Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Biotinylated carboxymethyl chitosan/$CaCO_3$ hybrid nanoparticles for targeted drug delivery to overcome tumor drug resistance

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Synthesis and characterization of FITC labeled polymers

1 mg of fluorescein isothiocyanate I (FITC) was added into 5 ml of $NaHCO_3$-Na_2CO_3 buffer solution (0.1 mol/l, pH=10) to obtain solution **A**. 50 mg CMC or BCMC was added into 5 ml $NaHCO_3$-Na_2CO_3 buffer solution (0.1 mol/l, pH=10) to obtain solution **B**. Solution **A** and solution **B** were mixed. After stirring 24 h under dark at room temperature, the product was placed in a dialysis bag (MWCO 8000-12000) and dialyzed against deionized water, and then freeze-dried to obtain FITC-CMC or FITC-BCMC. The FITC labeled polymers were detected by fluorescence spectrophotometer (Shimadzu，RF-5301PC) with an excitation wavelength at 488 nm.

Western blot analysis of P-gp expression

Cells were seeded in 24-well plates at a density of 5×10^5 cells per well and then incubated for 24 h. After washing with PBS for 3 times, the cells were lysed and re-suspended in SDS sample buffer containing 1% β-mercaptoethanol. Total protein extracts were subjected to SDS-PAGE. After electrophoresis, the proteins were transferred to PVDF membranes (Millipore). To block non-specific binding sites, the membranes were treated for 1 h with TBST buffer containing 5% milk. Subsequently the membranes were incubated with the primary antibody, rabbit polyclonal anti-P170 antibody (1/1000 dilution), overnight at 4 ºC. After washing, the membranes were incubated with the secondary antibody, HRP-labeled goat anti-rabbit IgG (1/10000 dilution), for 1 h. As a control, the expression of GAPDH in the cells was also

measured.

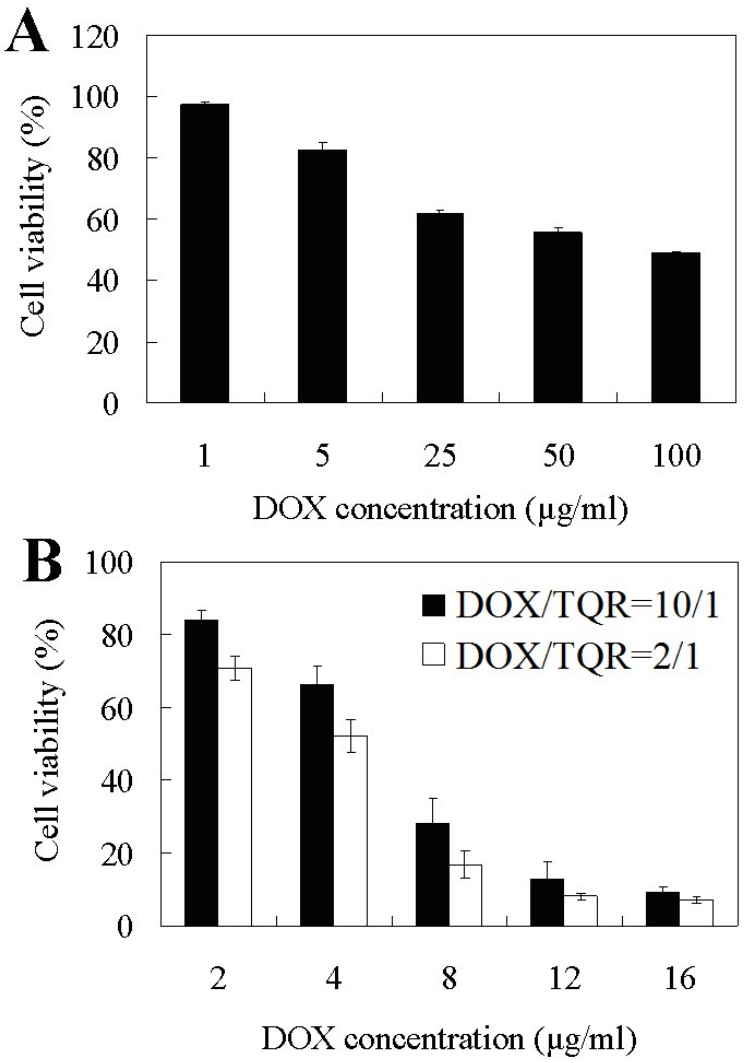

Figure S1. Cell viability of MCF-7/ADR cells treated by (A) free DOX, and (B) the mixture of free DOX and free TQR with a particular DOX/TQR weight ratio for 48 h.

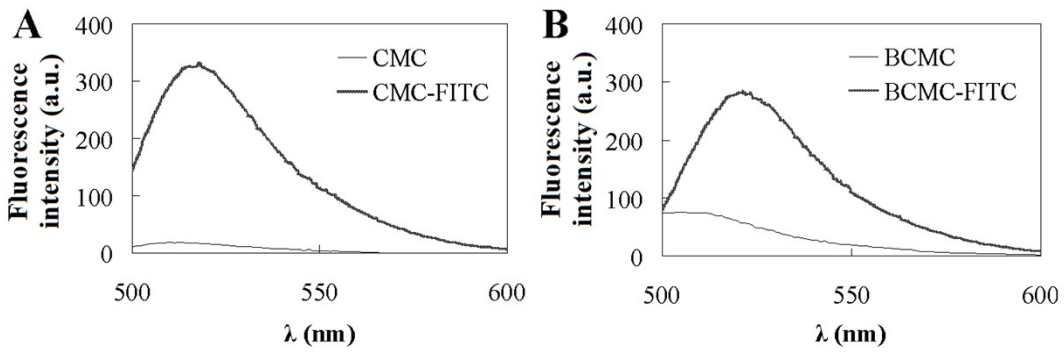

Figure S2. The fluorescence emission spectra of (A) CMC, and (B) BCMC before and after FITC labeling.

With an excitation wavelength at 488 nm, an emission peak appears among 500~600 nm in both CMC-FITC and BCMC-FITC, indicating the successful FITC labeling. These FITC labeled polymers were used to prepare hybrid nanoparticles to study the cellular uptake of both biotinylated nanoparticles and non-biotinylated nanoparticles.

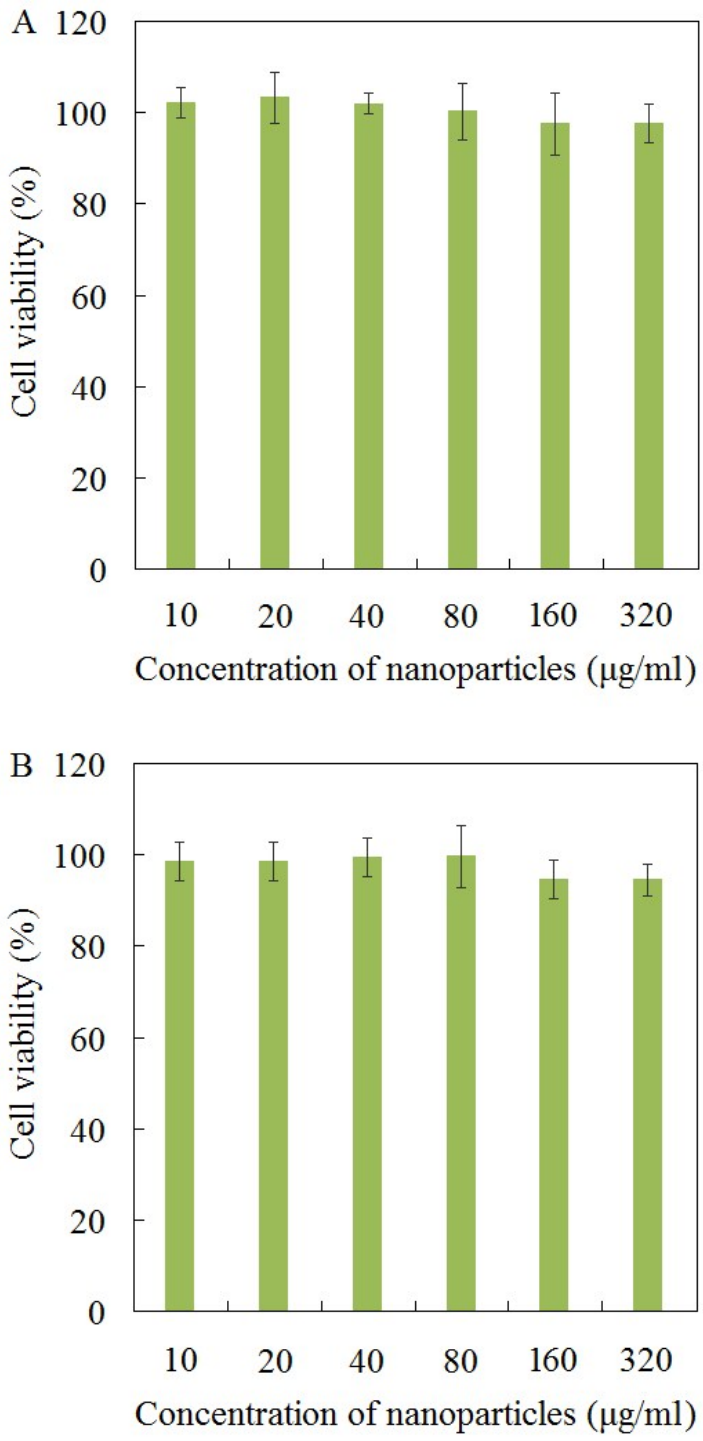

Figure S3. Cell viability of HeLa cells treated by (A) CMC/$CaCO_3$ blank nanoparticles and (B) BCMC/$CaCO_3$ blank nanoparticles for 48 h.

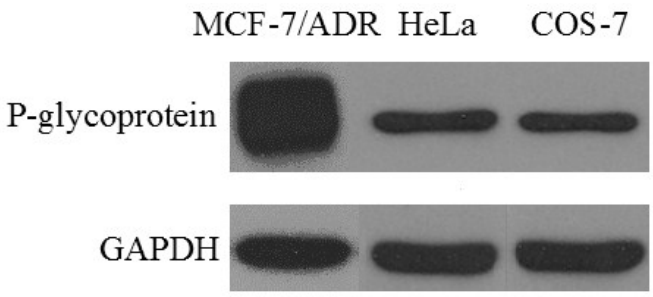

Figure S4. Expressions of P-glycoprotein in different cells determined by Western blot analysis. GAPDH was used as a control.

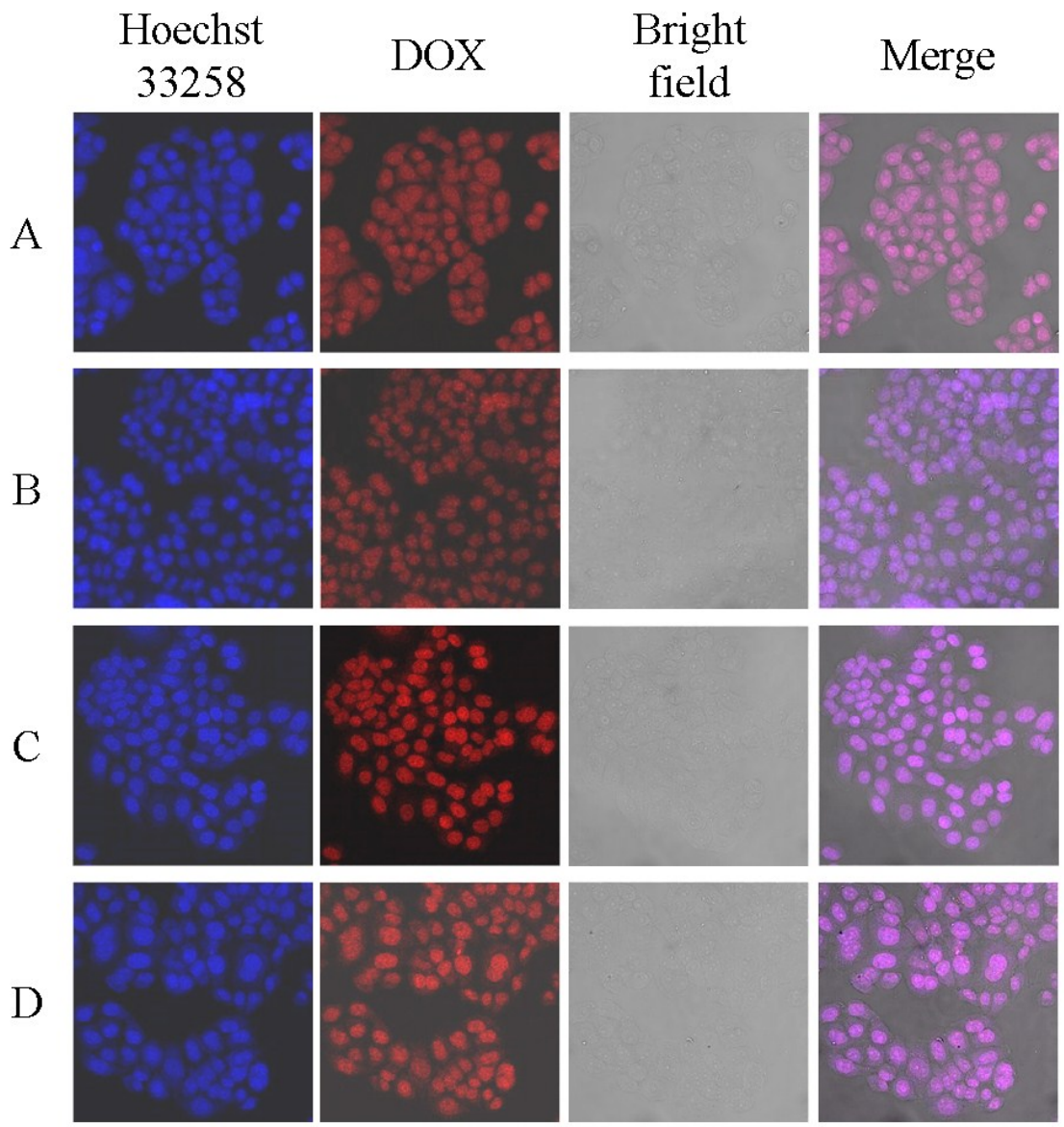

Figure S5. Confocal images of HeLa cells after being treated by (A) free DOX, (B) CMC/$CaCO_3$/DOX, (C) BCMC/$CaCO_3$/DOX, and (D) BCMC/$CaCO_3$/DOX/TQR for 4 h. The cell nuclei were stained with Hoechst 33258. The images were obtained under magnification of 400.

It should be noted that, being different from the result in drug resistant MCF-7/ADR cells with overexpressed P-gp, the DOX concentration in HeLa cells treated by free DOX was higher than that treated by CMC/$CaCO_3$/DOX nanoparticles. This was due

to the fact that the cell uptake of CMC/$CaCO_3$/DOX nanoparticles without targeting ligands was limited in a relatively short time period (4 h). Although nanoparticles entered the cells by “stealth endocytosis” to prevent DOX molecules from being recognized by membrane transporters, this factor could not counterbalance with the limited cell uptake of nanoparticles without targeting ligands since P-gp expression in HeLa was not very high and the “stealth endocytosis” did not play a dominant role.