epi/Etpoil@XG was 0.5 ml Etpoil emulsified Epi with 1 ml MC (0.06 g ml^{-1}) and 1 ml XG (0.02 g ml^{-1}), respectively. The volume of each group was fixed at 1.5 ml and the concentration of Epi was unified.

Each group was placed in a sealed dialysis bag (10 kDa), and the dialysis bag was immersed in a centrifuge tube containing 40 ml PBS (10 mM) with pH value=7.4 at 37°C. The centrifuge tubes were fixed in a shaker with 80 rpm/min. A total of 1 ml PBS containing released Eip was taken out every specific time, and 1 ml freshly prepared PBS was added to the tube to maintain the total volume. The Epi was determined through a fluorescence spectrophotometer. The drug release rate was calculated by F/F_0, where F_0 and F represent the fluorescence intensity at 620 nm of the 6 groups and the obtained PBS solution containing released Epi at each specific time point, respectively. The excitation wavelength was 484 nm.

In vivo measurements

Two adult female New Zealand White rabbits weighing 2.5-3.0 kg were purchased from the Experimental Animal Research Center, The Southern Medical University (Guangzhou, China). They were allowed to acclimatize for at least 5 days before use and were kept in a closed system without an isolator. The experimental protocols were approved by the Southern Medical University animal care and use committee guidelines. After the injection of 3% pentobarbital sodium (1 ml/kg) in the marginal ear vein, the hair was removed from the auricle by shaving the skin with a pair of clippers. Tumor pieces were obtained from non-necrotic, well vascularized portions of

aseptic VX2 cells bearing tumors of the hind paw. The fresh tumor tissue was cut into the long strip shape at a size of 1×1×3 mm pieces, and implanted into a small subcutaneous pocket using an 18-gauge co-axial introducer needle. Both tumor tissue pieces were used within 2 h. The sizes of the two rabbit tumors were recorded with a caliper. When the tumor volumes reached ~20×20×20 mm, the rabbits were ready for the following embolization experiments.

First, the rabbits were anesthetized by injection with 3% pentobarbital sodium (1 ml/kg) from the marginal ear vein. Then, the auricles were shaved and disinfected, and a cutaneous incision was made parallel and slightly lateral to the distal end of central auricular artery. The artery of the tumor can be found under the illumination of a flashlight. Several drops of 2% lidocaine were injected to prevent arterial spasm. The 21G needle with outer catheter was inserted into the distal end of central auricular artery, and the needle and catheter were fixed with elastic adhesive tapes to prevent inadvertent displacement. Then, the contrast media (Iodixanol Injection; GE Healthcare) was injected to map the tumoral vasculature. Next, 0.5 ml of Epi/Etpoil@MC/XG was injected to one tumor artery and the other was injected with saline (0.5 ml; pH=7), the injection process and the effect of embolization was monitored through X-ray. After injection for 1 h, the rabbits were sacrificed. The two tumors were removed and inspected under the CT scan. Finally, the tumor issues were fixed in formalin. Sections were embedded in paraffin and histologically evaluated after staining with hematoxylin and eosin (H&E).

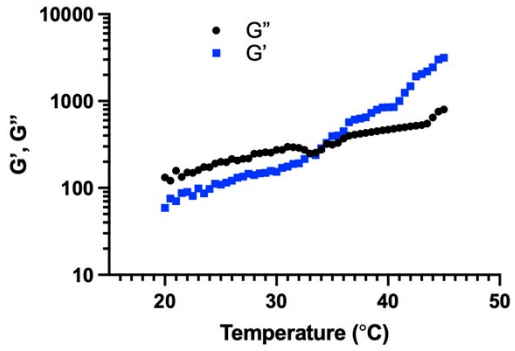

Figure S1. The temperature-dependent G' and G'' changes of MC solution (12 wt%).

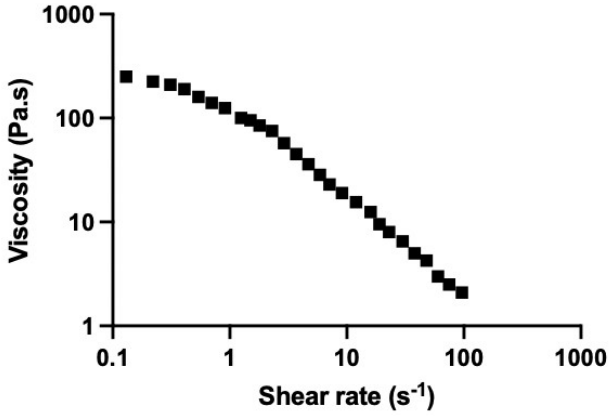

Figure S2. The viscosity changes of Epi/Etpoil@MC/XG between the low shear rate and high shear rate at 37°C.

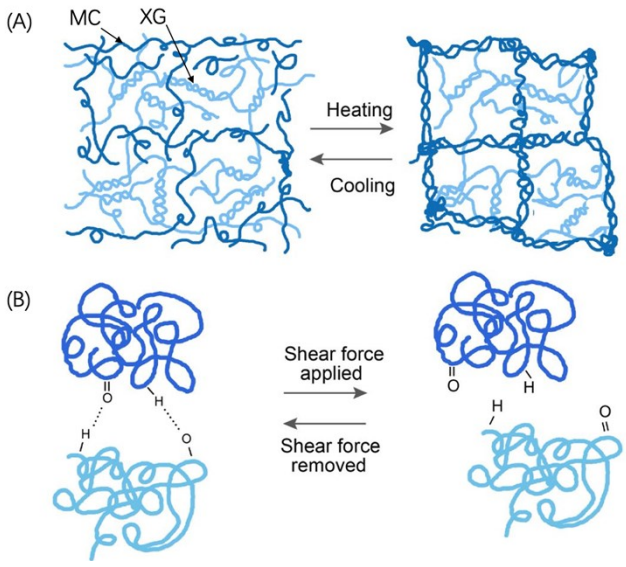

Figure S3. The illustration of (A) mechanism for the formation of hydrogel and its thermal responsive ability, and (B) thixotropic property of XG.