Supporting Information

Redox-sensitive Hyaluronic Acid-Paclitaxel Conjugate Micelles with High Physical Drug Loading for Efficient Tumor Therapy

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1. Materials

Sodium hyaluronic acid (HA, molecular weights 11 kDa) was purchased from Freda Biochem Co., Ltd. (Shandong, China). 1-Ethyl-3 (3-dimethylaminopropyl) carbodiimide (EDC), N-Hydroxysuccinimide (NHS), deoxycholic acid, dithiothreitol and glutathione were purchased from Aladdin Reagent Database Inc. (Shanghai, China). N-hydroxysulfosuccinimide (sulfo-NHS) and paclitaxel (PTX) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and Chongqing Melian Pharmaceuticals Co., Ltd. (Chongqing, China), respectively. Cystamine and adipic dihydrazide were purchased from TCI Development Co., Ltd. (Shanghai, China). The fluorescence probe, tetramethyl rhodamine isothiocyanate (TRITC), Nile red (NR) and Cy7 Mono NHS ester (Cy7-NHS) was obtained from Beijing Fanbo Science and Technology Co., Ltd. (Beijing, China). All other chemicals were of analytical grade and were used without further purification.

2. Methods

Structure characterization of HA-ss-PTX and HA-PTX conjugates

The chemical structures of HA-ss-PTX conjugates and HA-PTX conjugates were characterized by ^{1}H NMR and FTIR spectra. ^{1}H NMR spectra were acquired on a Bruker (AVACE) AV-500 spectrometer. HA-ss-PTX and HA-PTX conjugates were dissolved in the mixed solution of D_2O/CD_3OD (1:1, v/v). HA was dissolved in only D_2O. PTX and 2'-Suc-PTX were dissolved in $CDCl_3$. FTIR measurements were performed on a Bruker Equinox-55 FTIR spectrometer with a disk of KBr. The chemically conjugated amount (DL_c, wt%) of PTX in conjugates was estimated by UV absorbance at 227 nm in the mixed solution of acetonitrile/H_2O

(80:20, v/v).

3. Results and Discussion

^{1}H NMR spectra of PTX, 2'-Suc-PTX, HA, HA-ss-PTX and HA-PTX conjugates were shown in Figure S1A. Firstly, the structure of 2'-Suc-PTX was confirmed through the characteristic peaks at 2.60 ppm (-COC*H*$_2$C*H*$_2$CO-) of Suc and 4.40 ppm which was belong to the 7-H of PTX. More importantly, the chemical shift of 2'-H in 2'-Suc-PTX (5.99 ppm) was different from that in PTX (4.70 ppm). However, this difference did not appear in that of 7-H. These two results collectively indicated that the esterification proceeded at the site of 2'-H. The characteristic peaks of HA appeared at 2.01 ppm (acetyl (-NHCOCH$_3$)), 3.0-4.0 ppm (glucosidic H (10H)) and 4.40-4.60 ppm (anomeric H (2H)). As compared with the spectrum of HA, the aromatic signals at 7.0-8.2 ppm in the spectra of HA-ss-PTX and HA-PTX belonged to the typical benzene ring of PTX. These results demonstrated that both HA-ss-PTX and HA-PTX conjugates were successfully synthesized.

The chemical structure of HA-ss-PTX and HA-PTX was further confirmed by the FTIR spectra. As shown in Figure S1B, after 2'-Suc-PTX was introduced to HA, a new band at 1690 cm−1 was observed in spectra of HA-ss-PTX (Figure S1B(b)) and HA-PTX (Figure S1B(c)), which were assigned to the ester bond vibration of 2'-Suc-PTX. In addition, the bands appeared at 1430 cm−1, 710 cm−1 in Figure S1B(b, c) were attributed to the –C=C– stretching mode of benzene ring of PTX, indicating 2'-Suc-PTX was successfully linked to the backbone of HA.

The degree of substitution (DS) of HA-CYS and HA-ADH was determined by the method of ^{1}H NMR described before [36], and DS value was calculated to be ~12% and ~18%, respectively.

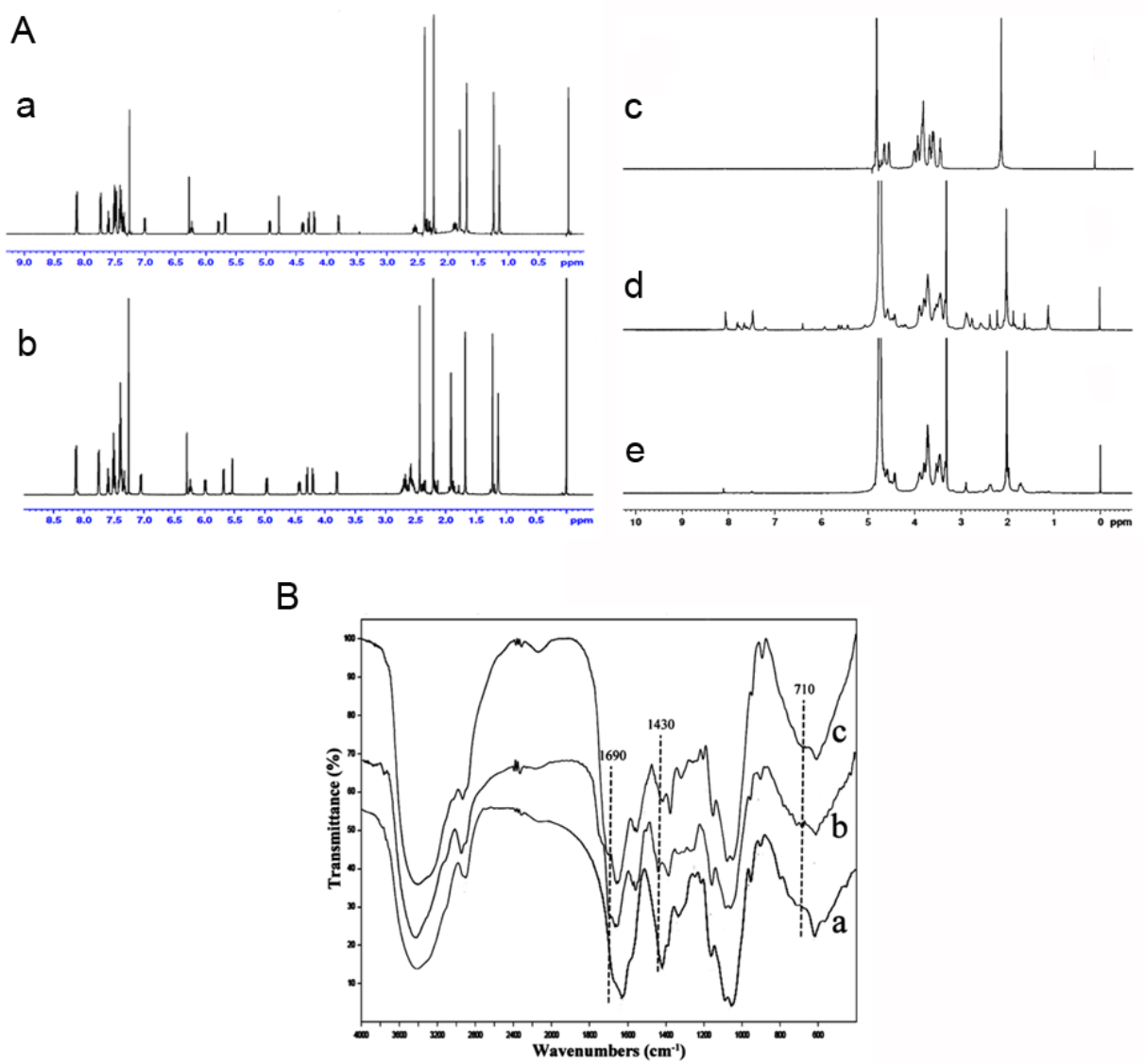

Figure S1. ^{1}H NMR spectra (A) of PTX (a), 2'-Suc-PTX (b), HA (c), HA-ss-PTX conjugates (d) and HA-PTX conjugates (e). FTIR spectra (B) of HA (a), HA-ss-PTX conjugates (b) and HA-PTX conjugates (c).

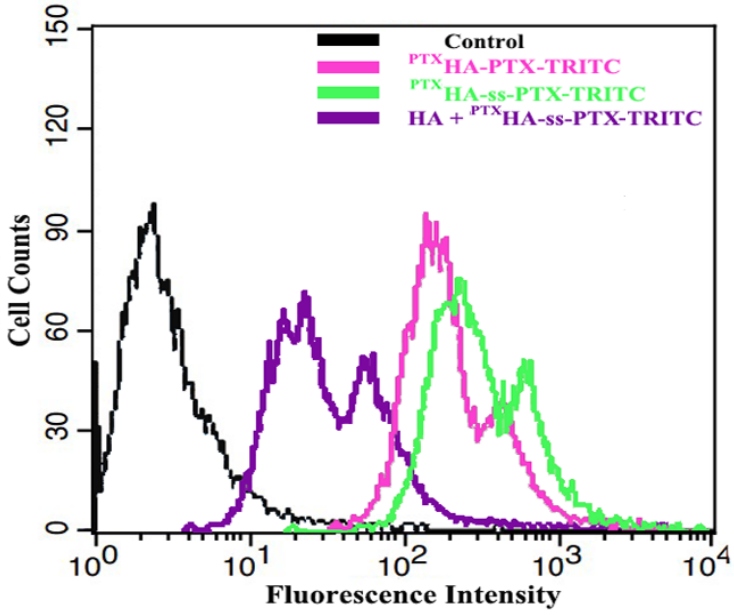

Figure S2. Flow cytometric curves obtained by flow cytometry. MDA-MB-231 cells were incubated with

^{PTX}HA-PTX-TRITC micelles or ^{PTX}HA-ss-PTX-TRITC micelles for 4 h in the presence and absence of free HA in the medium.