Supporting Information

Tumor-Adhesive and pH-Degradable Microgels by Microfluidics and Photo-Crosslinking for Efficient Anti-Angiogenesis and Enhanced Cancer Chemotherapy

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Materials

Acryloyl chloride (98%), ethylene glycol vinyl ether (98%), triethylamine (Et_3N, 99%), succinic anhydride (SA, 99%), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 99%), docetaxel (DTX, 98%), N-hydroxysuccinimide (NHS, 99%), 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (I2959, 98%), *p*-toluenesulfonic acid monohydrate (PTSA, 98%) and 3-hydroxytyramine hydrochicride (dopamine; DA 98%) were purchased from Energy Chemical (Shanghai, China) and used as received. Bevacizumab (Bev) was obtained from TheraMabs Bio-technology Co. Ltd (Shanghai, China). Polyvinyl alcohol (PVA, 87.0-89.0% hydrolyzed, M_n = 16000) was purchased from Acros. Cyanine5 amine (Cy5) was purchased from RuiXi Biological Technology Co. Ltd (Xi'an, China). For cell culture, 4T1-Luc cells were incubated in Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum, 4 mM glutamine, 100 U/mL penicillin and 0.1 mg/mL streptomycin at 37 °C with 5% CO_2. The medium and supplements were purchased from Life Technologies (Nanjing, China). The mice were obtained from the Model Animal Research Centre of Nanjing University (Nanjing, China). All the animal experiments were carried out in compliance with the Animal Management Rules (Ministry of Health, People's Republic of China) and the guidance for Care and Use of Laboratory Animals (China Pharmaceutical University). Microfluidic chips were fabricated with poly(dimethyl siloxane) (PDMS) material using a standard soft lithographic protocol.[1] In brief, the elastomer kit of Sylgard 184 with a weight ratio of PDMS/crosslinker at 10/1 (DowCorning) were poured onto a silicon wafer patterned with SU-8 photoresist (the inner channel pattern as shown in Scheme 1). After solidifying at 70 °C for 1 h, the PDMS chips were bonded onto the glass slides by oxygen plasma, and then treated with Aquapel® (PMG, Pittsburgh, PA, USA) for 30 s to form the hydrophobic channel surface inside. The solution was removed by air drying to obtain the microfluidic device chips with the height and width of outer/core channel at 100 μm and 150 μm, respectively.

Vinyl ether acrylate (VEA) and VEA-functionalized PVA (PVA-VEA) were prepared according to the method reported in a previous work.[2] Carboxyl modified PVA-VEA (PVA-VEA-COOH) was synthesized by adding PVA-VEA and SA (molar ratio of OH/SA: 17/1) into DMSO and stirring at room temperature overnight in the presence of catalytic Et_3N referring to our previous work.[3] Then the reaction solution was dialyzed against methanol and concentrated. Finally, PVA-VEA-COOH was isolated by precipitation in ice-cold diethyl ether. DA-decorated PVA-VEA-COOH (PVA-VEA-COOH(DA)) was prepared by adding PVA-VEA-COOH and DA (molar ratio of COOH/DA: 2/1) into DMSO using EDC and NHS

as catalysts under N_2 protection, and stirring at room temperature overnight via carbodiimide chemistry. Subsequently, the polymer solution was dialyzed against methanol and PVA-VEA-COOH(DA) was isolated by precipitation in ice-cold diethyl ether. The degrees of VEA, COOH and DA functional groups on PVA were estimated by ^{1}H NMR using a Bruker ACF-300Q spectrometer (USA).

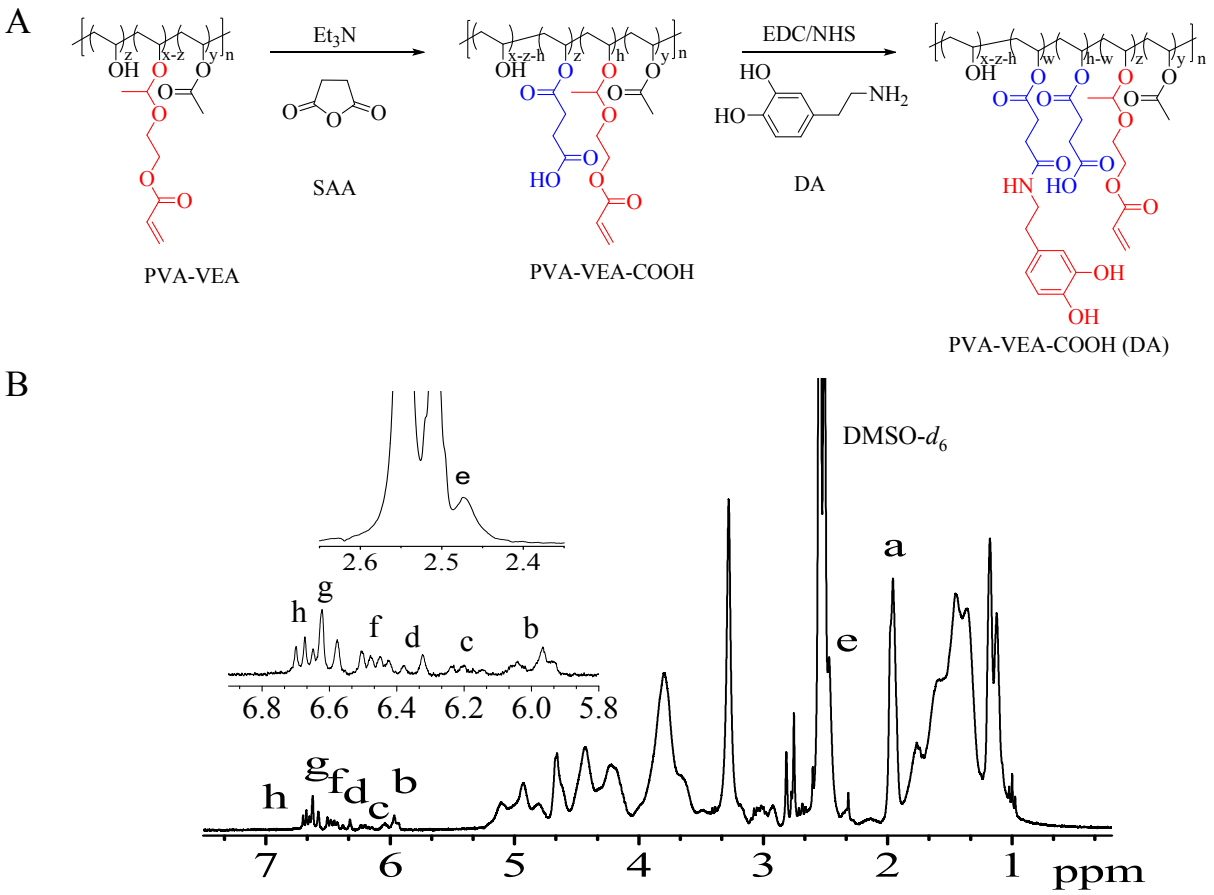

Figure S1. (A) Synthesis of VEA/carboxyl/dopamine multi-functionalized PVA (PVA-VEA-COOH(DA)). Conditions: (i) SAA, Et_3N, room temperature, overnight, DMSO; (ii) DA, EDC, NHS, room temperature, overnight, DMSO. (B) ^{1}H NMR spectra of PVA-VEA-COOH(DA) (400 MHz, DMSO-d_6).

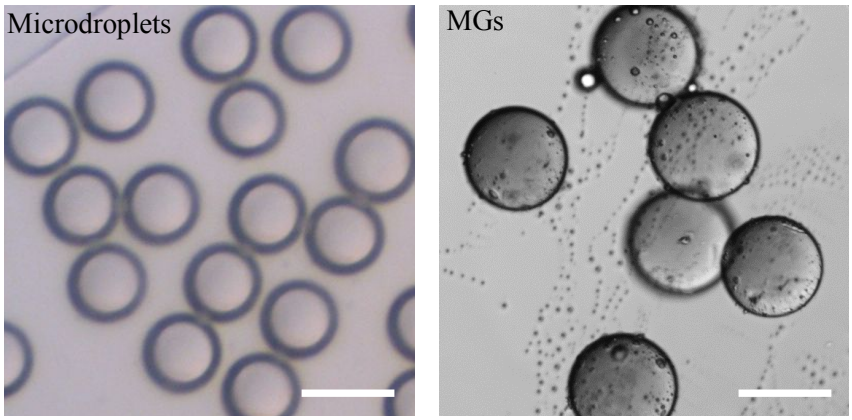

Figure S2. Size distribution of non-functionalized PVA microdroplets and microgels visualized by microscopy. PVA microgels were prepared by a combination of microfluidics and photo-crosslinking (conditions: Q_{oil} = 8.0 µL/min, Q_{PVA} = 1.0 µL/min, scale bar: 200 µm).

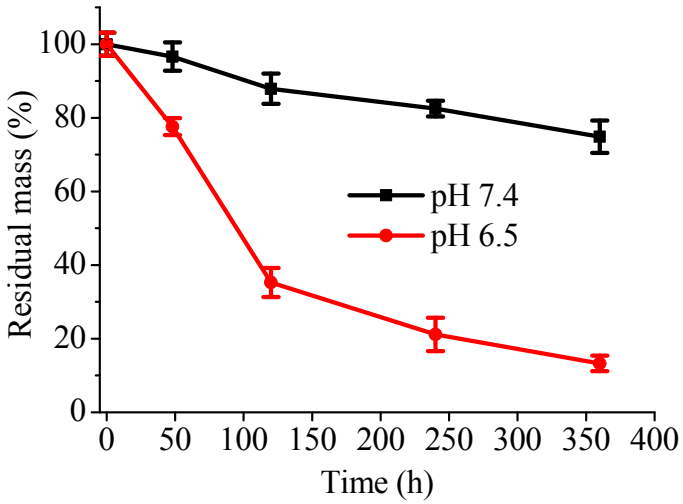

Figure S3. Degradation of DMGs incubated with pH 7.4 and 6.5 determined by weight loss. Each point represents the mean ± SD (n = 3).

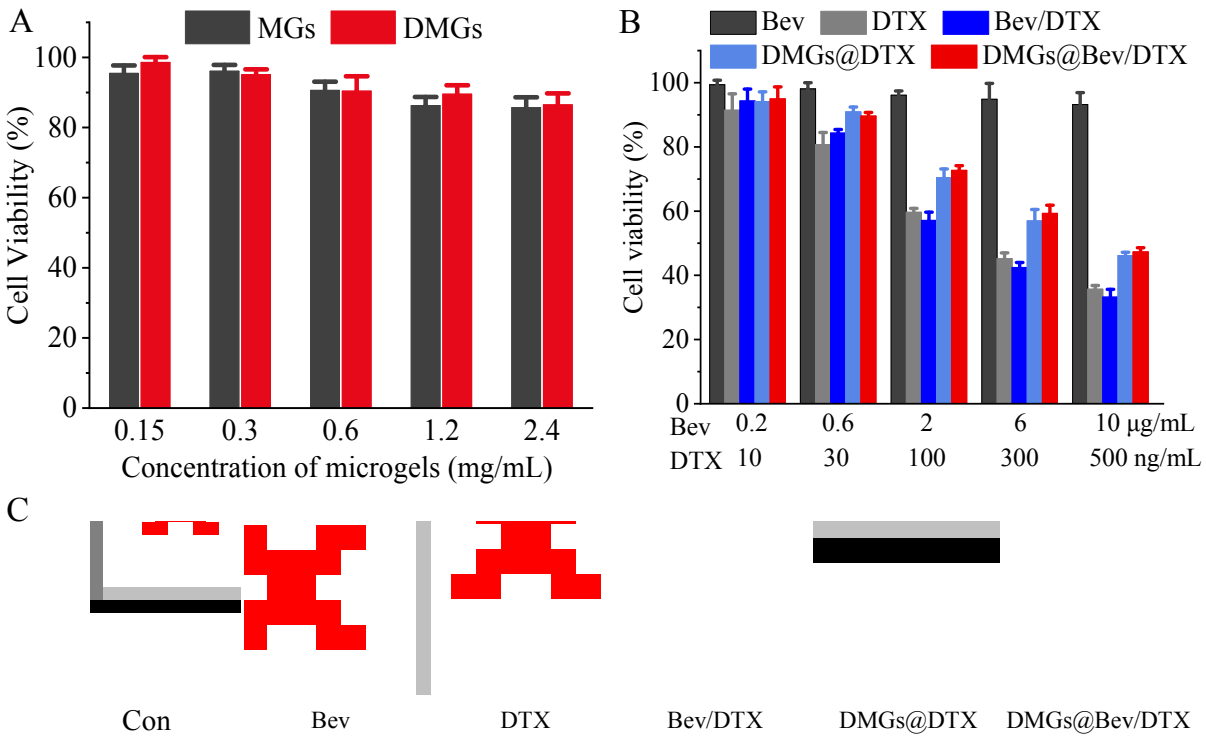

Figure S4. Cytotoxicity test of blank MGs and DMGs (A) and Bev/DTX encapsulated DMGs at different concentrations (B) in 4T1-Luc cells following 48 h incubation by MTT assay (each point represents the mean ± SD, n = 3). (C) fluorescence images of 4T1-Luc cells stained with Calcein-AM/PI after 48 h incubation with DMGs@Bev/DTX at 10 μg/mL Bev and 500 ng/mL DTX (scale bar: 50 μm). Cells cultured without any treatments were used as a control.

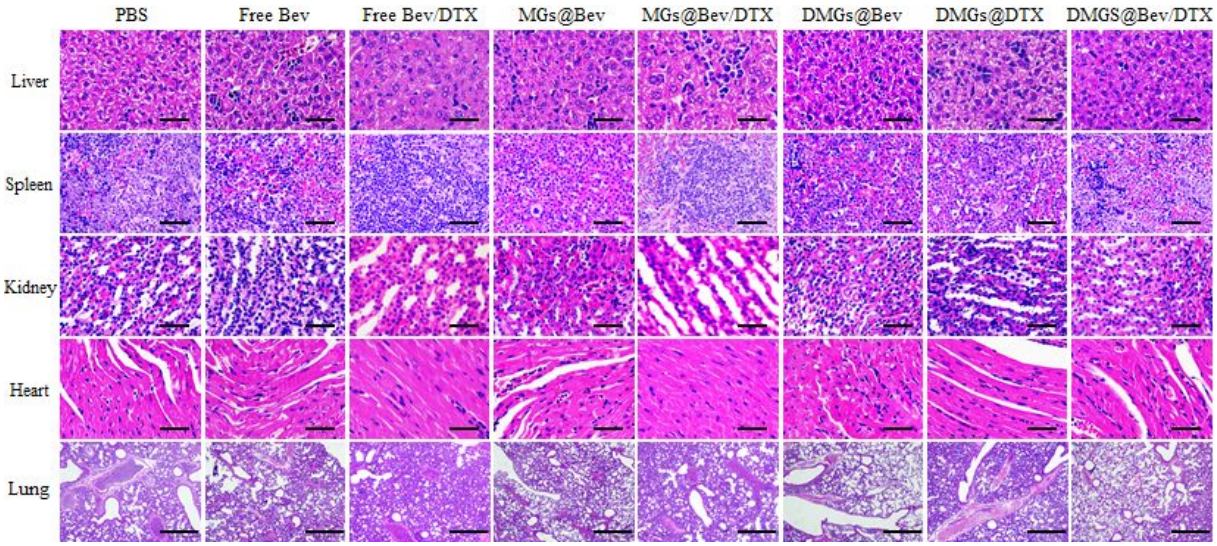

Figure S5. H&E staining images of livers, spleens, kidneys, hearts and lungs of 4T1-Luc tumor-bearing mice after different treatments for 10 days (scale bar of lung: 100 μm; others: 10 μm).

Reference

1. Steinhilber, D.; Rossow, T.; Wedepohl, S.; Paulus, F.; Seiffert, S.; Haag,R.; A microgel Construction Kit for Bioorthogonal Encapsulation and pH-Controlled Release of Living Cells, *Angew. Chem. Int. Ed.* **2013**, *52*, 13538–13543.
2. Chen, W.; Hou, Y.; Tu, Z.; Gao, L.; Haag, R. pH-Degradable PVA-Based Nanogels via Photo-Crosslinking of Thermo-Preinduced Nanoaggregates for Controlled Drug Delivery. J. Control. Release **2017**, *259*, 160-167.
3. Qiao, H.S.; Chen, X.M.; Chen, E.P.; Zhang, J.M.; Huang, D.C.; Yang, D.Q.; Ding, Y.C.; Qian, H.L.; Feijen, J.; Chen, W. Folated pH-Degradable Nanogels for Simultaneous Delivery of Docetaxel and IDO1-Inhibitor in Enhancing Cancer Chemo-Immunotherapy, *Biomater. Sci.* **2019**, *7*, 2749-2758.