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Supporting Information

Dendritic Catiomer with MOF Motif for Construction of Safe and Efficient Gene Delivery Systems

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1. Experiments:

1.1 Chemicals and materials:

All reagents were obtained from the commercial sources and used without further purification. Polyethylenimine [average M_w ~25 kDa by LS, the number average molecular weight M_n ~10 kDa by GPC, branched], Zirconium (IV) chloride ($ZrCl_4$), 2-aminoterephthalic acid were purchased from Sigma-Aldrich (Shanghai, China). Glycidyl methacrylate (GMA), 2-bromoisobutyryl bromide (BiBB), bipyridyl were purchased from Shanghai Adamas Reagent Co., Ltd (Shanghai, China). Copper (I) bromide was purchased from Heowns (Tianjin, China). Prior to use, tetrahydrofuran (THF) was distilled by refluxing over sodium, in the presence of benzophenone as an indicator. Triethylamine (TEA) was distilled from calcium hydride under argon. A Cell Counting Kit-8 (CCK8) was used in the cell cytotoxicity assay (Dongjindo, Japan). A Micro BCA Protein Assay Reagent Kit was purchased from Pierce Co., Inc. (Rockford, IL) and the luciferase assay kit was a product of Promega (USA).

1.2 Agarose Gel Electrophoresis

The agarose gel electrophoresis was performed to confirm the condensation ability of the nanohybrids to pDNA at varying N/P ratios. Generally, diverse proportion polyplex solution was loaded onto a 0.8% agarose gel containing ethidium bromide (EtBr). A constant voltage (85 V) was applied to the polyplex-loaded gel in tris-acetate buffer (pH=7.4) for 90 min. DNA retardation was analyzed using an UV illuminator (Gel Documentation System, Bio-Rad, Hercules, CA). In addition, the nanohybrids in resistance to polyion exchange to heparin were also evaluated by agarose gel retardation assay. The samples were allowed for 0.5 h polyion exchange with heparin at varying concentrations. The reaction solutions were electrophoresed on a 0.8% agarose gel containing ethidium bromide (EtBr) with tris-acetate buffer (pH = 7.4) running buffer at 85 V for 90 min. The pDNA retardation was visualized under UV illumination.[1]

1.3 Particle size and Morphology Measurement

The size and morphologies of UiO-PGMA-EA and UiO-PGMA-EA/pDNA were characterized using a SEM on a JSM-6700F type field emission scanning electron microscope (JEOL, south Korea). SEM samples were prepared by depositing solutions of the samples onto a clean glass slide. After evaporation of solvent, the samples were coated with a thin gold layer.

1.4 Cell viability assay

To determine the PEI and UiO-PGMA-EA cytotoxicity, a CCK8-based cell viability test was performed in 96-well plates.[2] A549 cells were seeded at a density of 5×10^3 cells per well and the cells were cultured for 24 h in serum containing culture medium (100 μL of medium per well). The samples were complexed at a pDNA concentration of 50 μg mL^{-1} with a rising N/P ratio (N/P=3, 5, 10, 15, 20). The complexes (10 μL) were added to each well. After incubation at 37 °C for 48 h, the cells in the culture medium of 100 μL were treated with CCK8 solution (10 μL) to measure the cell viabilities. The absorbance of the solution was measured at 450 nm using a microplate reader (Epoch, BioTek, Gene Company Limited) after 30 min incubation. The cell viability in each well was calculated from the obtained values as a percentage of the control wells. The results were presented as mean and standard deviation of eight samples.

1.5 In vitro transfection

Transfection assays were performed using the plasmid CAG-Luc as the reporter gene in A549 cell lines. Briefly, cells were seeded in 24-well plates at a density of 1×10^4 cells per well and cultured with 0.4 mL of culture medium for 24 h at 37 °C until the cells reached 70% confluency. The complexes (25 μg mL^{-1} pDNA) at varying N/P ratios were added into the culture plate. Followed by a further 48 h incubation, cells were washed twice with PBS and lysed in 100 μL of the cell culture lysis reagent. Luciferase activity was measured by a luciferin substrate (Promega Co. Cergy Pontoise, France) in a luminometer (FLX800, BioTek, Gene Co. Ltd.). The protein concentration of each

well was analyzed using a protein assay kit (Pierce). The results were expressed as relative light units (RLUs) per milligram of cell protein (RLU/mg protein). The gene expression results were presented as a mean and standard deviation of the measurement from the obtained four samples.

1.6 Dynamic light scattering (DLS) and zeta potential

The particle sizes and zeta potentials of the complexes in ultrapure water were determined by a Zetasizer Nano ZS90 instrument (Malvvern Instruments, Southborough, MA) at 25 °C. In brief, 600 μL of UiO-PGMA-EA/pDNA and PEI/pDNA polyplex (at varying N/P ratios) were prepared to DLS and zeta potential measurement.

1.7 Stability of UiO-PGMA-EA and UiO-PGMA-EA/pDNA system

Briefly, 500 μg UiO-PGMA-EA and UiO-PGMA-EA/pDNA (N/P=10) were dissolve in 1 mL of PBS (pH=7.4) with or without 5% BSA, respectively. The mixtures were incubated at 37 °C and particle sizes of particles were determined by DLS at various time intervals. The ratio of particle size change was calculated as t_i/t_o.

1.8 Nitrogen sorption measurements

N_2 adsorption and desorption isotherms were obtained using a Micromeritics Gemini instrument (Shanghai). Samples outgassing was performed for 5 hours at 120 °C. Specific surface areas were calculated from the adsorption data in the low-pressure range using the Brunauer-Emmett-Teller (BET) model. Pore size were determined

following the Barrett-Joyner-Halenda (BJH) method.

1.9 Other instruments and methods

X-ray diffraction (XRD) patterns of the samples were recorded on a Rigaku Ultima IV X-ray Diffractometer using Cu Kα radiation (40kV, 40mA) with scanning rate of $2\theta=10°$ min^{-1} in the range of 5-50 °. XPS signals were recorded on a VG ESCALAB 220i-XL spectrometer under ultra-high vacuum (6×10^{-9} mbar). FTIR spectroscopy was conducted on a BRUKER TENSOR 27 FTIR Spectrometer using the KBr disc method.

Statistical analysis

Statistic significance in comparing the groups of data in protein adsorption assay, cell viability and transfection efficiency between the UiO-PGMA-EA and PEI were conducted using Student's *t*-test.

2. Characterization of polymers in polymer-MOF

The chemical structures of UiO-PGMA and UiO-PGMA-EDA were characterized by ^{1}H NMR measurement (Figure S1.). Pertaining to the ^{1}H NMR spectrum of UiO-PGMA (Figure S1a), the peaks at δ of 4.3 and 3.8 ppm correspond to the methylene protons adjacent to the ester linkages (a, O=C-O-$\underline{CH_2}$-CH). The signal at δ of 3.2 ppm peaks at δ of 2.6 and 2.8 ppm can be assigned to the methylidyne protons [b, CH_2-$\underline{CH}$(O)-CH_2] and methylene protons of the epoxide ring [c, CH_2-CH(O)-$\underline{CH_2}$], respectively. Consistent with the theoretical ratio, the integration area ratio of peak a, b

and c was approximate 2:1:2, indicating that the epoxy groups remained intact throughout ATRP process. UiO-PGMA-EA was synthesized through the ring-opening reaction by EA from the precursor of UiO-PGMA. As shown in Figure S1b, peaks b and c characterized by the epoxide rings of UiO-PGMA disappeared post the ring-opening reaction with EA, and peaks at 3.8 and 4.3 ppm merged as a peak at δ of 4.0 ppm (d). Besides, the peak at δ of 3.8 ppm could be assigned to the methylene protons adjacent to the hydroxyl groups (e, h). The peak at δ of 3.0 ppm is attributable to the methylene protons (f, g, NH-CH_2). The above ^{1}H NMR spectra approved the ring opening reaction of oxirane rings in UiO-PGMA with EA.[3,4]

The N_2 adsorption-desorption isotherm are provided in Figure S5, the specific surface areas of NH_2-UiO-66 and UiO-BiBB post washing and activation treatment are 799 m^2/g and 708 m^2/g, respectively. Their pore sizes were determined to mainly distribute at the range of 0.8-1.0 nm, which are consistent with the previous report.[5,6] The marked decrease in surface area for UiO-PGMA indicated that the cavities of MOF were covered by the polymer shell. Herein, the accurate pore size of UiO-PGMA is not given due to the surface of UiO-PGMA was mainly composed of PGMA.

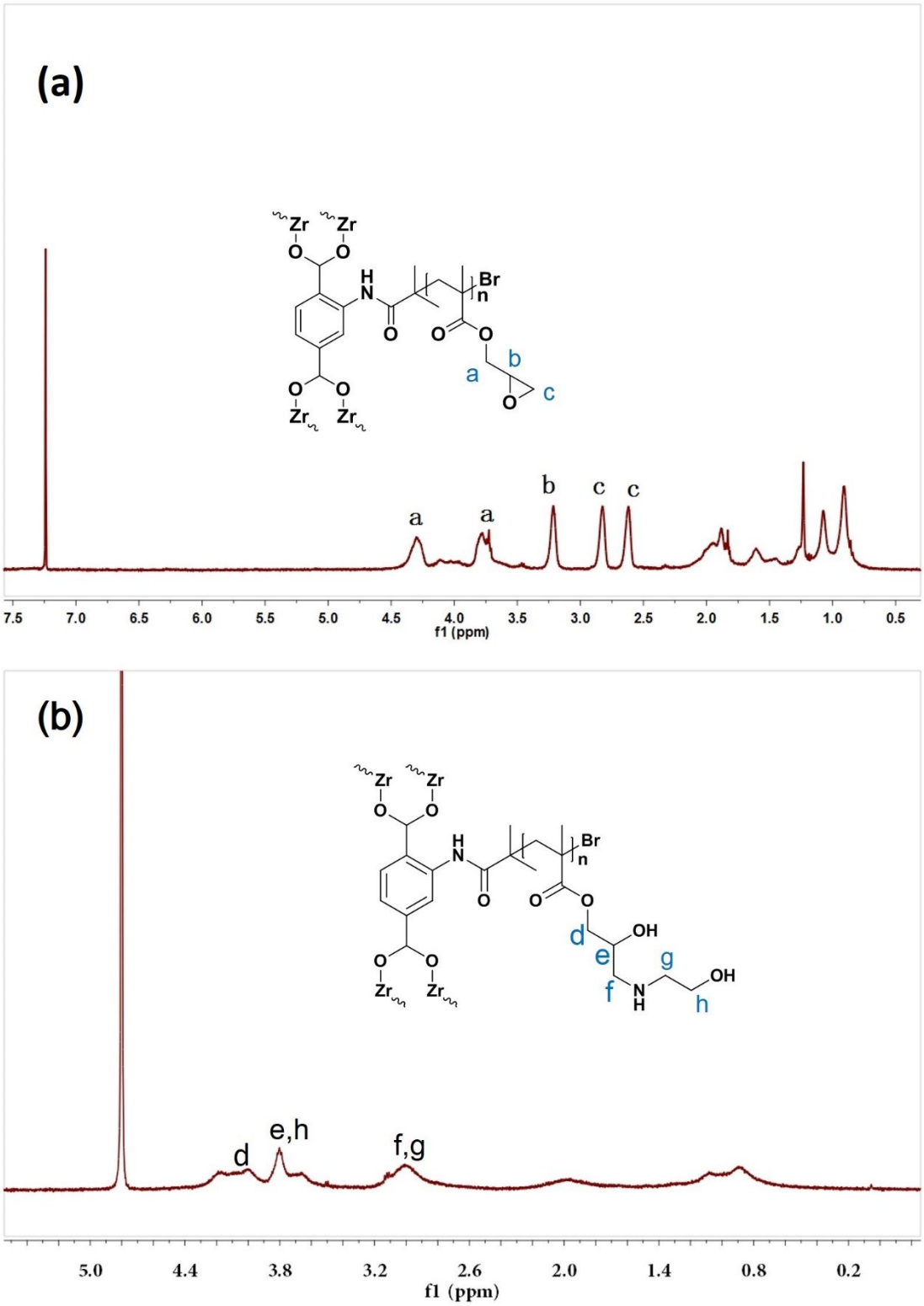

Figure S1. ^{1}H NMR spectra of UiO-PGMA (a) and UiO-PGMA-EA (b).

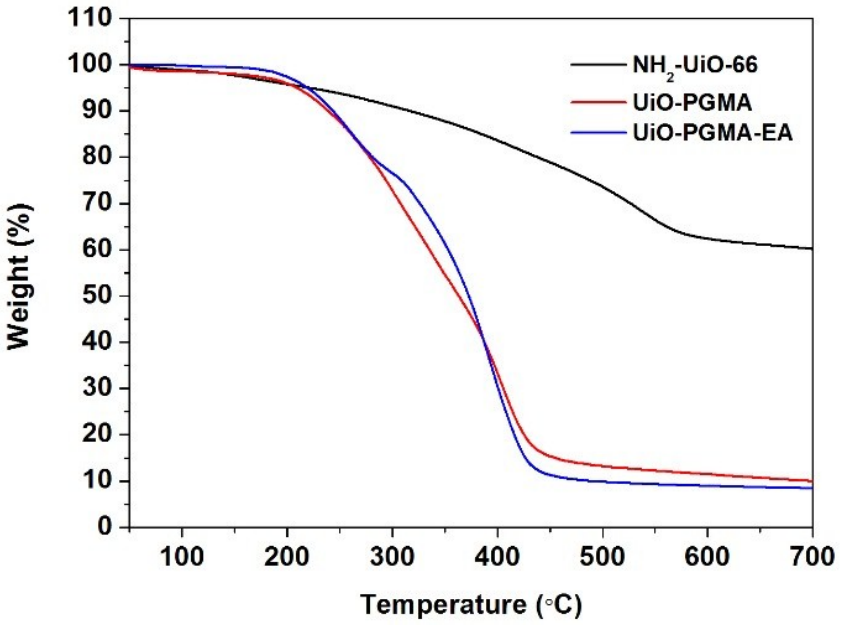

Figure S2. Thermogravimetric analysis of (1) NH_2-UiO-66 (2) UiO-PGMA and (3) UiO-PGMA-EA.

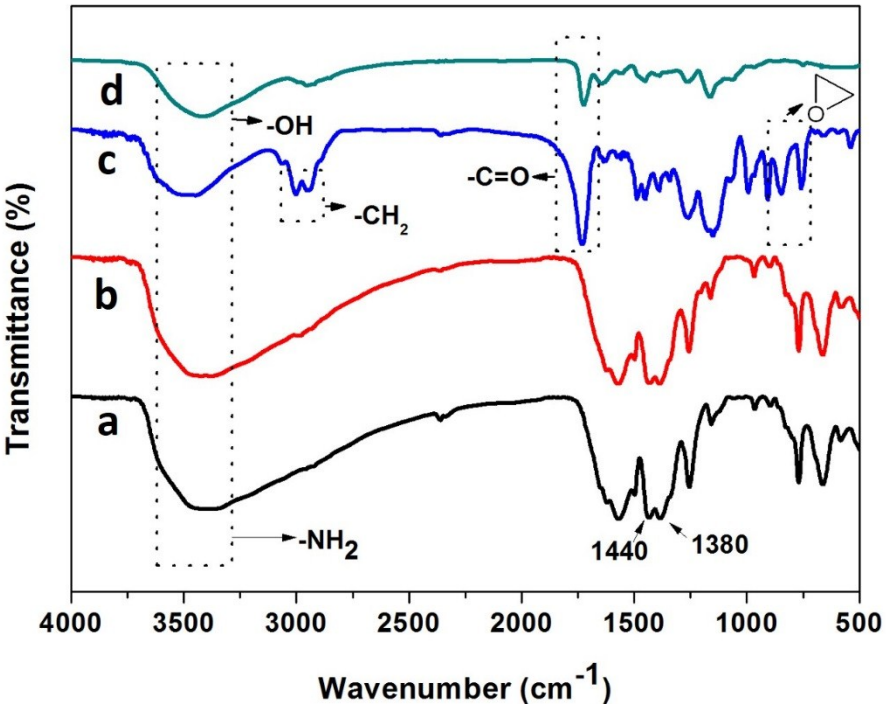

Figure S3. FT-IR spectra of NH_2-UiO-66 (a), UiO-BiBB (b), UiO-PGMA (c) and UiO-PGMA-EA (d).

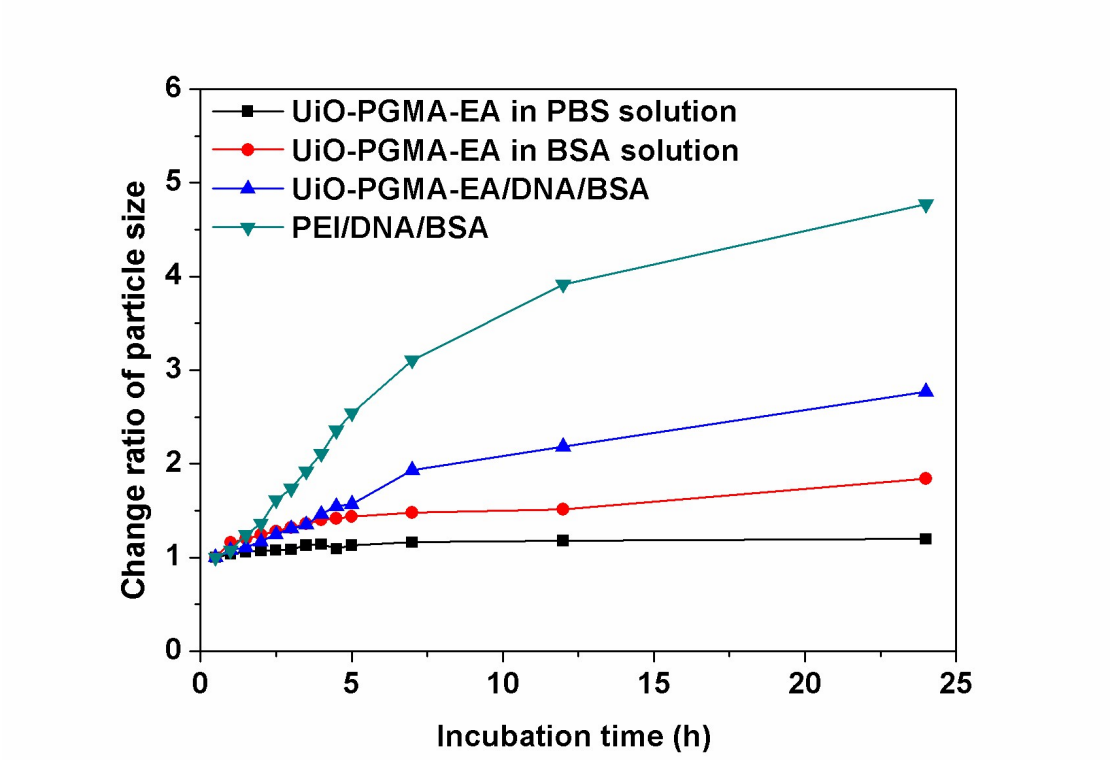

Figure S4. Stability of UiO-PGMA-EA and UiO-PGMA-EA/pDNA system at simulate physiological environment.

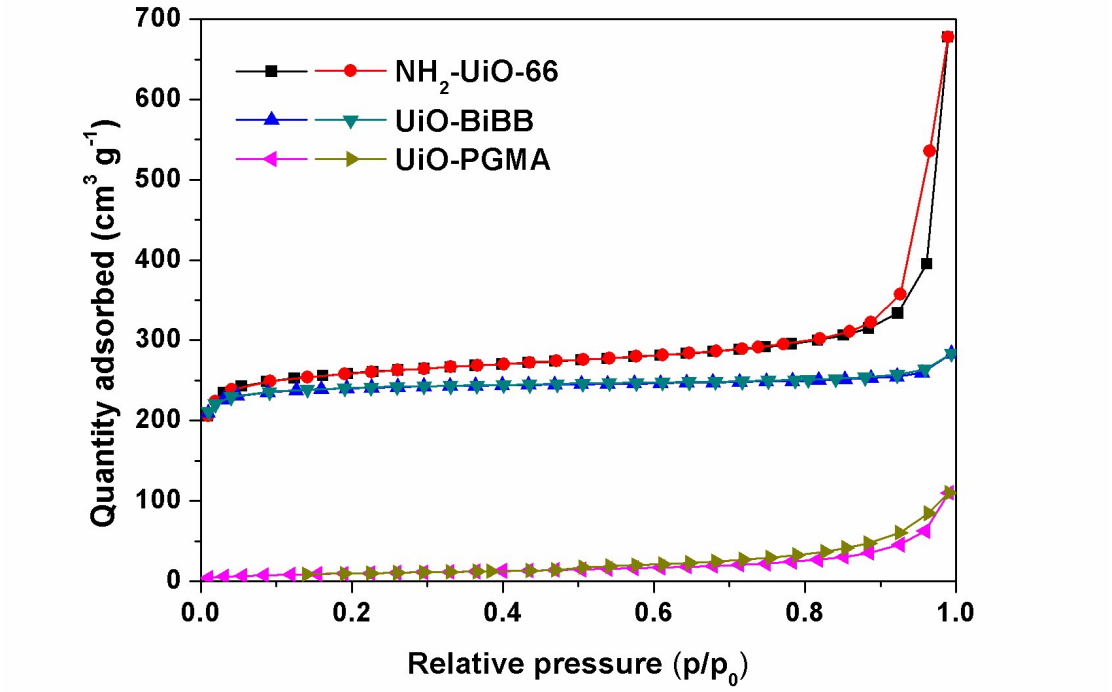

Figure S5. Nitrogen sorption isotherm of NH_2-UiO-66, UiO-BiBB and UiO-PGMA.

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