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# Electronic Supplementary Information (ESI)

for

# Fabrication of aminated poly(glycidyl methacrylate)sbased polymers for co-delivery of anticancer drug and p53 gene

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#### **Experimental section**

Materials. The following agents were purchased and used without further purification: ethanol amine (EA, 99%, Adamas), 1-amino-2-propanol (AP, 92%, TCI) N-(2hydroxyethyl)ethylenediamine (HA, 99%, Alfa Aesar), agarose (99%, Sinopharm Chemical Reagent), pyrene (99%, Sigma-Aldrich), 2-hydroxyethyl disulfide, (Sigma-Aldrich), 4dimethylamino pyridine (DMAP, 99%, 9 Ding chemistry), N,N'-diisopropylcarbodiimide (DIC, 98%, Aladdin), poly(ethylene glycol) methacrylate (PEGMA,  $M_{\rm p}$ =475 g/mol, 99%, Sigma-Aldrich), doxorubicin hydrochloride (DOX·HCl, 99%, Beijing Zhongshuo Pharmaceutical Technology Development), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide (MTT, 98%, Sigma-Aldrich). Azodiisobutyronitrile (AIBN, A. R., Enox) was purified by recrystallization from ethanol. ɛ-Caprolactone (ɛ-CL, 92%) was purified using reduced pressure distillation. Glycidyl methacrylate (GMA, 99%, Sigma-Aldrich) was passed through a column of activated basic alumina to remove the inhibitors. Milli-Q water (18.2 M $\Omega$  cm at 25 °C) was generated using a water purification system (Simplicity UV, Millipore). The other part including solvents such as dimethyl N,N-dimethylformamide (DMF) was dried over sodium sulfate anhydrous (Na<sub>2</sub>SO<sub>4</sub>, 99%, Enox) for 24 h and distilled before use. Dichloromethane  $(CH_2Cl_2)$  were dried over  $CaH_2$  and purified before use.

**Characterizations.** <sup>1</sup>H NMR was recorded on the 400 MHz spectrometer (INOVA-400) using deuterated chloroform (CDCl<sub>3</sub>)/deuterium oxide (D<sub>2</sub>O) as the solvent and tetramethylsilane (TMS) as internal standard. The number-average molecular weight ( $M_n$ ) and polydispersity index (D) of PGMA, PCL-*ss*-CPDB and PCL-*ss*-P(PEGMA-*co*-GMA) using a Waters 1515 gel permeation

chromatography (GPC) instrument with a PL gel 5.0  $\mu$ m bead size guard column (50 × 7.5 mm), followed by two linear PL gel columns (500 Å and Mixed-C), and a differential refractive index detector. THF was used as the eluent at a flow rate of 1.0 mL min<sup>-1</sup> at 30 °C.

#### Synthesis of PGMA homopolymer.

The PGMA hopolymer was synthesized *via* RAFT polymerization. All magnetic stirring bars and glass ware used in the experiments were dried at 120 °C for 24 h and cooled under vacuum to eliminate the moisture before use. Briefly, CPDB (50 mg, 0.18 mmol), GMA (1.996 g, 14.1 mmol), and azobis(isobutyronitrile) (AIBN, 14.24 mg, 0.009 mol) were added into the flask together with 8 mL of DMF. After being degassed through three exhausting-refilling nitrogen cycles, the mixture was stirred under a nitrogen atmosphere at 70 °C for 12 h. Then, the flask was cooled to terminate the polymerization, the raw product was subsequently dialyzed (MWCO 7000) against Milli-Q water for 24 h, with the purpose of removing the unreacted monomers. The final product PGMA homopolymer was obtained by lyophilization method. (Yield: 88.8%)

#### Synthesis of PGEA, PGAP, PGDA and PGHA homopolymers.

PGEA, PGAP, PGDA and PGHA homopolymers were obtained by postmodification with ethanol amine (EA), 1-amino-2-propanol (AP) 3-(dibutylamino)propylamine (DA) and *N*-(2hydroxyethyl)ethylenediamine (HA), respectively. For example, synthesis of PGHA: 120 mg of PGMA and 0.5 mL of HA were added into flask with 5 mL of DMF, and then was carried out in an oil bath at 70 °C for 6 h. The crude products were dialyzed against deionized water with dialysis membrane (MWCO 7000 Da) against Milli-Q water for 24 h. The final product PGHA homopolymer was obtained by lyophilization menthod. (Yield: 95.8%). PGEA and PGAP were obtained using similar method. PGEA:  $\delta$  4.07 ppm (c,  $-COCH_2CH(OH)-$ ),  $\delta$  3.73 ppm (b,  $-CH_2NHCH_2CH_2OH$ ), δ 3.73 (b', -COCH<sub>2</sub>C<u>*H*</u>(OH)-), and δ 3.73 ppm (a, -CH<sub>2</sub>NHC<u>*H*<sub>2</sub>CH<sub>2</sub>OH); PGAP: δ 3.95-4.04 ppm (c+b, -COC<u>*H*<sub>2</sub>C<u>*H*</u>(OH)-, δ 2.66 ppm (-(OH)CHC<u>*H*<sub>2</sub>NHC<u>*H*<sub>2</sub>-), and δ 1.18 ppm (-NHCH<sub>2</sub>CH(OH)C<u>*H*</u><sub>3</sub>); PGHA: δ 4.06 ppm (c, -COC<u>*H*<sub>2</sub>CH(OH)-), δ 3.72 ppm (b, -(OH)C<u>*H*</u>CH<sub>2</sub>NH-), δ 3.72 ppm (b', -NHCH<sub>2</sub>C<u>*H*</u><sub>2</sub>OH), and δ 2.81 ppm (a, -C<u>*H*</u><sub>2</sub>NHC<u>*H*</u><sub>2</sub>C<u>*H*</u><sub>2</sub>NHC<u>*H*</u><sub>2</sub>CH<sub>2</sub>OH).</u></u></u></u></u>

#### Synthesis of CPDB-ss-OH

The CPDB-ss-OH double-head initiator was prepared by esterification reaction. A flask was charged with 2-hydroxyethyl methacrylate (5.46 g, 35.4 mmol), DIC (0.88 g, 7.0 mmol), DMAP (214 mg, 1.75 mmol), and 10 mL of CH<sub>2</sub>Cl<sub>2</sub>. This suspension was stirred until the solids dissolved. Then, the solution of CPDB (1.0 g, 3.5 mmol) was added dropwise into the flask, which was further stirred at 30 °C for 24 h. The produced white solids were removed by filtration. Subsequently, CH<sub>2</sub>Cl<sub>2</sub> was removed by rotary evaporation. The raw product was purified by column chromatography with mixing solvent of CH<sub>2</sub>Cl<sub>2</sub>/ethyl acetate (5/1, v/v), removal of the solvent by rotary evaporation and drying in a vacuum oven at 50 °C for 24 h. A reddish brown product was obtained. (CPDB-ss-OH, 0.95 g, yield: 61.9%) HO-ss-*i*BuBr: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>,  $\delta$  ppm), 4.40 (g, –(O)COC<u>H</u><sub>2</sub>CH<sub>2</sub>S–), 3.89 (i, –SCH<sub>2</sub>C<u>H</u><sub>2</sub>OH), 2.87-2.96 (h+h<sup>2</sup>, –CH<sub>2</sub>C<u>H</u><sub>2</sub>SSC<u>H</u><sub>3</sub>CH<sub>2</sub>–), 2.62-2.73 (e+f, –(CN)C(CH<sub>3</sub>)C<u>H</u><sub>2</sub>C<u>H</u><sub>2</sub>–), 2.456 (l, –SCH<sub>2</sub>CH<sub>2</sub>O<u>H</u>), and 1.95 (d, –CN)C(C<u>H</u><sub>3</sub>)CH<sub>2</sub>–).

#### Synthesis of PCL-ss-CPDB homopolymer

The macroinitiator PCL-*ss*-CPDB was prepared by ROP with different feed ratios of  $\varepsilon$ -CL using Sn(Oct)<sub>2</sub> as catalyst. A typical procedure was carried out as follows: To a dried round flask, CPDB-*ss*-OH (51.3 mg, 0.122 mmol) was added into flask, the water of CPDB-*ss*-OH was further removed by toluene azeotrope method. Then,  $\varepsilon$ -CL (698 mg, 4.84 mmol) and Sn(Oct)<sub>2</sub> (24.6 mg,

0.061 mmol) were added under a nitrogen atmosphere. The reaction mixture was kept under stirring at 110 °C for 6 h. After the polymerization, the product was precipitated three times in diethyl ether. Finally, the precipitate was collected and dried under vacuum to a constant weight at 40 °C. (PCL-*ss*-CPDB, 453.5 mg, yield: 65.9%) PCL<sub>40</sub>-*ss*-CPDB:  $\delta$  4.40 ppm (f, -(O)COC<u>H</u><sub>2</sub>CH<sub>2</sub>S-),  $\delta$  3.67 ppm (f', -SCH<sub>2</sub>C<u>H</u><sub>2</sub>OH),  $\delta$  2.93 ppm (e, -CH<sub>2</sub>C<u>H</u><sub>2</sub>SSC<u>H</u><sub>2</sub>CH<sub>2</sub>-),  $\delta$  2.62-2.73 ppm (g+h, -(CN)C(CH<sub>3</sub>)C<u>H</u><sub>2</sub>C<u>H</u><sub>2</sub>-),  $\delta$  1.95 ppm (i, -(CN)C(C<u>H</u><sub>3</sub>)CH<sub>2</sub>-),  $\delta$  4.06 ppm (d, -(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)<sub>n</sub>-OH),  $\delta$  2.31 ppm (c, -(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)<sub>n</sub>-OH),  $\delta$  1.65 ppm (b, -(CH<sub>2</sub>C<u>H</u><sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)<sub>n</sub>-OH),  $\delta$  1.38 ppm (a, -(C<u>H</u><sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>)<sub>n</sub>-OH).

#### Synthesis of PCL-ss-P(PEGMA-co-GMA) copolymer

The PCL-ss-P(PEGMA-co-GMA) copolymer was synthesized via RAFT polymerization. All magnetic stirring bars and glass ware used in the experiments were dried at 120 °C for 24 h and cooled under vacuum to eliminate the moisture before use. Briefly, PCL-ss-CPDB (100 mg, 0.020 mmol), GMA (282 mg, 1.98 mmol), PEGMA (136.mg, 0.29 mmol) and azobis(isobutyronitrile) (AIBN, 3.2 mg, 0.020 mol) were added into the flask together with 8 mL of DMF. After being degassed through three exhausting-refilling nitrogen cycles, the mixture was stirred under a nitrogen atmosphere at 70 °C for 12 h. Then, the flask was cooled to terminate the polymerization, the raw product was subsequently dialyzed (MWCO 12000~14000 Da) against Milli-Q water for 48 h, with the purpose of removing the unreacted monomers. The final product PCL-ss-P(PEGMA-co-GMA), yield: 58.6%)

The polymerization degree of PCL-*ss*-CPDB was calculated according to the <sup>1</sup>H NMR analysis by the following eqn S1.

$$\frac{A_i}{A_d} = \frac{3}{2x}$$
(S1)

where  $A_i$  and  $A_d$  were the integral values of the peaks i and d in Fig. 7, respectively.

The polymerization degrees of PCL-*ss*-P(PEGMA-*co*-GMA) were calculated according to the <sup>1</sup>H NMR analysis by the following eqn (S2) and (S3):

$$\frac{A_{\rm h}}{A_{\rm d}} = \frac{2x}{z} \tag{S2}$$

$$\frac{A_{\rm h}}{A_{\rm e}} = \frac{2x}{3y} \tag{S3}$$

where  $A_h$ ,  $A_d$ , and  $A_e$  were the integral values of the peaks h, d, and e in Fig. 8, respectively. The x, y, and z represent the polymerization degrees of  $\epsilon$ -CL, GMA and PEGMA, respectively.

## Synthesis of PCL-ss-P(PEGMA-co-GHA) copolymer

PCL-ss-P(PEGMA-co-GHA) copolymers were obtained by post-modification with N-(2hydroxyethyl)ethylenediamine (HA). Briefly, 100 mg of PGMA and 0.5 mL of HA were added into flask with 8 mL of DMF, and then was carried out in an oil bath at 70 °C for 10 h. The crude products were dialyzed against deionized water with dialysis membrane (MWCO 12000~14000 Da) against Milli-Q water for 24 h. The final product PCL-ss-P(PEGMA-co-GHA) copolymer was obtained by lyophilization menthod. (Yield: 91.5%).

# Self-assembly behavior of PCL-ss-P(PEGMA-co-GHA) (PGHAP).

The critical aggregation concentration (CAC) value was determined by the fluorescence probe method using pyrene as the hydrophobic probe. Typically, a predetermined pyrene solution in acetone was added into a series of ampoules, respectively. Acetone was evaporated and replaced with PGHAP nanoparticles at different concentrations in the range of 500 to  $2 \times 10^{-4}$  mg L<sup>-1</sup>. The final concentration of pyrene in each ampoule was  $6 \times 10^{-6}$  mol L<sup>-1</sup>. The samples were sonicated for 20 min, stirred at room temperature for 48 h, and analyzed on fluorescence spectrophotometer (Cary Eclipse, Agilent Technologies) at the excitation wavelength of 335 nm and an emission wavelength of 350 to 550 nm, with both bandwidths set at 2.5 nm. From the pyrene emission spectra, the intensity ratio ( $I_3/I_1$ ) of the third band (382 nm,  $I_3$ ) to the first band (371 nm,  $I_1$ ) was analyzed as a function of polymer concentration. The CAC value was defined as the point of intersection of the two lines in the plot of fluorescence versus polymer concentration.

The average particle size  $(\overline{D}_z)$  and size polydispersity index (size PDI) of the polymeric micelles and DOX-loaded micelles were determined using DLS instrument (Zetasizer Nano ZS, Malvern), while the morphology was observed on a TEM instrument (HT7700, Hitachi) operated at 120 kV.

## Preparation of PCL<sub>40</sub>-ss-P(PEGMA<sub>7</sub>-co-GHA<sub>80</sub>) (PGHAP) nanoparticles

Samples for DLS and TEM were prepared as follows:  $PCL_{40}$ -ss- $P(PEGMA_7$ -co- $GHA_{80})$  (12.5 mg) was dissolved in DMSO (2 mL), and subsequently, Milli-Q water (15 mL) was added dropwise of 2 mL/h by micro-injection pump at room temperature. Then, the mixture was subsequently dialyzed (Molecular weight cut-off:  $12 \sim 14$  kDa) against Milli-Q water for 24 h, and nanoparticle could be obtained by diluting with Milli-Q water. The concentration of polymeric nanoparticles is 0.5 mg L<sup>-1</sup>. It can be named PGHAP nanoparticle.

#### **Encapsulation of DOX**

DOX-loaded PGHAP nanoparticle was prepared by a similar procedure to that for blank PGHAP nanoparticle. In brief, DOX was loaded into nanoparticle by the dialysis method. Briefly, 12.5 mg of copolymer was dissolved in 2 mL of DMSO in a 50 mL round-bottomed flask, followed by adding 2 mL of DOX/DMSO stock solution. 10 mL of Milli-Q water was then added dropwise under moderate stirring, followed by dialysis (Molecular weight cut-off: 12~14 kDa) against Milli-Q water for 24 h at room temperature to remove DMSO and free DOX. Finally, the DOX-loaded PGHAP nanoparticle solution was diluted to 25 mL with Milli-Q water to a desired concentration.

The drug loading content (DLC) and drug loading efficiency (DLE) were determined by UVvis spectrophotometry at 480 nm. To determine the drug loading level, a small portion of DOXloaded micelles was withdrawn and diluted with DMF to a volume ratio of DMF/H<sub>2</sub>O = 9/1. The amount of DOX encapsulated was quantitatively determined by UV-vis and the calibration curve used for drug loading characterization was established by the intensity of DOX with di□erent concentrations in DMF/H<sub>2</sub>O=9/1 (v/v) solutions. The DLC was defined as the weight ratio of entrapped DOX to that of the DOX-loaded micelles. The DLE of DOX was obtained as the weight ratio between DOX incorporated in assembled micelles and that used in fabrication.

$$DLC(wt\%) = \frac{\text{weight of loaded drug}}{\text{weight of polymer}} \times 100$$
(S4)  
$$DLE(wt\%) = \frac{\text{weight of loaded drug}}{\text{weight of drug in feed}} \times 100$$
(S5)

The *in vitro* DOX release profiles from the DOX-loaded PGHA nanoparticle were evaluated in a phosphate buffer solution (pH 7.4, pH 7.4+10 mM GSH), then placed in a dialysis bag (Molecular weight cut-off:  $12\sim14$  kDa). The whole bag was placed into 30 mL PB buffer and shaken (160 rpm) at 37 °C.

At specified time intervals 5 mL ( $V_e$ ) samples were taken and an equal volume of fresh buffer added to maintain the total volume. A fluorescence spectrophotometer with excitation at 480 nm and slit width at 5 nm over a wavelength range from 520 to 650 nm was carried out to determine the content of the released DOX. The cumulative release  $(E_r)$  was calculated using eqn S6.

$$E_r(\%) = \frac{V_e \sum_{1}^{n-1} C_i + V_o C_n}{m_{DOX}} \times 100$$
 (S6)

where  $m_{DOX}$  represents the amount of DOX in the micelle,  $V_0$  is the volume of the release medium ( $V_0 = 30 \text{ mL}$ ), and  $C_n$  represents the concentration of DOX in the nth sample. The *in vitro* release experiments were carried out in triplicate and the reported results were the average values with standard deviations.

PGEA/p53 gene, PGAP/p53 gene, PGHA/p53 gene, and PGHAP/p53 gene complexes at various weight ratios were formed by adding same amount of p53 gene into PGEA, PGAP, PGHA solution, and PGHAP nanoparticle at different concentrations and vortexing for 10 s. The complexes were then allowed to stand at room temperature for 30 minutes. The N/P ratio referred to the weight ratio of the copolymer to p53 gene in this study, and the calculation was performed based on Eqn S7.

N/P ratio=
$$\frac{M_1}{M_{p53}}$$
 (S7)

where  $M_1$  represent the weight of cationic polymer,  $M_{p53}$  represent the weight of p53 gene.

The average diameter, particle size distribution (size PDI) and zeta potential of the PGEA/p53 gene, PGAP/p53 gene, PGHA/p53 gene, PGHA/p53 gene, PGHAP, DPGHAP nanoparticles and DPGHAP/p53 gene complex were characterized by a dynamic light scattering instrument (DLS) (Zetasizer Nano ZS, Malvern, 90° collecting optics) at 25°C. The morphologies of PGHAP and DPGHAP nanoparticles were observed using a transmission electron microscope (TEM) instrument (HT7700, Hitachi) operated at 120 kV.

#### **Cell and Gene Culture**

L929 cells (mouse fibroblasts cells), A549 cells (human lung cancer cells) and H1299 cells (human lung cancer cells, derived from metastatic state, absence of p53 gene) were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin Solution at humidified atmosphere of 37 °C and 5% CO<sub>2</sub>. p53 gene, and p53-GFP gene were from Dr. Guanghui Wang at the College of Pharmaceutical Science of Soochow University.

## In Vitro Cytotoxicity Assay

First, a standard MTT assay was employed to evaluate the cytotoxicity of several PGEA, PGAP, PGHA solution, and PGHAP polymeric nanoparticle against L929 cells, A549 cells and H1299 cells. Afterwards, a standard MTT assay was employed to evaluate the cytotoxicity of PGEA/p53, PGAP/p53, PGHA/p53, PGHAP/p53, and DPGHAP/p53, using free DOX and free p53 gene as controls, respectively. A549 cells and H1299 cells were seeded into 96-well plates at a density of  $6 \times 10^3$  cells per well in 100 µL of RPMI 1640 medium containing 10% serum and 1% penicillin-streptomycin solution, and incubated under the humidified atmosphere of 37 °C and 5% CO2 for 12 h. Then the nanoparticles at different concentrations were added into wells and incubated with another 48 h. Then, 25 µL of MTT solution (5 mg mL<sup>-1</sup> in PBS) prepared in advanced was added into each well. After incubation for 4 h, the RPMI 1640 medium was removed and 150 µL DMSO was added into each well to dissolve the produced purple formazan. Finally, the optical density (OD) value at 570 nm of each well was measured using a microplate reader (Bio-Rad 680, USA). The optical density (OD) at 570 nm of each well was measured on a microplate reader (Bio-Rad 680). The cell viability was calculated according to the following eqn S8:

Cell viability (%) = 
$$\frac{OD_{treated}}{OD_{control}} \times 100$$
 (S8)

where  $OD_{treated}$  and  $OD_{control}$  represented the OD values of the treated wells in the presence of samples and the control wells in the absence of samples. Data were presented as the average values with standard deviations.

#### In Vitro Transfection

*In vitro* transfection was performed in A549 cells. Cells were seeded in  $\Phi$ 35 mm glass bottom cell culture dishes at the amount of 15 × 10<sup>4</sup> cells and incubated for 12 h in 1 mL of RPMI 1640 medium at 37 °C under 5% CO<sub>2</sub> atmosphere. PGEA, PGAP, and PGHA polymeric solution were blent with the DNA of green fluorescence protein (p53-GFP, 6 µg mL<sup>-1</sup> in PBS) at various N/P ratios for 30 min at 25 °C to form PGEA/p53-GFP, PGAP/p53-GFP, and PGHA/p53-GFP complexes. Subsequently, the samples were added to each well, gently mixed, and incubated for 6 h. After that, the RPMI 1640 medium containing samples were removed and 1 mL of fresh medium was added. Then the cells were incubated for 48 h at 37 °C and 5% CO<sub>2</sub> atmospheres. Finally, the results of transfection were captured by a live cell imaging system (CELL'R, Olympus).

#### **Measurement of Cellular Uptake**

The cell endocytosis and intracellular release behaviors of the nanoparticles were visualized with the live cell imaging system (CELL'R, Olympus) using A549 cells. Firstly, A549 cells were seeded in  $\Phi$  35 mm glass bottom cell culture dish at the amount of 15 × 10<sup>4</sup> cells for 12 h. Then the medium was removed and the cells were washed three times

with PBS and stained with the H 33342 (10 mg L<sup>-1</sup>) for 30 min, followed by washing with PBS three times. Subsequently, the medium was replaced by fresh RPMI 1640 medium containing the DPGHAP/p53-GFP complex (7.5 mg L<sup>-1</sup> of DOX, 5 mg L<sup>-1</sup> of p53-GFP gene). The culture dish was mounted in the incubation system of the live cell imaging system at 37 °C under 5% CO<sub>2</sub> for 6 h. The images were then captured at excitation wavelength of TRITC (red, 480 nm), DAPI (blue, 340 nm) and GREEN (520 nm, green).



Fig. S1 <sup>1</sup>H NMR spectrum of CPDB.



Fig. S2 GPC curve of PGMA.



Fig. S3 The pictures of polymeric solubility of PGDA, PGEA, PGAP, and PGHA homopolymers.



Fig. S4 FT-IR spectra of (A) PGEA, (B) PGAP, and (C) PGHA.

Element content (%)	PGEA	PGAP	PGHA
C%	67.88	69.2	69.01
N%	4.53	4.81	7.74
O%	27.59	25.99	23.25

Table S1 Element contents of PGEA, PGAP and PGHA

<sup>a)</sup> Element content of PGEA, PGAP and PGHA were measured by XPS.



Fig. S5 Zeta potentials of (A) PGEA, (B) PGAP, and (C) PGHA aqueous solution.



Fig. S6 Zeta potentials of (A) PGEA/DNA, (B) PGAP/DNA, (C) PGHA/DNA, and (D) PEI/DNA complexes at

various N/P ratios. (N/P ratio = 0, 0.5, 1, 2, 3, and 4).



**Fig. S7** Agarose gel electrophoreses of PGEA/DNA (a), PGAP/DNA (b), PGHA/DNA (c), and PEI/DNA (d) complexes at selected N/P ratio (N/P ratio=6) following incubation with negatively-charged heparin sodium at different contents. (Lane 1 is DNA control, lane 2-8 correspond to heparin sodium concentration of 0.1, 0.3, 0.5,



0.7, 0.8, 0.9, and 1.0 mg/mL, respectively).

Fig. S8 Cell viabilities of (A) A549 cells and (B) H1299 cells treated with free p53, PGEA/p53, PGAP/p53,

and PGHA/p53 complexes, respectively, at different p53 gene concentrations for 48 h of incubation.



Fig. S9 <sup>1</sup>H NMR spectrum of CPDB-ss-OH in CDCl<sub>3</sub>.



Fig. S10 <sup>1</sup>H NMR spectrum of PCL<sub>40</sub>-ss-CPDB in CDCl<sub>3</sub>.



Fig. S11 GPC traces of PCL<sub>40</sub>-ss-CPDB and PCL<sub>60</sub>-ss-CPDB polymers.



Fig. S12 GPC traces of PCL<sub>40</sub>-ss-CPDB polymer and PCL<sub>40</sub>-ss-P(PEGMA<sub>7</sub>-co-GMA<sub>80</sub>) copolymer.



Fig. S13 XPS spectra of PCL<sub>40</sub>-ss-P(PEGMA<sub>7</sub>-co-GHA<sub>80</sub>): (a) Survey scan, (b) C 1s, (c) N 1s, and (d) O 1s.



Fig. S14 Intensity ratios  $(I_3/I_1)$  as a function of Log C of PGHAP nanoparticles in Milli-Q water.



**Fig. S15** (A) agarose gel electrophoreses of DPGHAP/DNA complexes at different N/P ratios, (Lane 1 is DNA control, lane 2-7 correspond to N/P ratio of 1, 2, 3, 4, 5, and 6, respectively) and (B) agarose gel electrophoreses of DPGHAP/DNA complexes at selected N/P ratio (N/P ratio=6) following incubation with negatively-charged heparin sodium at different contents. (Lane 1 is DNA control, lane 2-7 correspond to heparin sodium concentration of 0.1, 0.3, 0.5, 0.7, 0.9, and 1.0 mg/mL, respectively)