Supporting Information

Natural Polysaccharide-incorporated Hydroxyapatite as Size Changeable, Nuclear Targeting Nanocarriers for Efficient Cancer Therapy

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1. Experimental methods

1.1 Drug loading and release study.

Drug loading content (DLC) and drug loading efficiency (DLE) were calculated according to the following formula:

DLC (wt%) =(weight of loaded DOX/ weight of HA-ALG/DOX NPs) ×100%

DLE (%)= (weight of loaded DOX/ weight of feeding DOX) ×100%

For the drug release test, dialysis bags (MWCO 10 kD) containing 1 ml of HA-ALD/DOX solution were soaked in tubes which contained 40 ml of PBS (pH 7.4 and pH 5.6) with moderate shake at 37 °C over 24 h. At the predetermined time points (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 12, 24), 1 ml dialysis fluid was collected and replaced with equal amount of fresh buffer. The concentration of DOX was measured by a UV-vis spectrophotometry at an excitation wavelength of 480 nm.

1.2 Live/dead fluorescent staining of the cells.

For fluorescent imaging, L929 cells were seeded in 24-well plate at the density of 2×10^4 cells per well and treated with the HA-ALG NPs (50, 100, 200 and 400 μg/ml). After 1, 3 and 5 days, the culture medium was removed and the attached cells were stained with fluorescein diacetate (FDA, Sigma, USA) for live cells (green) and propidiumiodide (PI, Sigma, USA) for

dead cells (red). After staining in dark for 20 min, the cells were observed by a confocal laser scanning microscopy (CLSM, Leica-TCS-SP5, Germany).

1.3 cell apoptosis

The cell apoptosis was evaluated using an Annexin V-FITC/PI apoptosis detection kit (Sigma-Aldrich). HeLa cells were seeded in a 6-well plate at the density of 1×10^5 per well. After 12 h of seeding, the HeLa cells were treated with HA-ALG NPs for 1 and 3 days. Then, the HeLa cells were gently trypsinized and washed with PBS. After being centrifuged, the HeLa cells were suspended in 500 μl of annexin binding buffer. Then, the cell suspensions were treated with 5 μl of annexin V FITC and 10 μl of PI in dark. Finally, fluorescence intensities of cells were analyzed by flow cytometer (CellLab Quanta SC, Beckman).

2. Results

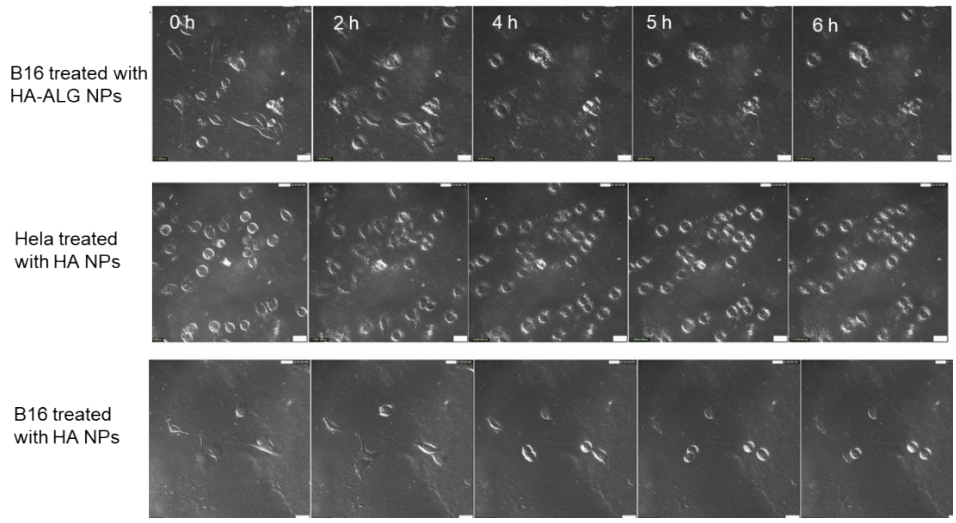

Figure S1. Time-lapse microscopy images of HeLa cells and B16 cells treated with HA NPs.

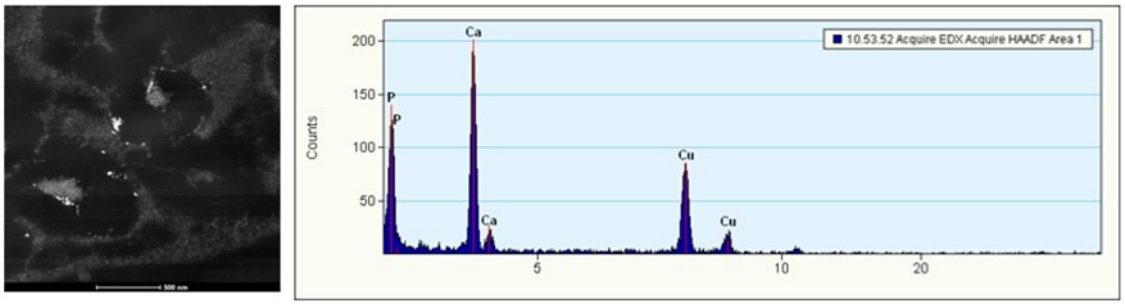

Figure S2. EDS results of HA-ALG NPs uptake by Hela cells.

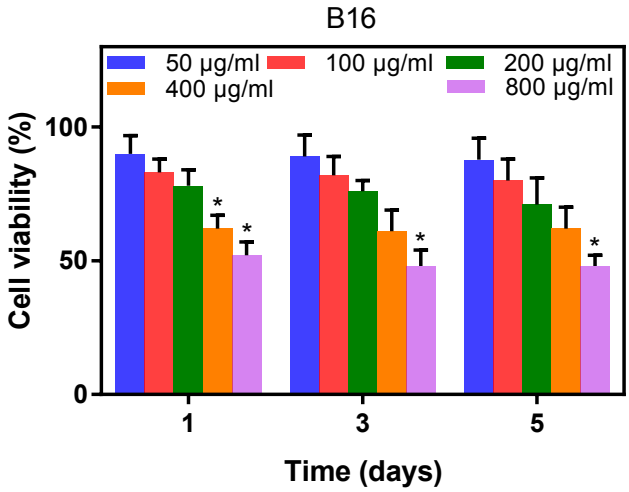

Figure S3. CCK-8 results of B16 cells treated with different concentrations of HA-ALG NPs.

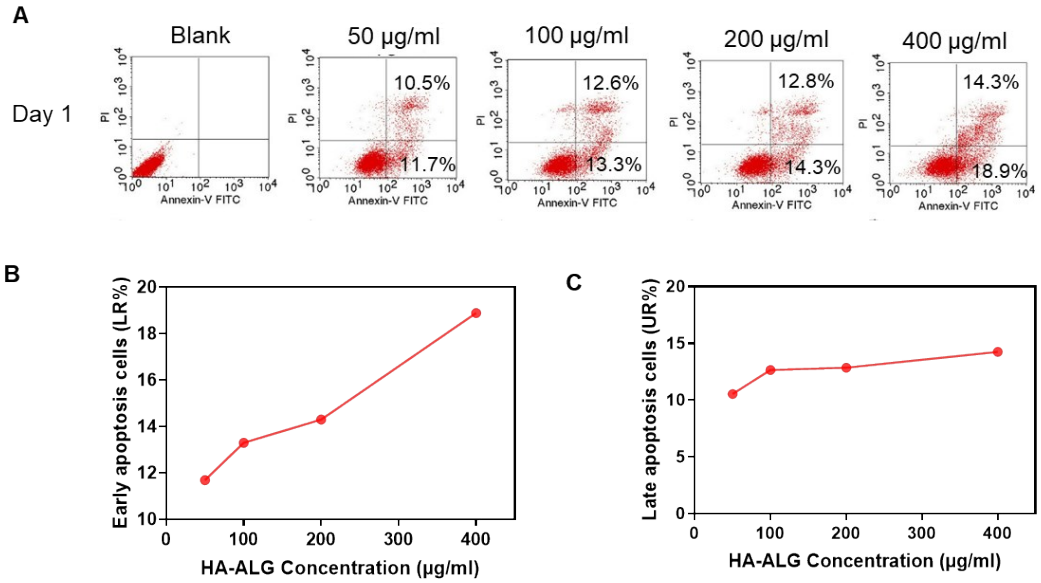

Figure S4. Flow cytometric analysis results of Hela cells treated with different concentrations of HA-ALG NPs for 1 and 3 days. (A) The flow chart of Hela cells treated with different concentrations of HA-ALG NPs. (B) The early apoptosis cells treated with different concentration of HA-ALG NPs. (C) The late apoptosis cells treated with different concentration of HA-ALG NPs.

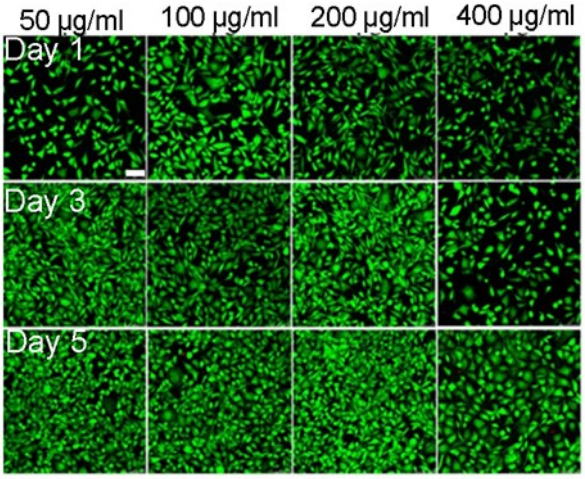

Figure S5. CLSM images of L929 cells treated with different concentrations of HA-ALG NPs stained with FDA (green) and PI (red). Scale bar is 75 μm and apply for all images.

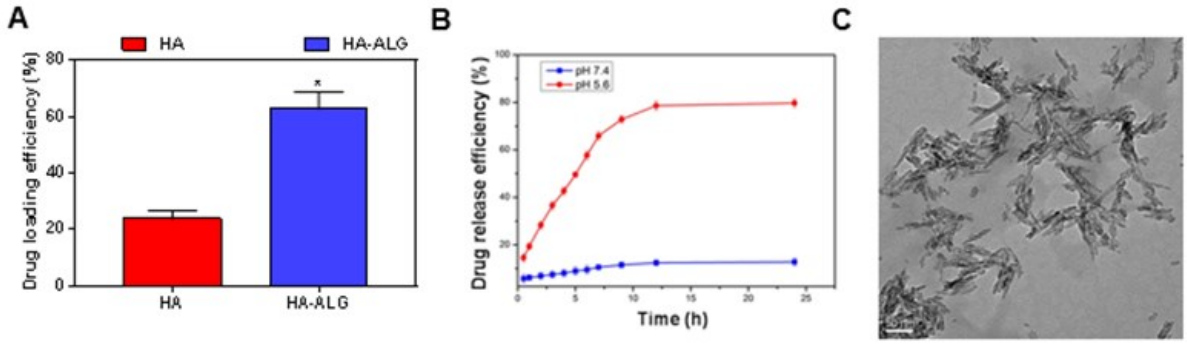

Figure S6. DOX loading and release profile of HA-ALG NPs. (A). Drug loading efficiency of HA and HA-ALG NPs. (B). Drug release efficiency of HA-ALG/DOX NPs at different pH. (C).TEM image of HA-ALG/DOX NPs.

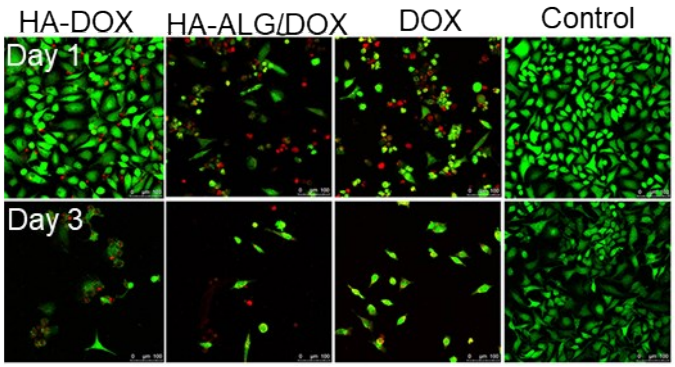

Figure S7. CLSM images of L929 cells treated with HA-DOX, HA-ALG/DOX NPs and DOX stained with FDA (green) and PI (red). Scale bar is 75 μm and apply for all images.

Movie S1. Tracking of Live HeLa Cells treated with HA-ALG NPs, Related to Figure 2. (scale bar, 25 μm, Timestamp in ms).

Movie S2. Tracking of Live B16 Cells treated with HA-ALG NPs, Related to Figure S1. (scale bar, 25 μm, Timestamp in ms).

Movie S3. Tracking of Live Hela Cells treated with HA NPs, Related to Figure S1. (scale bar, 25 μm, Timestamp in ms).

Movie S4. Tracking of Live B16 Cells treated with HA NPs, Related to Figure S1. (scale bar, 25 μm, Timestamp in ms).