Study on the Novel Drug Vehicle for Encapsulation of Hydrophobic Agent and MR Imaging

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Statement of Purpose: In order to achieve the concept that integration of diagnosis and therapy in one box, many drug delivery systems (DDSs) associating with imaging functions (i.e., image-guided DDSs) are developed to detect diseases, deliver therapeutic agents, and track the distribution of drug vehicles. As a diagnostic technique, magnetic resonance imaging (MRI) guided drug delivery systems can provide not only real-time structural information with high resolution but also functional information regarding living bodies in a non-invasive manner. In general, vehicles with particle size less than 150 nm could selectively accumulate in tumor tissue via the enhanced permeation and retention (EPR) effect. Hence, the purpose of this study was to develop a novel drug vehicle with targeted delivery and MR imaging functionality via encapsulating anti-cancer drug and iron oxide nanoparticles into carboxymethyl hexanoyl chitosan (CHC) polymeric micelles conjugated with folic acid (FA) molecules. The structure of the FA-conjugated CHC/SPIO micelles were analyzed by using transition electronic microscope (TEM). The MR imaging functionality and cytotoxicity of the CHC/SPIO micelles were also investigated.

Methods: Hydrophobic superparamagnetic iron oxide (SPIO) nanoparticles were prepared by the thermal decomposition method[1]. Our group has developed an amphiphilic chitosan derivative (carboxymethyl hexanoyl chitosan, CHC) using N,O-carboxymethyl chitosan (NOCC) as a precursor elsewhere[2]. FA conjugation was performed by using a modified procedure[3]. 5 mL of 0.01 M FA was mixed with 0.2 M EDC and MES buffer (pH 5.5) for 30 min. The resulting solution was then added in 0.5 M NHS in the room temperature. After a reaction time of 5 hr, the obtained solution was then mixed with 15 mL of CHC solution (1% v/v) in dark overnight to synthesize the FA- conjugated CHC molecules. Finally, the reaction mixture was dialyzed using dialysis bag (Mw cut-off: 14000 Da) followed by lyophilization. The lipophilic SPIO nanoparticles were loaded in the FA-conjugated CHC/SPIO micelles via using self-assembly route. FA-conjugated CHC solution was mixed with an anti-cancer drug (camptothecin; CPT/ DMSO 0.025 mg/mL) and hexane/SPIO suspension, which was followed by the sonication in an ice bath.

Results: We found that the SPIO nanoparticles [Figure 1 (a)] could successfully assemble to micelles with the incorporation of CHC [Figure (b)]. The magnetic properties of the FA-conjugated CHC/SPIO micelles were characterized using SQUID. Their magnetization-magnetic field strength (M-H) curve is shown in Figure 1 (c). No hysteresis loop was observed, which suggests that the FA-conjugated CHC/SPIO micelles demonstrated superparamagnetism. Hence, the proposed vehicle is expected to demonstrate MR T_2 image contrast. Figure 1 (d) shows that the characteristic UV-vis absorption peaks of FA (281 nm) was observed for the FA-conjugated micelles, which suggested that FA ligands have been grafted with CHC via amide reaction. Cell cytotoxicity of MDA-MB-231cancer cells cultured with CPT-loaded CHC/SPIO micelles and CPT-loaded FA-conjugated CHC/SPIO micelles were determined by MTT assay. As shown in Figure 2, cytotoxicity of the CPT-loaded FA-conjugated CHC/SPIO micelles were significantly lower than that of CPT-loaded CHC/SPIO micelles. This result indicated that CPT-loaded FA-conjugated CHC/SPIO micelles can effectively target cancer cells and enhance the delivery efficacy of anticancer drug. In vivo MR imaging of drug vehicles in the mice body was conducted to determine the vehicle distribution. As shown in Figure 3, the T_2 contrast of the MR image was enhanced in the liver in the first 15 min after injection .

Conclusions: A novel folic acid-conjugated drug vehicle was successfully developed to encapsulate hydrophobic agent, achieve in vitro targeted delivery and demonstrate in vivo MR imaging functionality.

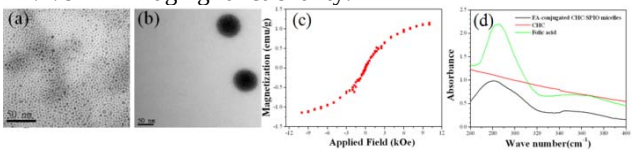

Figure 1. TEM images of (a) SPIO nanoparticles without incorporating with CHC; (b) FA-conjugated CHC/SPIO micelles; (c) M-H curve of FA-conjugated CHC/SPIO micelles and (d) UV-vis spectra of FA-conjugated CHC/SPIO micelles, CHC and folic acid.

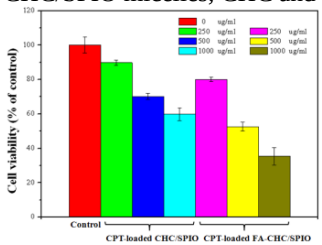

Figure 2. Cell cytotoxicity of CPT-loaded CHC/SPIO micelles and CPT-loaded FA-conjugated CHC/SPIO micelles cultured with MDA-MB-231cancer cells.

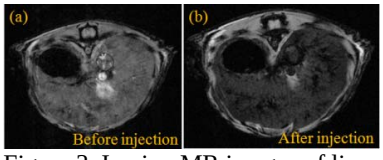

Figure 3. In vivo MR images of liver (a) before and (b) after injection with FA-conjugated CHC/SPIO micelles.

References:

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3. Setua S, et al. Biomaterials, 2010;31(4): 714-729.