Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Tumor-targeted and nitric oxide-generated nanogels of keratin and hyaluronan for enhanced cancer therapy

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METHODS

Cell Culture.

4T1 cell line (Mouse breast neoplasm cell), B16 cell line (Mouse melanoma cell), L929 cell line (Murine fibroblasts cell line) and NIH 3T3 cells (Mouse embryo fibroblasts cell line) were purchased from Chinese Academy of Science Cell Bank for Type Culture Collection (Shanghai, China). 4T1 cells and B16 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin, L929 cells and NIH 3T3 cells were cultured in DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. All cells were incubated at 37°C in a humidified 5% CO_2 incubator.

Animal tumor model

Female BALB/c mice (14 ± 2 g, 4-5 weeks old) purchased from Dashuo Experimental Animal Company (Sichuan, China) were selected to establish the xenograft 4T1 tumor model. The mice were given daily fresh diet with free access to water, kept at a temperature of 21 °C and relative humidity of 45%–65%, and acclimatized for at least 7 d prior to the experiments. All animal procedures were carried out according to the Institutional Animal Care and Use Committee of Sichuan University. 4T1 cells (5×10^7) in 75 μL PBS were injected subcutaneously into the right back of each nude mice. After approximately 1 week, the tumors were well established.

Histological examination and immunohistochemical analysis

The removed tumors, hearts, livers, spleens, and kidneys were fixed with 4% formalin and paraffin embedded to prepare tissue sections with a thickness of 5 μm. After deparaffinization, the tissue sections were stained with hematoxylin and eosin reagents (H&E)for histological examination; tumor sections were stained with Ki-67 and TUNEL. The images were captured using the Motic Images Advanced software (Motic China Group Co. Ltd.) and the positively-stained integrated optical density of Ki-67 was scaled *via* Image-Pro Plus 6.0 software (Media Cybernetics, Bethesda, MD). The ki-67 density in each image was calculated as the Ki-67-positive area over the total area.

FIGURE

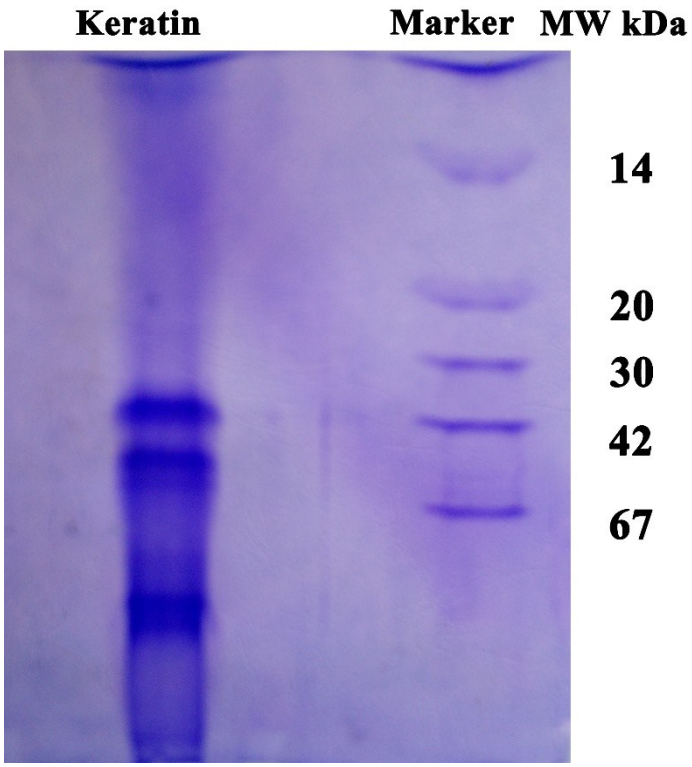

Figure S1. SDS-PAGE of human hair keratin.

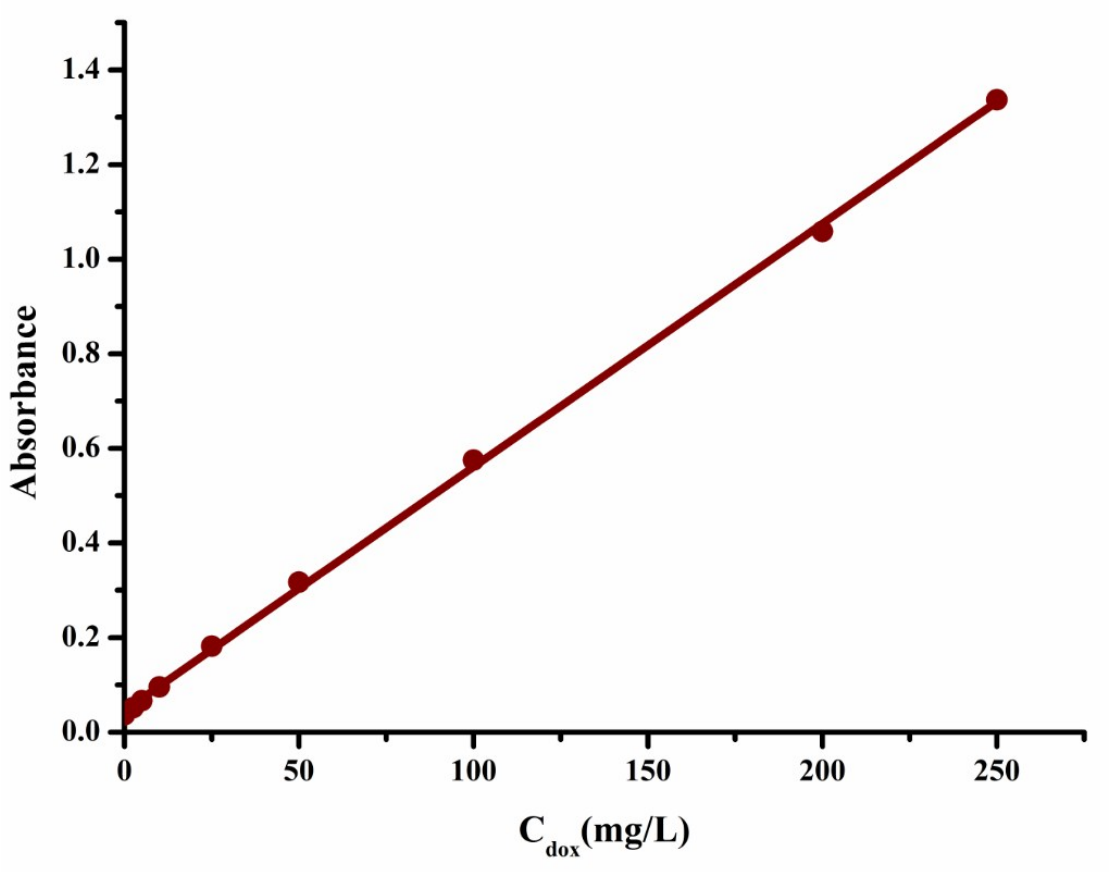

Figure S2. Calibration curve of DOX.HCl in PBS determined by UV-vis spectroscopy; Abs=0.0049 × (C) + 0.0135, R=0.9994; Abs is the absorbance and C is the concentration (mg/L).

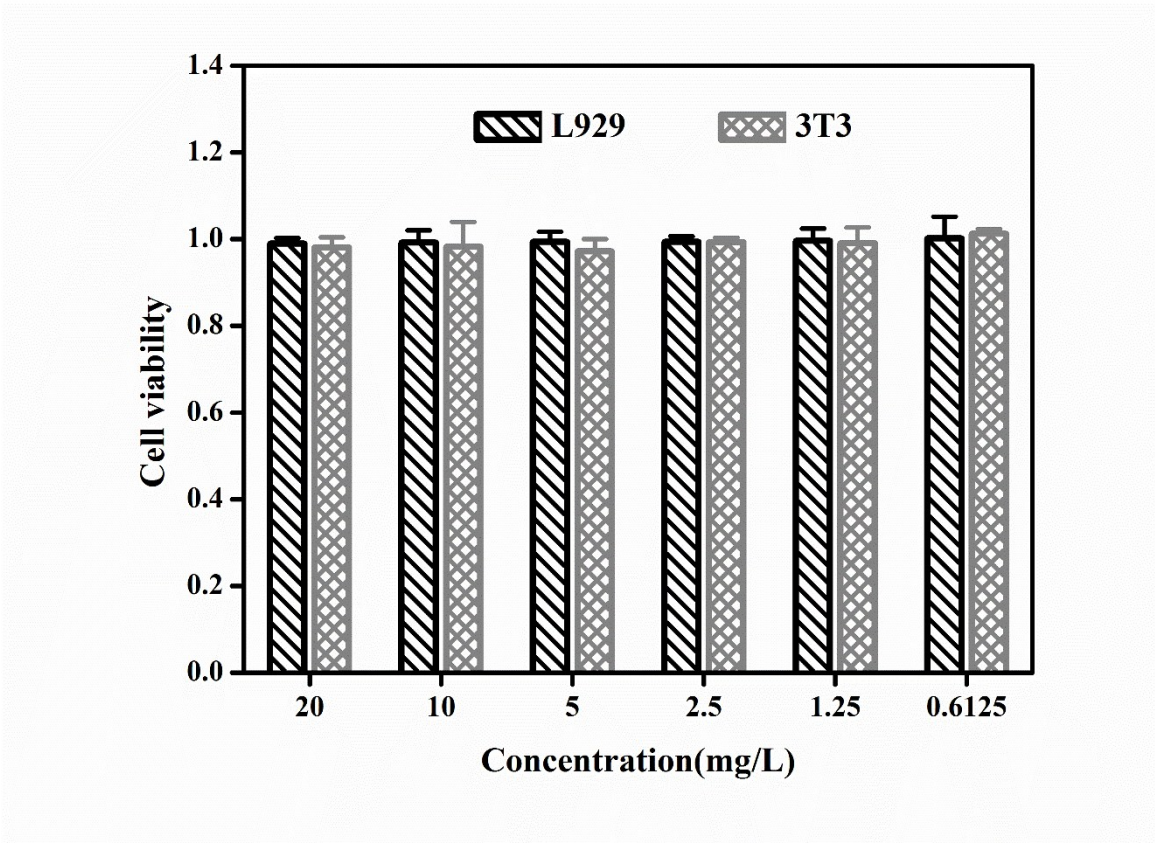

Figure S3. The cytotoxicity of KHA-NGs against L929 cells and NIH-3T3 cells.

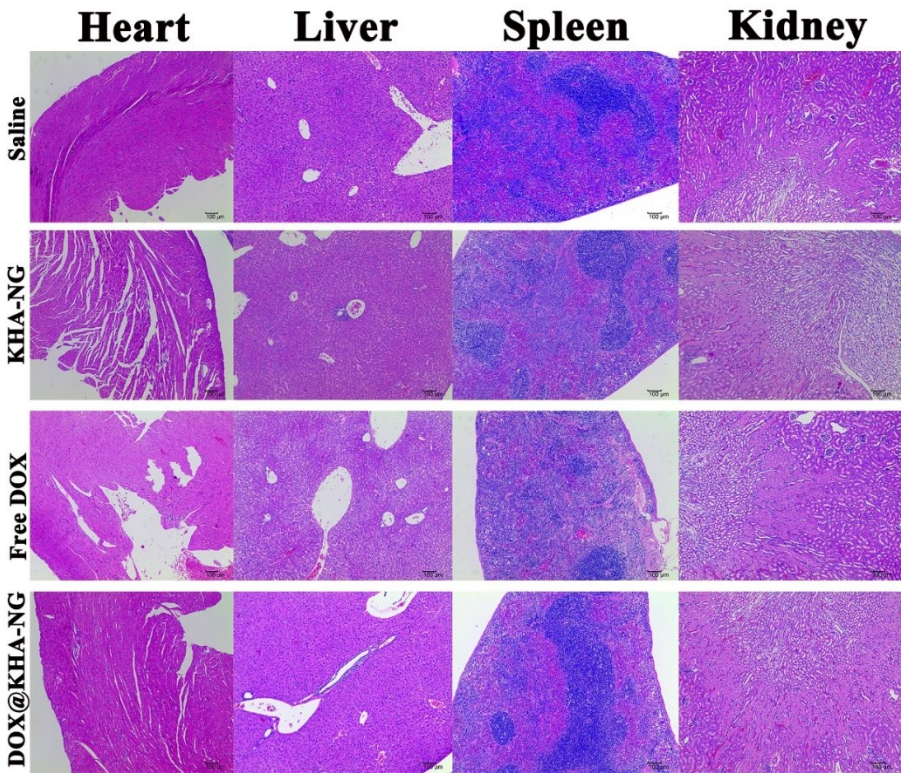

Figure S4. H&E stained images of major organs collected from mice after *i.v.* injection of Saline, KHA-NGs, DOX, DOX@KHA-NGs at day 19 treatment.

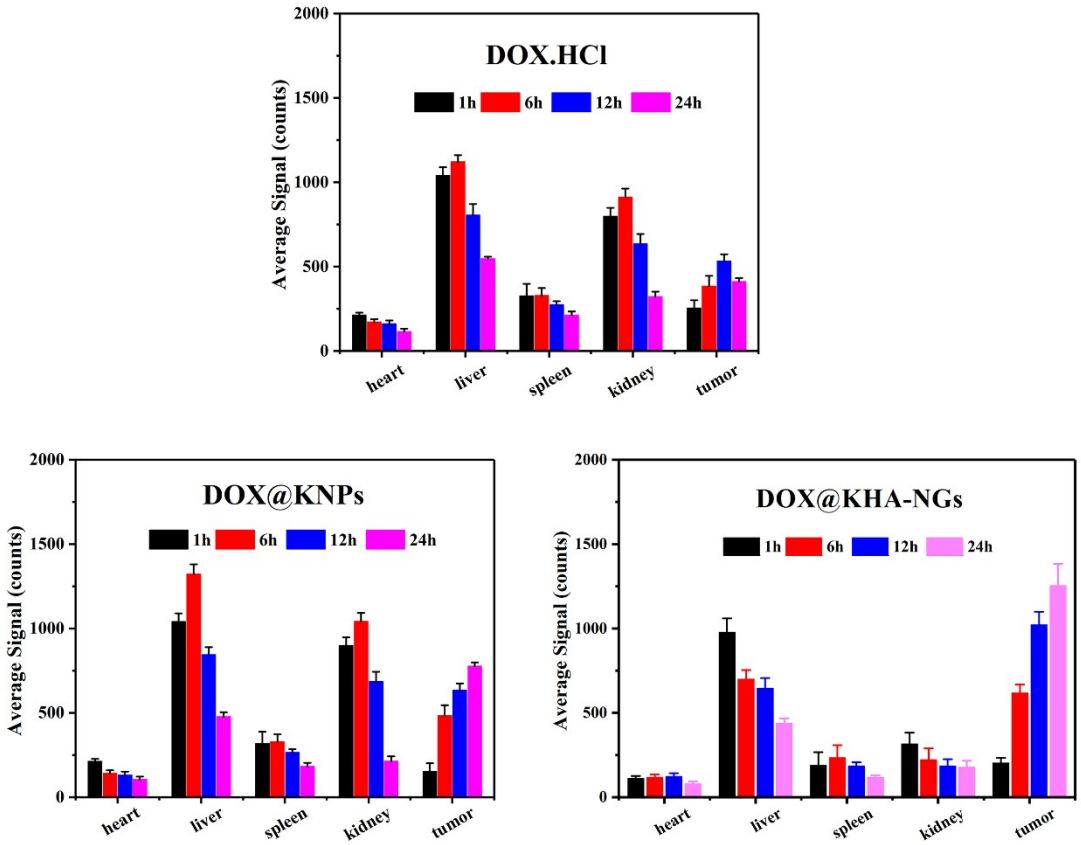

Figure S5. Semi-quantitative of DOX, DOX@KNPs and DOX@KHA-NGs distribution in major organs and tumors post intravenous injection for different time points.