Supplementary Information

Tuning the Properties of Polymer Capsules for Cellular Interactions

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Materials. HA (sodium salt) with molecular weights (M_w) of 47 kDa and 289 kDa were purchased from Shandong Freda Biopharmaceutical Co., Ltd. (China) and Lifecore Co (USA), respectively. PGA (sodium salt, M_w 15 kDa) was obtained from Alamanda Polymers (USA). PMA (30% solution, M_w 15 kDa) was purchased from Polysciences (USA). 8-PEG-A (tripentaerythritol core, M_w 20 kDa) was obtained from JenKem Technology (USA). PHPMA-A was synthesized according to our previous report.[1] AEMA, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM), pyridine (anhydrous, 99.8%), α-bromoisobutyryl bromide (98%), DEGDAA (75% aqueous solution), *N,N,N′,N′,N″*-pentamethyldiethylenetriamine (99%), copper(II) bromide (99%), sodium ascorbate (≥ 98%), hydrofluoric acid (HF), ammonium fluoride, sodium phosphate dibasic, sodium phosphate

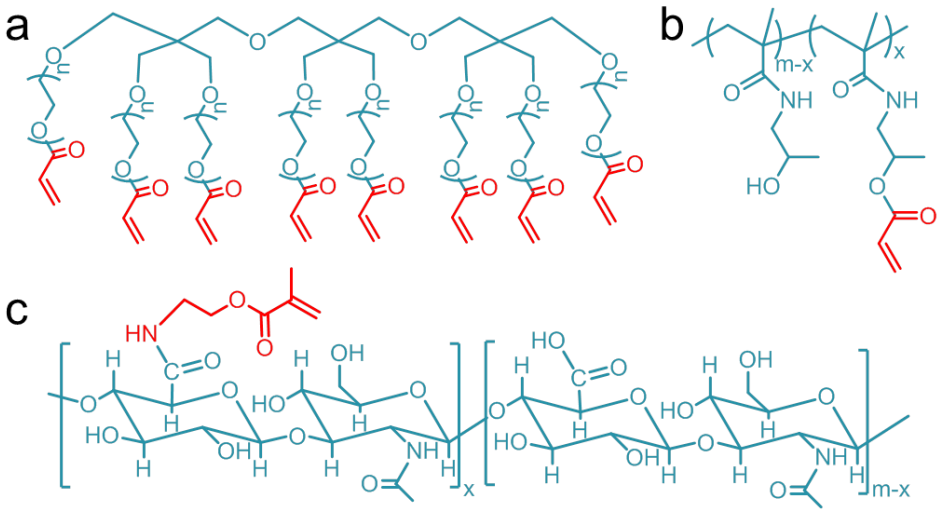
monobasic monohydrate, and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich and used as received. AF633 hydrazide reactive dyes, AF633 succinimidyl ester (AF633-NHS), AF488-WGA, and Hoechst 33342 were obtained from Invitrogen. Fluorescein (FITC)-labeled anti-CD44 antibody was purchased from Abcam (UK). SiO_2-NH_2 (50 mg mL^{-1}, average diameter 2.1 ± 0.1 μm, NH_2: >30 μmol g^{-1}) particles were obtained from Microparticles GmbH (Berlin, Germany). The water used in all experiments was obtained from an inline Millipore RiOs/Origin system (Milli-Q) and had a resistivity greater than 18.2 MΩ cm.

Characterization Methods. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy was conducted on a Varian Unity 400 MHz spectrometer, using deuterated water (D_2O) as the solvent and a sample concentration of ca. 4 mg mL^{-1}. DIC and fluorescence microscopy images of polymer capsules were obtained using an inverted Olympus IX71 microscope equipped with a DIC slider (U-DICT, Olympus), a UF1032 fluorescence filter cube, and a 100× oil immersion objective (Olympus UPFL20/0.5NA, W.D1.6). TEM images were taken using a FEI Tecnai TF20 instrument with an operation voltage of 200 kV. AFM experiments were performed with a JPK NanoWizard II BioAFM. Typical scans were performed in intermittent contact mode with MikroMasch silicon cantilevers (NSC/CSC). ζ-potentials of various polymer capsules were measured in PB (5 mM, pH 7.4) using a Malvern Zetasizer Nano-ZS.

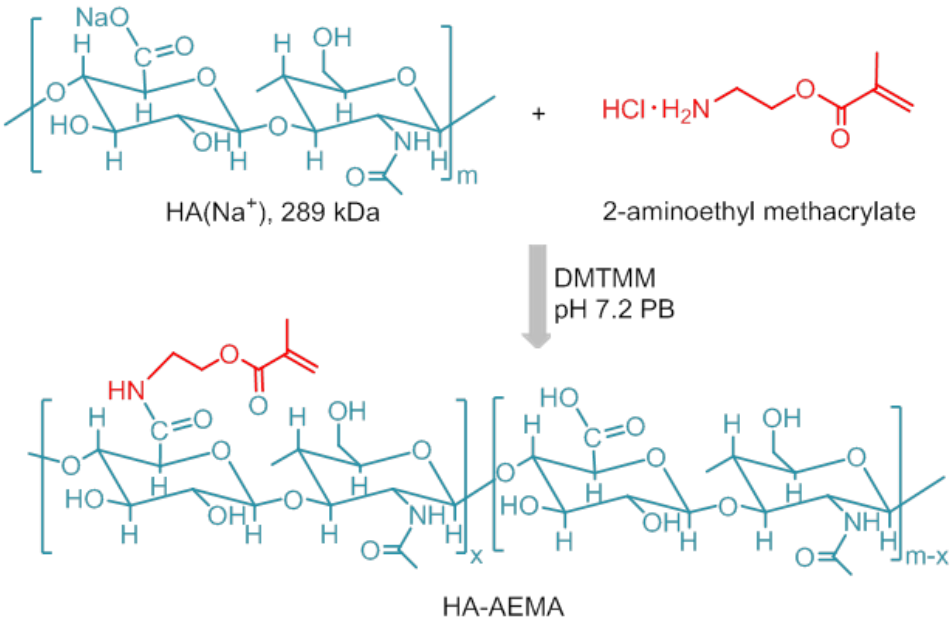
Cell Cultures. HeLa, Raw 264.7, THP-1, MDA-MB-231, and BT474 cells were purchased from ATCC (USA). HeLa, Raw 264.7 and MDA-MB-231 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) with the addition of 10% fetal bovine serum (FBS). THP-1 and BT474 cells were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) medium supplemented with 10% FBS. All cells were cultured at 37 ºC with 5% CO_2 and subcultured prior to confluence using trypsin.

CD44 Expression Study by Flow Cytometry. Cultured MDA-MB-231 and BT474 cells were harvested by 0.25% trypsin solution and washed with DPBS. A certain number (1×10^6) of cells were suspended in DPBS and incubated with FITC-labeled anti-CD44 antibody (at a final concentration of 10 μg mL^{-1}) on ice for 30 min. After incubation, cells were washed three time with DPBS. The percentage of cells that exhibited stronger fluorescence intensity than control cells (untreated cells) were analyzed with an Apogee A50-Micro flow cytometer (Apogee Flow Systems, UK).

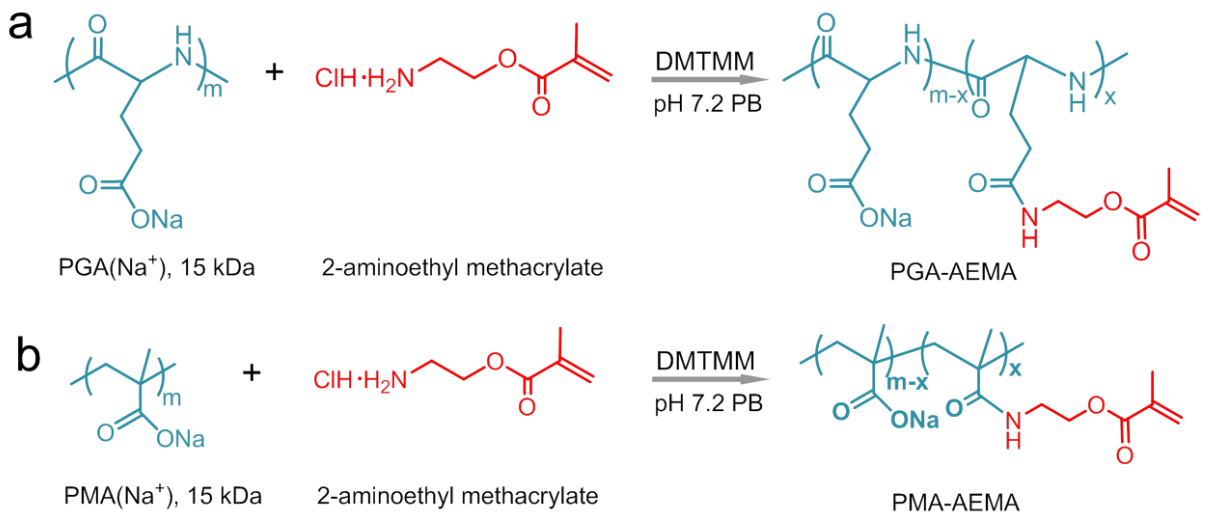
Cell Viability Analysis by XTT Assay. The cytotoxicity of polymer capsules toward HeLa and Raw 264.7 cells was evaluated *via* XTT assays. In brief, HeLa and Raw 264.7 cells were seeded into 96-well plates at a density of 5000 cells per well and allowed to adhere overnight at 37 °C with 5% CO_2. Following this, AF633-labeled polymer capsules were added at a capsule-to-cell ratio of 100:1 and incubated for 24 h. After treatment, culture media were replaced by 50 μL of fresh DMEM media as well as 50 μL of XTT solution (5 mL of 1 mg mL^{-1} XTT in DPBS + 100 μL DMSO + 200 μL of 0.15 mg mL^{-1} PMS in DPBS) and the cells were incubated for a further 5 h. Then, the absorbance at a wavelength of 450 nm was measured using a Cary 50 Bio UV-Visible Spectrophotometer with microplate reader. The relative cell viability (%) was determined *via* comparing the absorbance of untreated cells. Experiments were performed in quintuplicate and data are presented as the mean ± standard deviation.



Scheme S1. Chemical structures of a) 8-arm PEG-A, b) PHPMA-A, and c) HA_{47k}-AEMA macrocrosslinkers.



Scheme S2. Synthetic pathway for HA_{289k}-AEMA (M_w 289 kDa) macrocrosslinker.



Scheme S3. Synthetic pathways for a) PGA-AEMA and b) PMA-AEMA macrocrosslinkers.

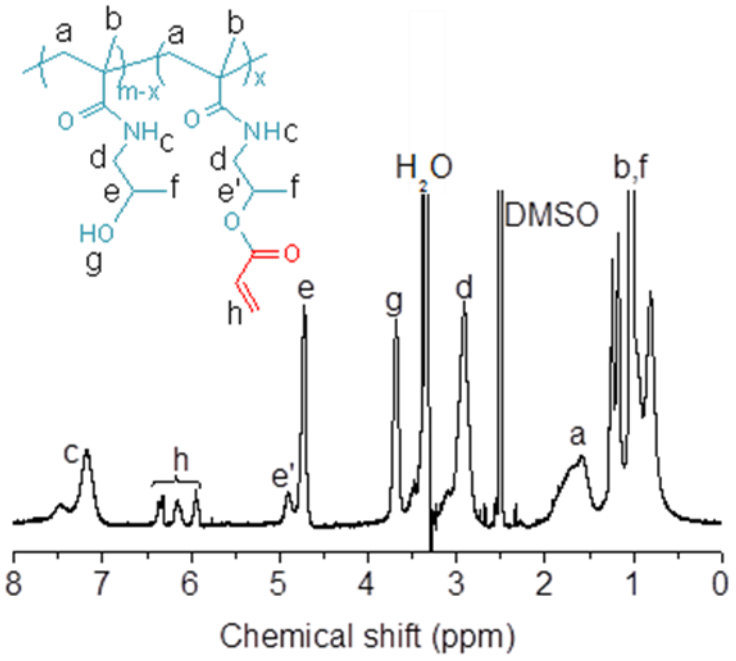


Figure S1. ^{1}H NMR spectrum of PHPMA-A macrocrosslinker (D_2O, 400 MHz).

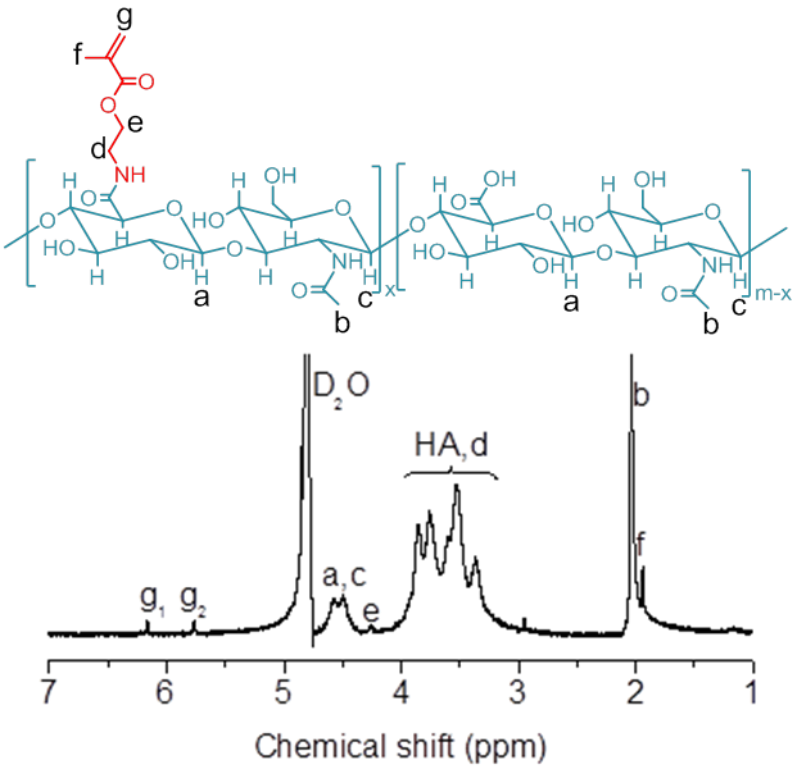


Figure S2. ^{1}H NMR spectrum of HA_{289k}-AEMA macrocrosslinker (D_2O, 400 MHz).

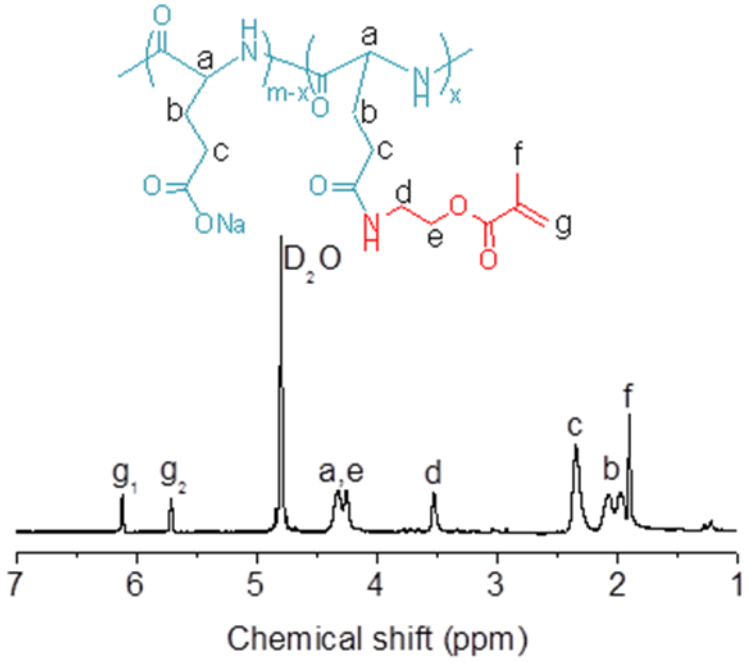


Figure S3. ^{1}H NMR spectrum of PGA-AEMA macrocrosslinker (D_2O, 400 MHz).

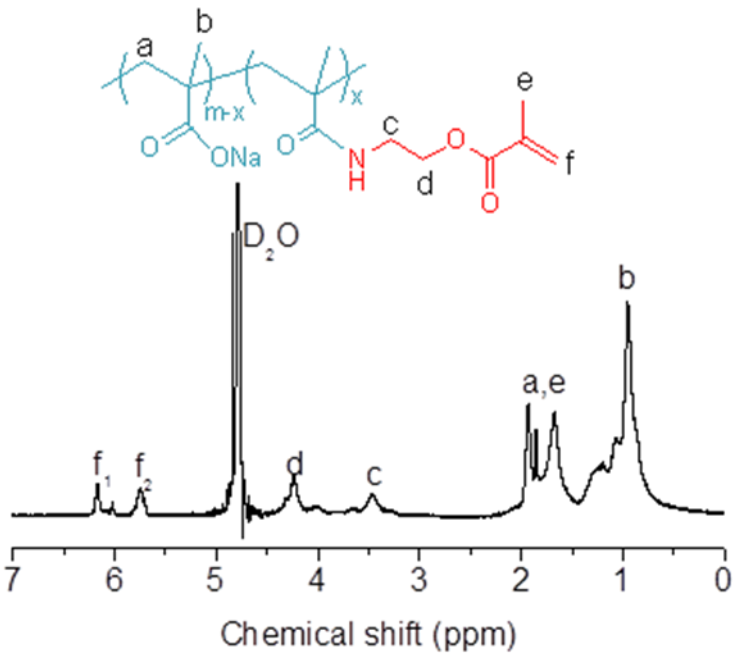


Figure S4. ^{1}H NMR spectrum of PMA-AEMA macrocrosslinker (D_2O, 400 MHz).

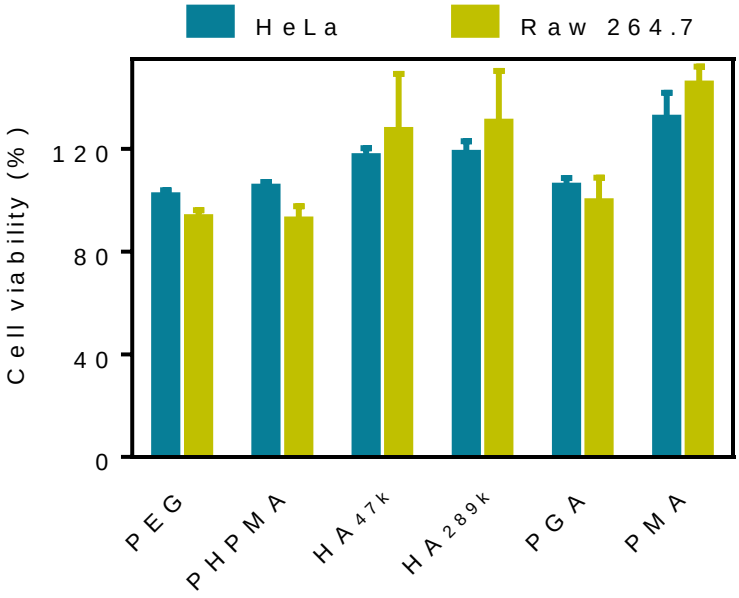


Figure S5. Cell viability of HeLa and Raw 264.7 cells after incubation with polymer capsules of different compositions (PEG, PHPMA, HA_{47k}, HA_{289k}, PGA, PMA) at a capsule-to-cell ratio of 100:1 for 24 h, as determined via XTT assay. The values were normalized to that of

untreated cells, which were set at 100%. Data are presented as the average ± standard deviation (n = 5).

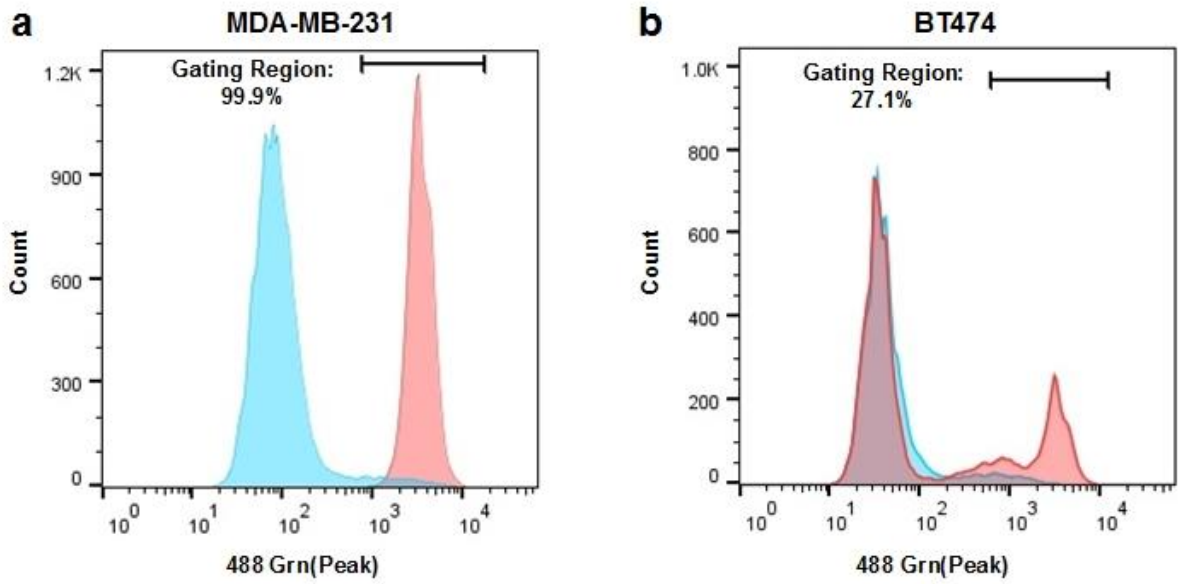


Figure S6. Overlay flow cytometry histogram showing a fluorescence shift of a) MDA-MB-231 and b) BT474 cells after stained with FITC-labeled anti-CD44 antibody. Untreated cells are denoted by the blue curve, while treated cells (stained with FITC-labeled anti-CD44 antibody) are denoted as the red curve. Gating region on the top showing the percentage of treated cells exhibited stronger fluorescence, compared to untreated cells.

Reference

(1) Wong, E. H. H., van Koeverden, M. P., Nam, E., Guntari, S. N., Wibowo, S. H., Blencowe, A., Caruso, F., and Qiao, G. G. (2013) Assembly of nanostructured films with hydrophobic subcompartments via continuous assembly of polymers. *Macromolecules 46*, 7789-7796.