

## Electronic Supplementary Material

### **Sequential delivery of bismuth nanoparticles and doxorubicin by injectable macroporous hydrogel for combined anticancer kilovoltage X-ray radio- and chemo-therapy**

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## Materials and methods

**Materials.** Anhydrous  $\alpha$ -D-glucose (>99+%), bismuth nitrate (98%), borane morpholine (97%), and 1,2-propanediol (99%) were purchased from Acros Inc. Mouse breast cancer cell line (4T1) was purchased from American Type Cell Culture (ATCC). Fetal bovine serum (FBS) was purchased from Thermo Fisher. Hyaluronic acid (HA, molecular weight = 400 kDa), dextran sulfate sodium salt (DS, molecular weight = 20 kDa, low sulfate content 8%-13%), methacrylic anhydride (MA), 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), ethylenediaminetetraacetic acid (EDTA), dimethyl sulfoxide (DMSO), nitric acid (HNO<sub>3</sub>), hydroxide sodium (NaOH), tetramethylethylenediamine (TEMED), ammonium persulfate (APS) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were obtained from Sigma. All chemicals were used without purification. Deionized (DI) water was obtained through an 18-M $\Omega$  (SHRO-plus DI) system.

### Synthesis of methacrylated hyaluronic acid (MAHA) and methacrylated dextran sulfate (MADS).

Methacrylated hyaluronic acid (MAHA) and methacrylated dextran sulfate (MADS) were synthesized following the previous reported reference.<sup>1</sup> In brief, 0.8 g HA (containing 2.08 mmol carboxylic acid groups) was dissolved in 40 mL of DI water. A five molar excess (relative to the hydroxyl groups in HA) of methacrylic anhydride (10.4 mmol, 1.6 g) was dropwise added to the solution under 0 °C. The reaction pH was adjusted to 8-9 by the addition of 4 M NaOH and the reaction was continued overnight at room temperature. After 24 h, the product was precipitated in cold ethanol, followed by washing it with ethanol three times and drying it under vacuum. The product was dissolved in DI water, and the solution was dialyzed using dialysis tube (molecular weight cut-off 10000) for 72 h. After dialysis, MAHA was lyophilized and stored at 4 °C. MAHA was dissolved in deuterium oxide (D<sub>2</sub>O) and characterized by <sup>1</sup>H NMR spectrum on 400 MHz Varian. <sup>1</sup>H NMR ( $\delta$ , D<sub>2</sub>O): 6.24 and 5.79 (2H, CH<sub>2</sub>=CCH<sub>3</sub>), 1.99 (3H, CH<sub>2</sub>=CCH<sub>3</sub>), 2.07 (3H, CH<sub>3</sub>CO). The degree of substitution of MAHA was assessed by <sup>1</sup>H NMR analysis according to eq 1, where  $I_{6.24}$  is the integral of proton resonance signal at  $\delta = 6.24$  ppm,  $I_{2.07}$  is the integral of proton resonance signals at  $\delta = 2.07$  ppm.

$$\text{Degree of substitution} = 3 \times \frac{I_{6.24}}{I_{2.07}} \times 100\%$$

(1)

1.0 g DS (containing 2.76 mmol hydroxyl groups) was dissolved in 10 mL of DMSO. A five molar excess (relative to the hydroxyl groups in DS) of methacrylic anhydride (13.8 mmol, 2.13 g) and trimethylamine (0.138 mmol, 13.94 mg) were dropwise added to the solution under 0 °C. After 24 h under 30 °C, the product was precipitated in cold ethanol, followed by washing with ethanol three times. After drying in vacuum, the product was dissolved in DI water, and the solution was dialyzed using dialysis tube (molecular weight cut-off 10 000) for 72 h. After dialysis, MADS was lyophilized and stored at 4 °C. MADS was dissolved in deuterium oxide (D<sub>2</sub>O) and characterized by <sup>1</sup>H NMR spectrum on 400 MHz Varian. <sup>1</sup>H NMR ( $\delta$ , D<sub>2</sub>O): 6.24 and 5.82 (2H, CH<sub>2</sub>=CCH<sub>3</sub>), 1.99 (3H, CH<sub>2</sub>=CCH<sub>3</sub>), 4.98 and 5.24 (H, H-1 in the repeating unit of dextran sulfate). The degree of substitution of MADS was assessed by <sup>1</sup>H NMR analysis according to eq 2, where  $I_{6.24}$  is the integral of proton resonance signal at  $\delta = 6.24$  ppm,  $I_{4.98}$  is the integral of proton resonance signals at  $\delta = 4.98$  ppm,  $I_{5.24}$  is the integral of proton resonance signals at  $\delta = 5.24$  ppm.

$$\text{Degree of substitution} = I_{6.24}/(I_{4.98} + I_{5.24}) \times 100\% \quad (2)$$

**Synthesis of Bi NPs.**  $\alpha$ -D-glucose (27 g) and 1,2-propanediol (42 mL) were added in a 250 mL round-bottom flask with vigorous stirring, followed by the addition of bismuth nitrate pentahydrate (243 mg) in 2 mL 1,2-propanediol solution. The temperature of the mixture was increased to 90 °C in nitrogen atmosphere for 30 min. Borane morpholine (154 mg) in 6.1 mL 1,2-propanediol solution was added to the mixture rapidly. After 2 min, the reaction was quenched by pouring in 100 mL of icy water. The obtained Bi NPs were collected by centrifugation and washed with DI water for 3 times, and then were filtered using 0.2  $\mu$ m syringe filter and stored at 4 °C. The yield of Bi NPs was determined to be 80% by using an inductively coupled plasma-mass spectroscopy (ICP-MS, Thermal Elemental X7, Thermal Fisher Scientific).

**Characterization.** Size and zeta potential of Bi NPs were detected by dynamic light scattering (DLS, Zeta sizer Nano ZS90). A JEOL JEM2100 transmission electron microscope (TEM) was operated at 200 kV to collect Bi NPs images. The crystalline structures of Bi NPs were analyzed using an X-ray diffractometer (XRD; Rigaku RINT 2000) operating with Cu K $\alpha$  radiation 40 kV, 100 mA. Scans were performed over the angular range 20-70° 2 $\theta$  at a scan rate of 0.25°/min at room temperature. The macroporous structure of hydrogel was investigated using scanning electron microscope (SEM, FEI Quanta 200). The average size of pores in macroporous hydrogels was calculated by averaging the diameters of the pores observed by SEM. Energy Dispersive X-ray spectroscopy (EDX) analysis was applied in connection with SEM for the elemental analysis. The elemental mapping was also recorded with the same spectrophotometer. The macroporous structure and pore size of HA-DS/DOX/Bi hydrogels were investigated by Confocal Laser Scanning Microscope (Zeiss LSM 510). Excitation wavelength was selected at 480 nm and fluorescence emission was collected at 575-585 nm.

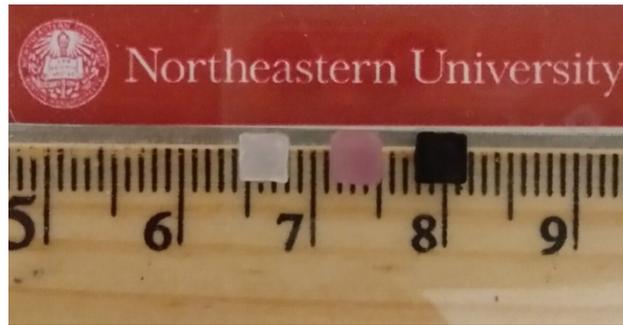
Young's modulus was determined using an Instron testing system (Instron 3342). Disc-shaped hydrogels (8 mm diameter, 5 mm height) were deformed between two parallel plates with a strain rate of 20% per minute. Engineering stresses and strains were recorded. The pore connectivity was evaluated using a water wicking technique, in which the interconnected porosity was calculated as the interconnected void volume over the total volume. To determine total volume, macroporous hydrogels and non-macroporous hydrogels were soaked in water for 1 h and weighed. A Kimwipe was then used to wick away water within interconnected pores, and the hydrogels were weighed once again. The interconnected void volume was calculated as the volume of water wicked from the gels. The swelling ratio was determined using a conventional gravimetric procedure. To investigate the swelling ratio of each sample, macroporous hydrogels and non-macroporous hydrogels were prepared and immersed in PBS. The equilibrium mass swelling ratio ( $Q_M$ ) was calculated by the following equation 3:

$$Q_M = \frac{M_s}{M_d}$$

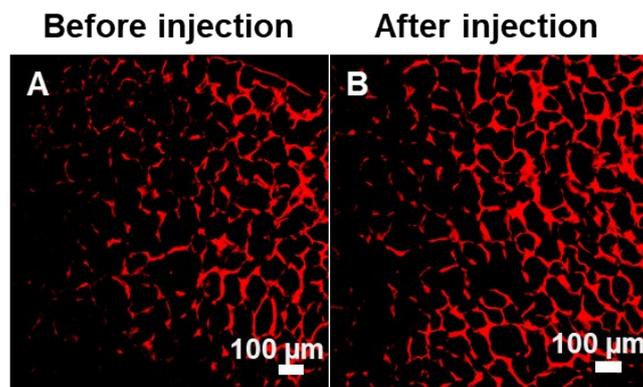
(3)

where  $M_s$  and  $M_d$  were fully swollen hydrogel and dried hydrogel weights, respectively. The swelling data were corrected by subtracting the soluble fraction of salt in PBS from the hydrogels.





**Figure S1.** The photographs of HA-DS macroporous hydrogel (white color), HA-DS/DOX macroporous hydrogel (pink color), and HA-DS/DOX/Bi macroporous hydrogel (black color). The Size of hydrogel: length 4 mm; width 4 mm; height 4 mm.



**Figure S2.** Confocal microscopy images of HA-DS/DOX macroporous hydrogel before (A) and after injection (B) through an 18-gauge needle.

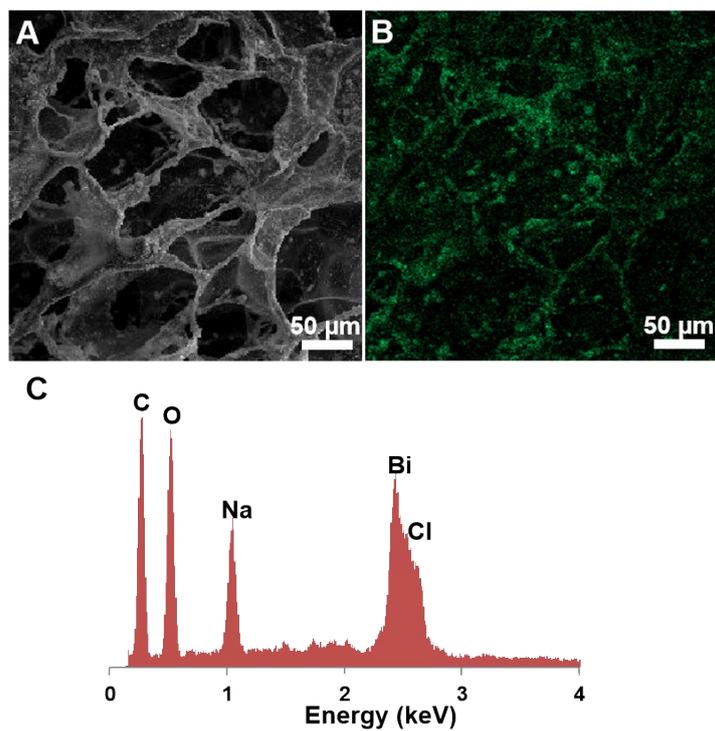


Figure S3. (A) SEM surface image of HA-DS/DOX/Bi macroporous hydrogel. (B) EDX mapping of Bi element of the corresponding HA-DS/DOX/Bi macroporous hydrogel (green color : Bi element). (C) EDX analysis of the corresponding HA-DS/DOX/Bi macroporous hydrogel.

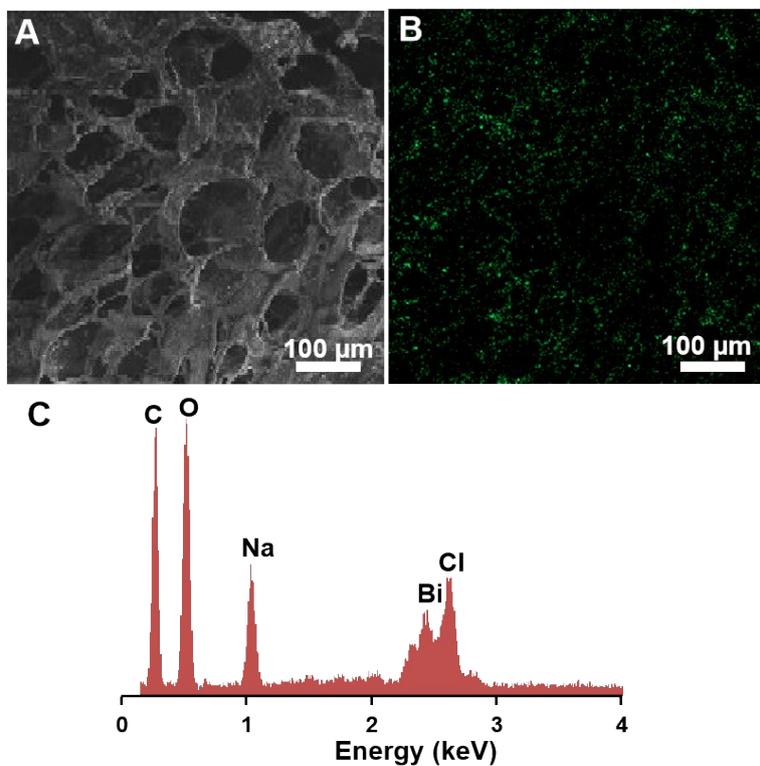
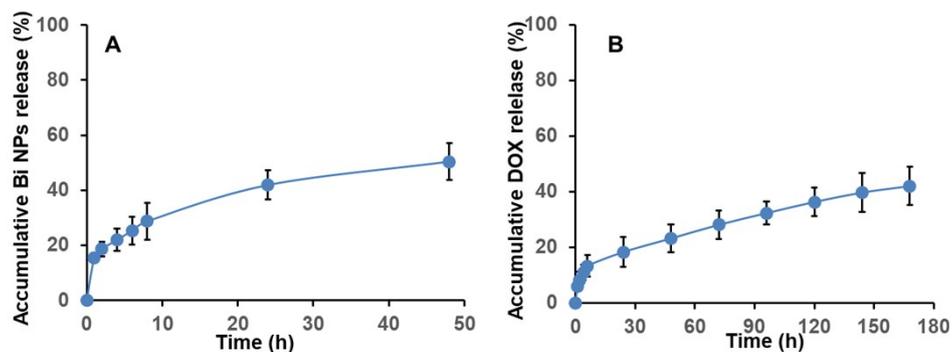
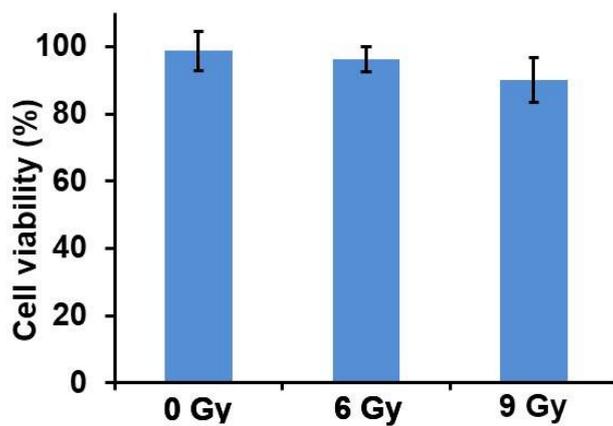


Figure S4. (A) SEM cross-sectional image of HA-DS/DOX/Bi macroporous hydrogel. (B) EDX mapping of Bi

element of the corresponding HA-DS/DOX/Bi macroporous hydrogel (green color : Bi element). (C) EDX analysis of the corresponding HA-DS/DOX/Bi macroporous hydrogel.



**Figure S5.** Accumulative Bi NPs (A) and DOX (B) release profiles of HA-DS/DOX/Bi non-macroporous hydrogel. Values represent mean and SD (n = 3).



**Figure S6.** Viabilities of 4T1 cells after treated with X-ray radiation at difference dose from 0 Gy to 9 Gy. Values represent mean and SD (n = 3)

## References

1. S. K. Seidlits, Z. Z. Khaing, R. R. Petersen, J. D. Nickels, J. E. Vanscoy, J. B. Shear and C. E. Schmidt, *Biomaterials*, 2010, **31**, 3930.

