Supplementary Information

Sequential Drug Release for Synergistic Cancer Treatment and Immunity Promotion

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Preparation of HPG-NCTD/CDDP Fluorescent Nanoparticles

To study the cellular internalization of HPG-NCTD/CDDP complexes, RB was incorporated into the nanoparticles as a fluorescence probe. HPG-NCTD/CDDP complexes solution and RB were added into PBS (7.4) according to the ratio of 100:7:25 and stirred for 24 h. The solution was transferred to dialysis bag (MWCO=1.0 kDa). Dialysis against to water was carried out for 7 days to remove the free RB. Some solutions were sucked out to lyophilize for concentration determination. The RB concentration in complex solution was roughly evaluated by colorimetric method compared to pure RB. The color of HPG-NCTD/CDDP dialyzed complexes solution (about 3-4 mg/mL) was close to 0.9 μg/mL RB. This result indicated that the content of RB in HPG-NCTD/CDDP complexes was very low and the presence of RB almost didn't affect the property of HPG-NCTD/CDDP complexes. Therefore, these nanoparticles could be used to evaluate the cellular uptake. The morphology of HPG-NCTD/CDDP fluorescent nanoparticles is given in Figure S1.

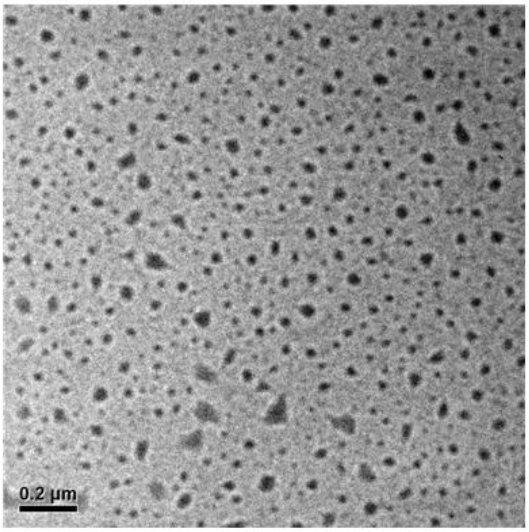

Figure S1. TEM image of HPG-NCTD/CDDP fluorescent nanoparticles.

Anti-Tumor Effects of HPG-NCTD Conjugate.

The *in vitro* anti-tumor effects of HPG-NCTD conjugate compared to NCTD were evaluated by MTT assay against BEL-7402 cells. The BEL-7402 cells were treated with HPG-NCTD and NCTD at different dose from 5 to 600 μmol/L, and the anticancer proliferation of NCTD and HPG-NCTD is shown in Figure S2. The required dose of NCTD for 50% cellular growth inhibition (IC_{50}) is about 55 μmol/L while the IC_{50} of HPG-NCTD is about 85 μmol/L. These results demonstrate that NCTD releases slowly from HPG and can reduce cytotoxicity to normal cells during its blood circulation.

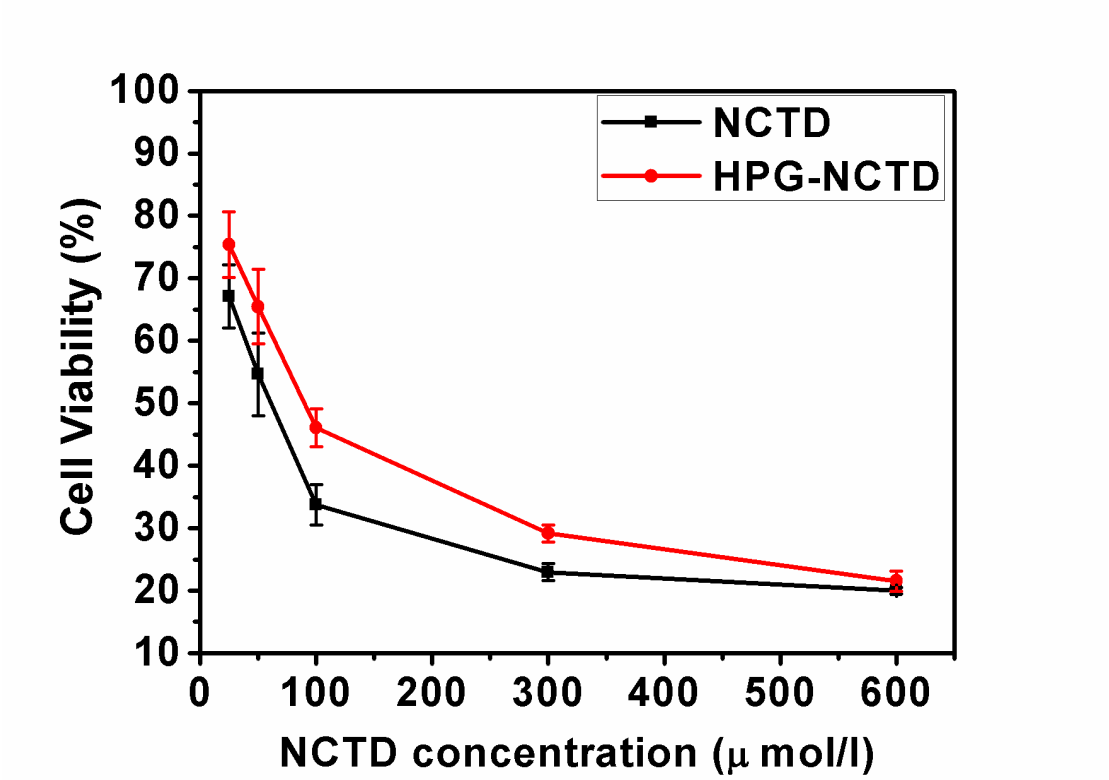

Figure S2. Cell viability of BEL-7402 cells against NCTD and HPG-NCTD after incubation for 48 h with different concentration (mean±SD, n=3).

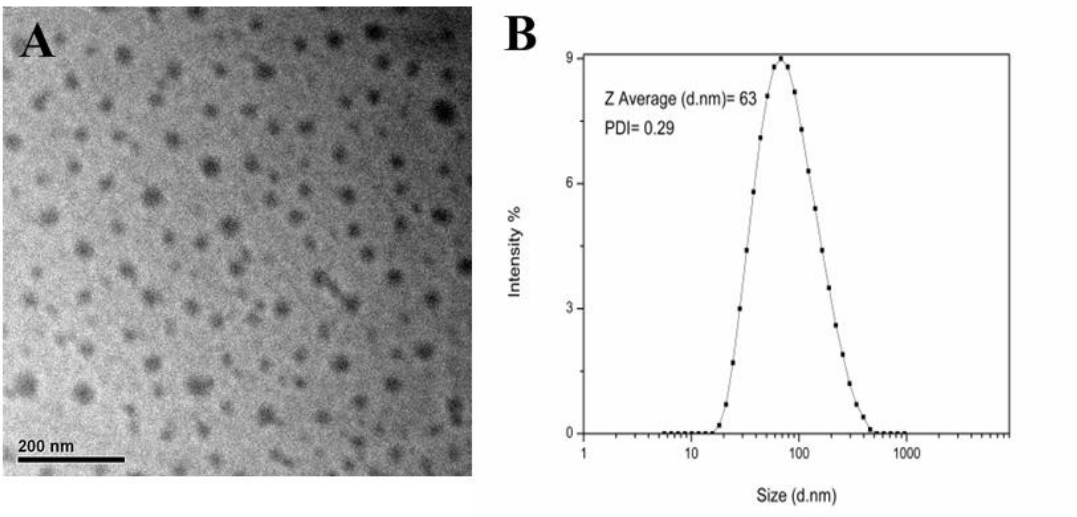

Figure S3. Characterization of HPG-NCTD morphology: (A) TEM image of self-assembled micelles; (B) The size distribution of HPG-NCTD determined by DLS.