Electronic Supplementary Material (ESI) for Biomaterials Science

Supporting Information

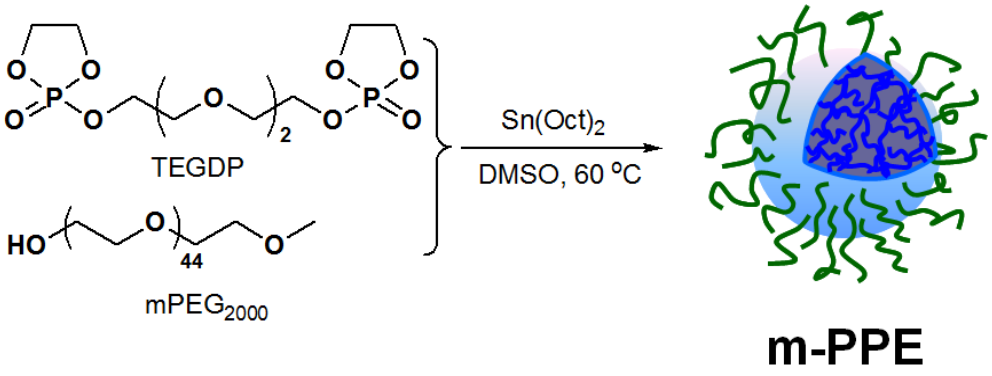
Enhanced Drug Delivery to Hepatocellular Carcinoma with Galactosylated Core-Shell Polyphosphoester Nanogel

Juan Wu, Tian-Meng Sun, Xian-Zhu Yang, Jing Zhu, Xiao-Jiao Du, Yan-Dan Yao, Meng-Hua Xiong, Hong-Xia Wang, Yu-Cai Wang and Jun Wang**

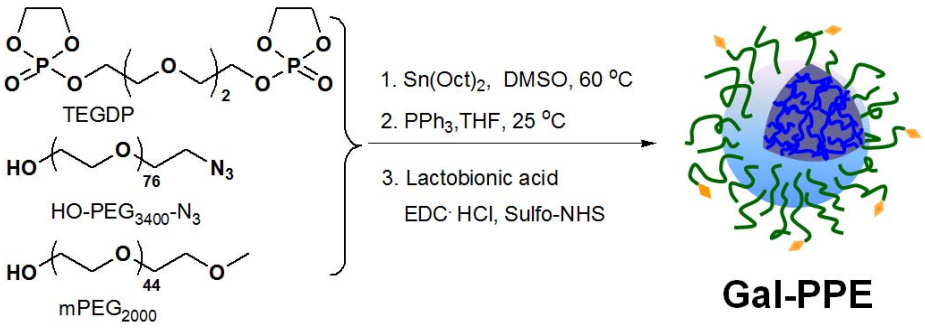
Isolation and culture of rat hepatocytes and Kupffer cells for *in vitro* cell internalization

Rat hepatocytes and Kupffer cells were isolated from the liver of Wistar rat under pentobarbitone anaesthesia. The portal vein of the rat was cannulated and perfused with HBSS (containing NaCl (8.00 g), KCl (0.40 g), Na_2HPO_4 (0.0475 g), KH_2PO_4, (0.06 g), and dextrose (1.0 g) per liter) containing 0.5 mM EDTA for 20 min at 37 °C to remove the blood in the liver. Then the perfusion solution was changed to 0.04% collagenase type IV (Sigma-Alderich) in HBSS and the liver was perfused for 20 min. The liver was removed from the rat and cut into small pieces, and were incubated with 0.02% collagenase type IV in HBSS containing 0.002% DNase I (Sigma-Alderich) for 30 min at 37 °C with gentle shake. The cell suspension was filtered through sterile nylon filter (100 μm) to remove undigested tissue and connective tissue. The cell suspension was centrifuged at 50×g for 3 min at 4 °C and the hepatocytes and Kupffer cells were purified from the pellet and suspension respectively. To obtain hepatocytes, the pellet cells were layered onto a 40% Percoll (Sigma-Alderich) in a 50 ml conical centrifuge tube and centrifuged at 600×g for 10 min at 4 °C. The cells in the pellet were placed in the culture dish in RPMI-1640 medium containing antibiotic and antimycotic and 10% FBS after washing with HBSS. To obtain Kupffer cells, the suspension cells were layered onto a 25%/50% two-step Percoll gradient in a 50 ml conical centrifuge tube and centrifuged at 1800×g for 15 min at 4 °C. The cells on the middle layer were placed in the culture dish in RPMI-1640 medium containing

antibiotic and antimycotic and 10% FBS after washing using HBSS. To obtain a pure monolayer culture of Kupffer cells, the non-adherent cells were removed by replacing the culture medium after 15 min culture.



Scheme S1. Synthesis of non-galactosylated m-PPE.



Scheme S2. Synthesis of galactosylated Gal-PPE.

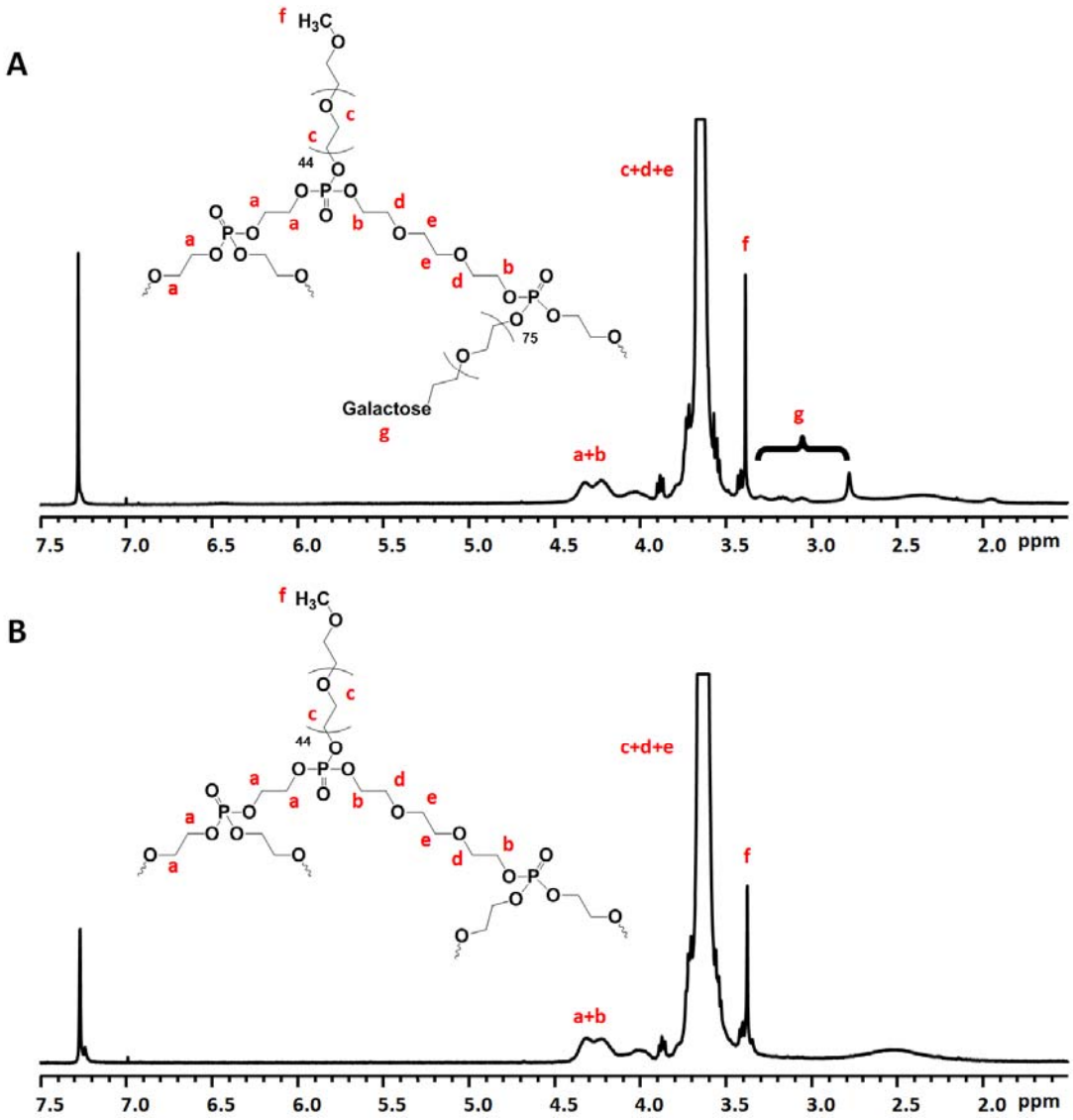


Fig. S1. ^{1}H NMR analyses of (A) Gal-PPE and (B) m-PPE. The resonances appeared at 2.7-3.3 ppm in the spectrum of Gal-PPE indicates successful conjugation of lactobionic acid to Gal-PPE.

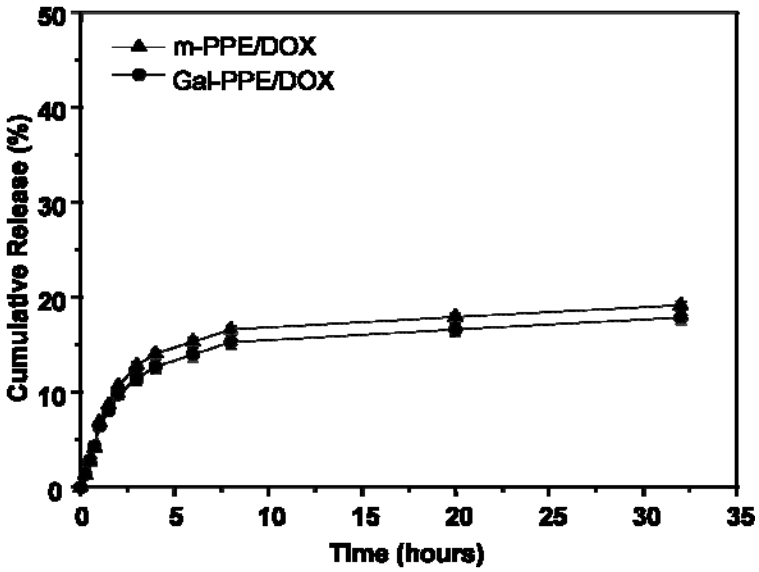


Fig. S2. Release profiles of doxorubicin from m-PPE/DOX and Gal-PPE/DOX in phosphate-buffered saline (PBS, 0.02 M, pH=7.4) at 37 °C. m-PPE/DOX or Gal-PPE/DOX (1.5 mL) was introduced into a dialysis membrane tubing and incubated in 25 mL of PBS in a shaking water bath at 37 °C in dark. At predetermined intervals, buffer was drawn and replaced with an equal volume of fresh buffer. The concentration of doxorubicin was measured by HPLC.

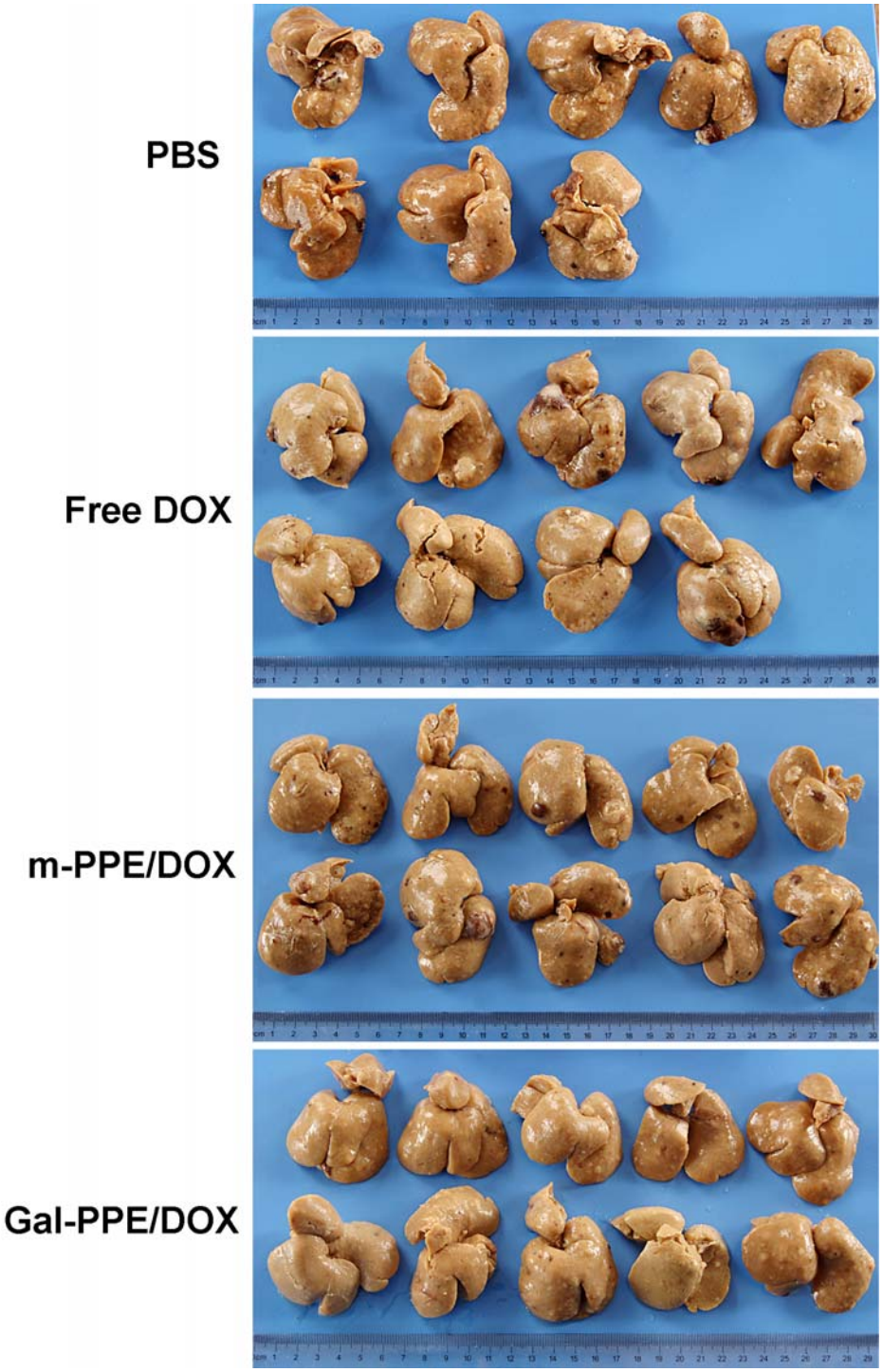


Fig. S3. Macroscopic views of liver lobes of HCC-bearing rats after treatments with PBS, free DOX, m-PPE/DOX or Gal-PPE/DOX. Two and one of rats died in PBS and free DOX treatment groups during the treating period, respectively. Rats were administered every four days at equivalent DOX dose at 1 μg/g of body weight by tail intravenous injections for eight times.