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Aptamer anchored di-polymer shells capped mesoporous carbon as drug carrier for bi-trigger targeted drug delivery

Yang Zhang[a,c#], Yan-Qin Chang[a#], Lu Han[a], Yue Zhang[a], Ming-Li Chen[a], Yang Shu[*b], Jian-Hua Wang[*a]

[a]*Research Center for Analytical Sciences, College of Sciences, Northeastern University, Box 332, Shenyang 110189, China. E-mail: jianhuajrz@mail.neu.edu.cn; Fax: +86 24 83676698; Tel: +86 24 83688944*

[b]*Institute of Biotechnology, College of Life and Health Sciences, Northeastern University, Shenyang 110189, China. E-mail: shuyang@mail.neu.edu.cn*

[c]*Institute for Biosensing, and College of Chemistry and Chemical Engineering, Qingdao University, 308 Ningxia Road, Qingdao 266071, China*

[#]*Yang Zhang and Yan-Qin Chang contributed equally to this work.*

Electronic Supplementary Information

Experimental

Chemicals

The MUC1 aptamer (Apt) with a sequence of (5'-GCAGTTGATCCTTTGGATACCCTG G-3') and the primers are synthesized and purified by Shanghai Sangon Company. Poly(ethylene oxide)-block-poly (propylene oxide)-block-poly(ethyleneoxide) triblock copolymer Pluronic F127 (PEO106PPO70PEO106, Mw 12600), formalin solution (37 wt%), polyethylenei mine (PEI), N-hydroxysuccinimide (NHS), 1-ethyl-3-[3-dimethyl- aminopropyl] carbodiimide hydrochloride (EDC), 4,6-diamidino-2-phenylindole (DAPI) ,doxorubicin (DOX) and hydroxymethyl aminomethane (Tris) are purchased from Sigma-Aldrich (St. Louis,USA). Cystamine dihydrochloride is obtained from Yuanye Bio-Technology Co. Ltd (Shanghai, China). Poly(acrylic acid) (PAA), ammonium persulfate (APS), 2-(N-morpholino)ethanesulfonic acid (MES), sulfuric acid, sodium hydroxide, hydrochloric acid, acrylamide, N,N'-Methylenebisacrylamide, carbamide, ethylenediaminetetraacetic acid disodium salt (EDTA-Na) and boric acid are products from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). RIPA Lysis Buffer is obtained from Beyotime (Shanghai, China). Nucleic acid gel stain (10,000×H_2O), DMEM (high glucose), trypsin, penicillin/streptomycin, fetal bovine serum, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit are received from Nanjing KeyGEN Biotech (Nanjing, China). The 6×loading buffer is the product from Takara Biotechnology Co. Ltd. (Dalian, China). N,N,N',N'-Tetramethylethylenediamine (TEMED) is obtained from Aladin (Shanghai, China). These reagents are at least of analytical-reagent grade and used without any further treatment.

Apparatus and characterization

Scanning electronic microscopy (SEM) images are obtained on a Ultra Plus scanning electronic microscope (SEM, Zeiss, Germany). High resolution transmission electron microscopy (HR-TEM) images are recorded on a JEM-2100 F microscope (JEOL, Japan) operated at 200 kV. Small-angle X-ray scattering (SAXS) measurements are

performed on a Nanostar U SAXS system (Bruker, Germany) using Cu Ka radiation (40 kV, 35 mA). The d-spacing values are calculated by the formula of d = 2p/q. Fourier transform infrared spectra (FT-IR) are obtained on a Nicolet 6700 spectrophotometer (Thermo Electron, USA). Nitrogen adsorption/desorption isotherms are measured at 77 K with a ASIQCTV00000-6 (Quantachrome, USA). Before the adsorption/desorption measurements, the samples are degassed in a vacuum oven at 100°C for 10 h. X-ray photoelectron spectroscopy (XPS) analyses are performed on an ESCALAB 250 surface analysis system (Thermo Electron, USA). Raman spectra are recorded by a Horiba XploRA microscopic confocal Raman spectrometer (HORIBA Jobin Yvon, France). The zeta potentials of the nanocarriers in aqueous solution are measured on a ZEN3600 Nano Zetasizer (Malvern, UK). Fluorescence spectra are recorded on a F-7000 fluorescence spectrophotometer (Hitachi High Technologies, Japan). UV-vis absorption spectra are recorded using a U-3900 UV spectrophotometer (Hitachi High Technologies, Japan). Cell imaging is performed on a fluorescence confocal microscope (FV1200, Olympus, Japan).

Preparations of MCNs, ss-MCN and PAA-ss-MCN

Preparation of MCNs: 0.6 g of phenol, 2.1 mL of formalin aqueous solution (37 wt%) and 15 mL NaOH (0.1 M) aqueous solution are mixed at 70 °C for 30 min; 0.96 g of triblock copolymer Pluronic F127 (Mw 12600, PEO106PPO70PEO106) dissolved in 15 mL H_2O is added subsequently. After stirring for 2.5 h, 50 mL water is added to dilute the solution and the reaction is stopped until a precipitate is observed. The reaction mixture is transferred into an autoclave, diluted with 260 mL H_2O and kept at 130 °C for 8 h. The product is collected by centrifugation at 10000 rpm for 10 min, washed with distilled water and dried at room temperature. Finally, the product is carbonized at 600 °C in N_2 atmosphere for 3 h.

Disulfide crosslinked MCNs (ss-MCN) are prepared by firstly impregnating 50 mg MCNs into 20 mL mixed solution containing ammonium persulfate (1.75 M) and sulfuric acid (2 M). The mixture is stirred for 24 h at 40 °C. The oxidized MCNs (ox-MCN) are washed with distilled water until the supernatant becomes neutral.

Afterwards, 0.1 g ox-MCN is activated using EDC (10 mg mL^{-1}) and NHS (10 mg mL^{-1}) in a MES buffer (50 mM, pH 6.0) for 20 min at room temperature with continuous stirring. pH value of the solution is then adjusted to 7.5 followed by addition of 1.0 g cystamine dihydrochloride, and the mixture is stirred at 25 ^{o}C for 24 h. The obtained product is centrifuged, washed and dried for further use.

PAA-ss-MCN preparation: 10 mL PAA solution (1%, v/v) is activated using EDC (5 mg mL^{-1}) and NHS (5 mg mL^{-1}) in MES buffer for 20 min at room temperature with continuous stirring, and the mixture is adjusted to pH 4.8 by 0.1 M HCl. Afterwards, 30 mg ss-MCN is added and pH value of the reaction mixture is adjusted to 7.0 followed by continuous stirring for 24 h at room temperature. The product is obtained by washing with distilled water for three times, centrifuging at 5000 rpm for 5 min, and finally collected by freeze drying.

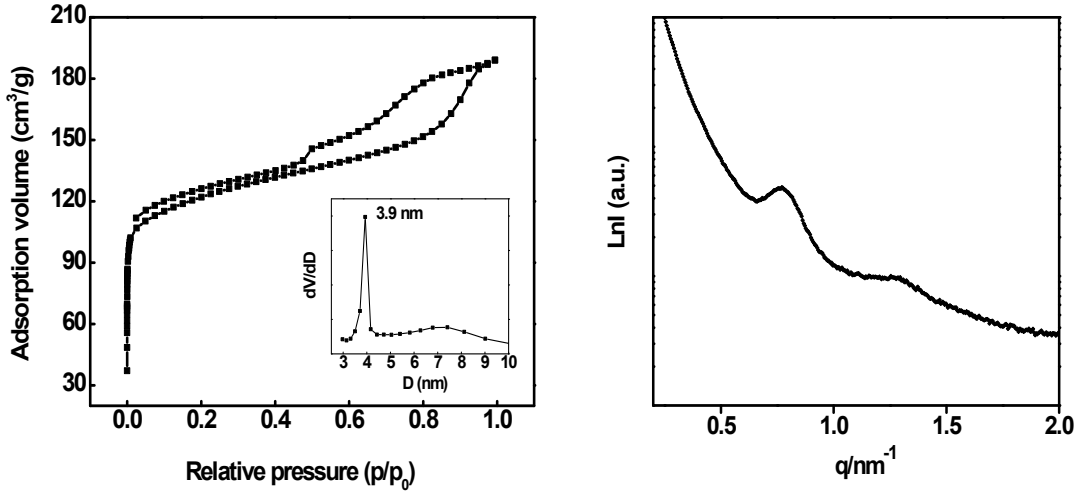

Fig. S1. (A). N_2 sorption/desorption isotherms and the corresponding pore size distribution curve (inset) of the bare ox-MCN; (B) Small-angle X-ray scattering (SAXS) of ox-MCN.

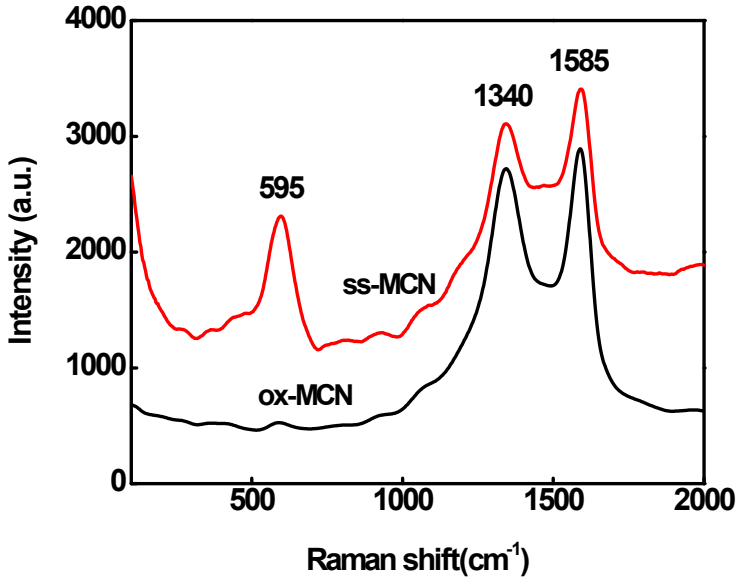

Fig. S2. Raman spectra of ox-MCN and ss-MCN.

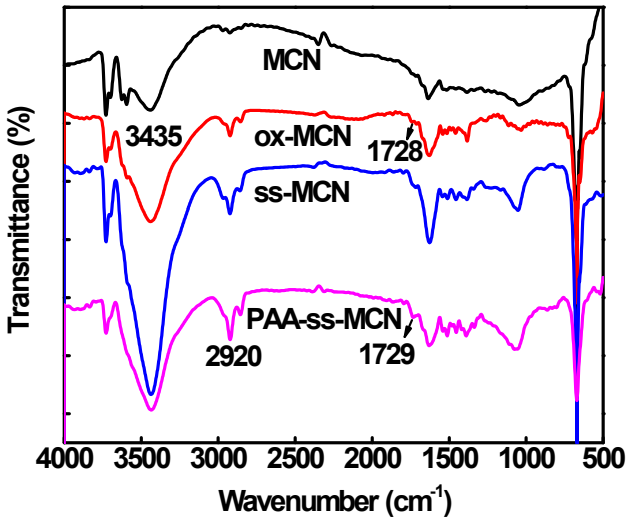

Fig. S3. FT-IR spectra of MCNs, ox-MCN, ss-MCN and PAA-ss-MCN.

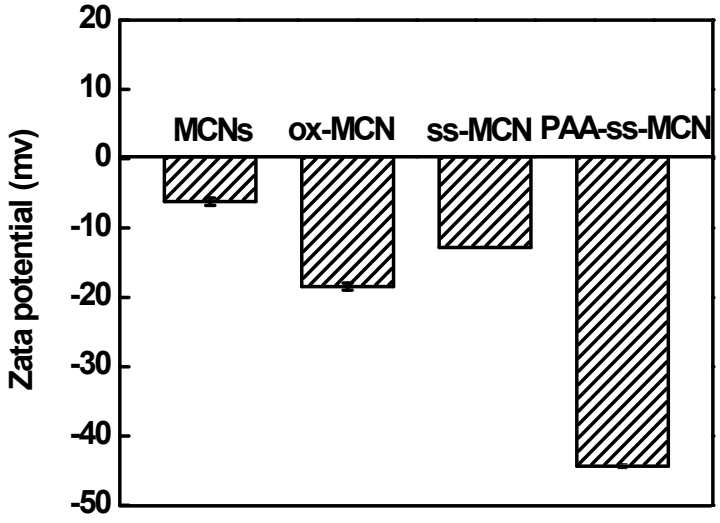

Fig. S4. Zeta potentials of MCNs, ox-MCN, ss-MCN, PAA-ss-MCN.

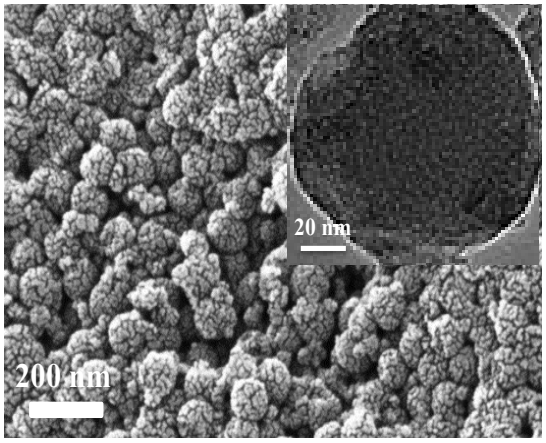

Fig. S5. SEM and HR-TEM images of the mesoporous carbon nanoparticles capped with PAA/PEI double polymer (DP-MCN).

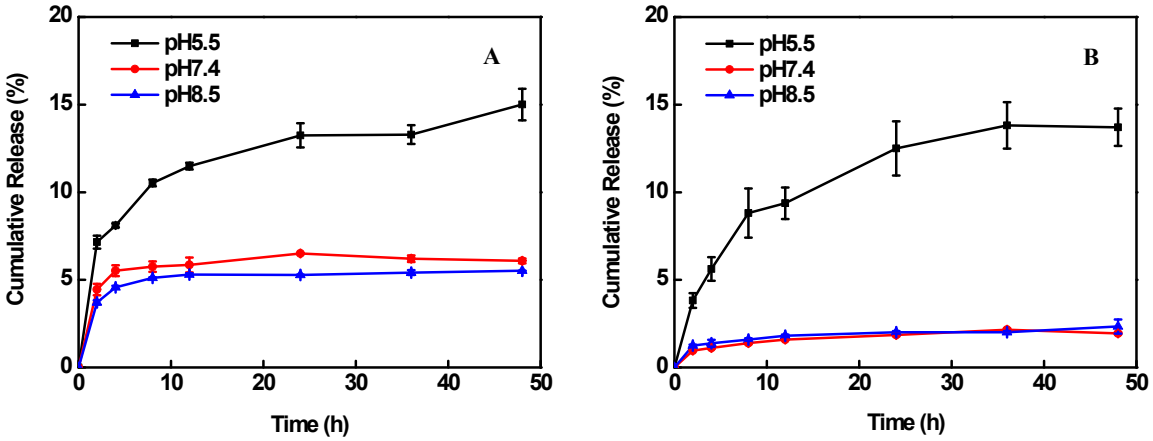

Fig. S6. The release of DOX from the PAA-DOX-MCN (A) and DP-DOX-MCN (B) nanocomposite at pH 5.5, 7.4 and 8.5.

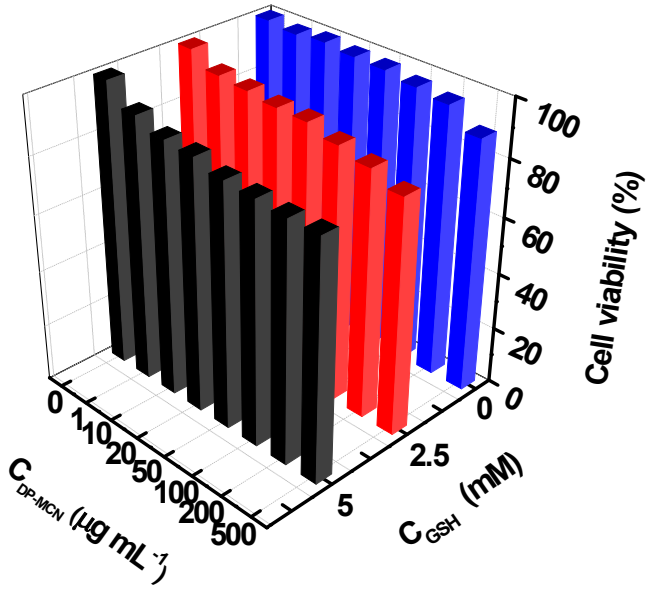

Fig. S7. Cell viability after incubation with DP-MCN under different concentrations (0, 1, 10, 20, 50, 100, 200, 500 μg mL^{-1}) for 24 h. HeLa cells are pretreated with 2.5 mM and 5 mM GSH for increasing the intracellular level of GSH, with un-treated HeLa cells as a reference.

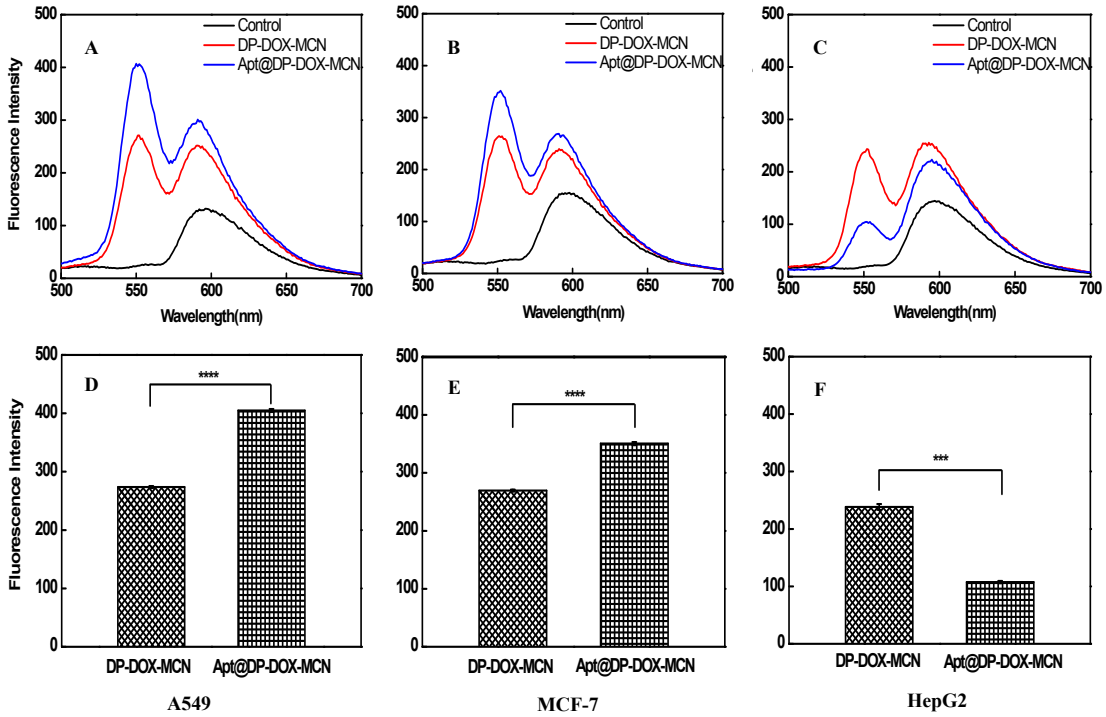

Fig. S8. Evaluation of the cellular uptake of DOX for the MUC1-positive cells A549 and MCF-7 and MUC1 negative cells HepG2 monitored by fluorescence spectrophotometry. A549 (A and D), MCF-7 (B and E) cell lines positive for MUC1 expression and HepG2 (C and F) cell line negative for MUC1 expression are treated with DP-DOX-MCN and Apt@DP-DOX-MCN at 37℃, 5% CO_2 for 2 h and quantitative uptake is further investigated by fluorescence spectrophotometry. Ex/Em=470 nm/552 nm; Data are expressed as mean ± SD (n=3), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.