Supporting Information for

Synthesis of PEG-Armed and Polyphosphoester Core-Crosslinked Nanogel by One-Step Ring-Opening Polymerization

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Materials. 2-Chloro-2-oxo-1,3,2-dioxaphospholane (COP) was synthesized by a method described previously,¹ and distilled under reduced pressure before use. Triethylamine was refluxed with phthalic anhydride, then with potassium hydroxide, and distilled. Triethyleneglycol (2-[2-(2-hydroxyethoxy)ethoxy]ethanol) was dried over CaH₂ over night and distilled under reduced pressure before use. Toluene and 1, 4-dioxane were refluxed over sodium, tetrahydrofuran (THF) was dried over potassium-sodium and distilled just before use. Monomethoxy poly(ethylene glycol) with Mn=5000 (mPEG₅₀₀₀, Acros Organics) was dried by azeodistillation of anhydrous toluene. Stannous octoate (Sn(Oct)₂, Sinopharm Chemical Reagent Co., Ltd., China) was purified according to a method described in literature.² Briefly, Sn(Oct)₂ was first fractionally distilled under vacuum and then azeotropic distillation with xylene under reduced pressure twice and finally subjected to a fractionated distillation again (140 °C, 20 Pa). Milli-Q water (18 MΩ) was prepared using a Milli-Q Synthesis System (Millipore, Bedford, MA, USA). The anticancer drug doxorubicin hydrochloride (DOX) is a product of Zhejiang Hisun Pharmaceutical Co, Ltd. Phosphodiesterase I from Crotalus atrox and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma Chemical Co. All other solvents and reagents were used as received.

Synthesis of 3, 6-dioxaoctan-1, 8-diyl bis(ethylene phosphate) (TEGDP). Under magnetic stirring at -5 °C, to a solution of COP (44.24 g, 0.32 mol) in 200 mL of anhydrous THF was added dropwise a mixture of triethyleneglycol (23.29 g, 0.16 mol) and triethylamine (31.42 g, 0.32 mol) in 100 mL of anhydrous THF over a period of 2 h. The mixture was maintained at -5 °C for additional 4 h. The precipitate triethylammonium chloride was then filtered off using a Schlenk funnel with dried silica gel.
The filtrate was concentrated under vacuum, washed with 50 mL of toluene twice, and dried under vacuum overnight to obtain the product TEGDP with 33% yield.

\[ ^1\text{H-NMR}, \quad ^{13}\text{C-NMR}, \quad ^{31}\text{P-NMR} \] spectra shown in Figure 1S supported the structure. \[^1\text{H-NMR}\ \text{(in CDCl}_3\text{, ppm)}: 4.36 \text{ (a, 8H, -OCH}_2\text{CH}_2\text{OP-)}, 4.15 \text{ (b, 4H, -POCH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-)}, 3.67 \text{ (c, 4H, -POCH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-)}; \quad ^{13}\text{C-NMR}\ \text{(in CDCl}_3\text{, ppm)}: 66.21 \text{ (a, -OCH}_2\text{CH}_2\text{OP-)}, 67.83 \text{ (b, -POCH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-)}, 70.07 \text{ (c, -POCH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-)}, 70.68 \text{ (d, -POCH}_2\text{CH}_2\text{O-CH}_2\text{CH}_2\text{O-)}; \quad ^{31}\text{P-NMR}\ \text{(in CDCl}_3\text{, ppm)}: 13.84. \]

**Synthesis of the nanogel.** mPEG\textsubscript{5000} (2.5 g, 0.5 mmol) and TEGDP (1.8 g, 5 mmol) and 5 mL dioxane were added into a fresh flamed and nitrogen purged round-bottomed flask in a glove box with H\textsubscript{2}O and O\textsubscript{2} contents less than 0.1 ppm, then Sn(Oct\textsubscript{2}) (20 mg, 0.05 mmol) was added. The mixture was allowed to maintain at 80 °C for 12 h. The product was dried under vacuum and dissolved in distilled water. It was further dialyzed (Spectra/Por\textsuperscript{®}, MWCO=15,000) against Milli-Q water for 3 days. The nanogel was obtained after lyophilization.

**Monomer conversion determination.** Monomer conversion was monitored according to a method similar to that for EEP conversion determination in our previous study.\textsuperscript{3} Samples (50 µL) were collected and diluted with 1 mL of chloroform, filtered with 100 nm of membrane filter, then immediately analyzed by gel permeation chromatography. The GPC system is composed of a Waters 1515 pump and a Waters 2414 refractive index detector equipped with Waters Styragel\textsuperscript{®} High Resolution columns (1×HR4, 1×HR2 and 1×HR1, effective molecular-weight range 5 000-500 000, 500-20 000, 100-5 000, respectively). Chloroform was used as mobile phase at the flow rate of 1 mL min\textsuperscript{-1} at 40 °C. The monomer concentrations at different reaction time were determined by height comparison of peak with an elution volume at 28.2 mL to a standard curve with known EEP concentrations using Waters Breeze HPLC software.

**Characterization of the nanogel.** Bruker AV300 NMR spectrometer was used for \[^1\text{H}, \quad ^{13}\text{C} \] and
$^{31}$P-NMR spectra measurements. Deuterated chloroform ($\text{CDCl}_3$) containing 0.03 v/v % tetramethylsilane (TMS) was used as the solvent. Phosphoric acid (85%) was used as the external reference for $^{31}$P-NMR analyses. Elementar Vario ELIII was used to measure the content of C and H elements of the nanogel. AAnalyst 800 spectrometer was used to measure the content of P element of the nanogel.

The size and size distribution of the nanogel were measured by dynamic light scattering (DLS) carried out on a Malvern Zetasizer Nano ZS90 with a He-Ne laser (633 nm) and 90° collecting optics. Sample was prepared at a concentration of 1 mg mL$^{-1}$. Measurements were carried out at 25 °C, and data were analyzed by Malvern Dispersion Technology Software 4.20.

Transmission electron microscopy (TEM) was performed on a Hitachi model H-800 transmission electron microscope with an accelerating voltage of 200 KV. The samples were prepared by pipetting a drop of the nanogel in aqueous solution (1 mg mL$^{-1}$) onto a 230 mesh copper grids coated with carbon and allowing drying in air before measurements.

**Pyrene Fluorescence Measurements.** The pyrene stock solution prepared with acetone was added into volumetric flasks, then the acetone was evaporated and subsequently a predetermined amount of nanogel stock solutions and Milli-Q water were added to each flask, while the concentrations of nanogel were ranging form $3.90 \times 10^{-3}$ to 1 mg mL$^{-1}$ and the concentration of pyrene was fixed at $6.0 \times 10^{-7}$ mol L$^{-1}$. The excitation spectra were recorded at 20 °C on a Shimadzu RF-5301PC spectrofluorophotometer with $\lambda_{em}$ at 390 nm and a slit width of 3 nm.

**Doxorubicin hydrochloride loading and release.** DOX was loaded into nanogel by directly mixing nanogel (10 mg mL$^{-1}$) with DOX at various concentrations (1 and 5 mg mL$^{-1}$) in Milli-Q water. The mixture was stirred for two days. The DOX-loaded nanogel was collected by centrifugation at 37,000 g for 30 min. The concentration of DOX in the supernatant was determined by HPLC analyses. The content of DOX loaded into nanogel was calculated by subtracting the amount of DOX in supernatant.
from the total amount used for loading.

HPLC analyses were performed using a Waters HPLC system consisting of Waters 1525 binary pump, Waters 2475 fluorescence detector, 1500 column heater and a Symmetry C18 column. HPLC grade acetonitrile-water (50:50, v/v) with pH 2.7 adjusted by HClO$_4$ was used as the mobile phase at 30 °C with a flow rate of 1.0 mL min$^{-1}$. Fluorescence detector was set at 460 nm for excitation and 570 nm for emission and linked to Breeze software for data analysis. Linear calibration curves for concentrations in the range of 6.25-100 µg/mL were constructed using the peak areas by linear regression analysis.

The drug loading content (DLC) and efficiency (DLE) were calculated by the following equations:

$$DLC\% = \frac{\text{amount of DOX in nanogel}}{\text{amount of DOX - loaded nanogel}} \times 100\%$$

$$DLE\% = \frac{\text{amount of DOX in nanogel}}{\text{total amount of DOX for drug loading}} \times 100\%$$

Release profiles of DOX from the nanogel in vitro were studied in a release medium (0.02 mol L$^{-1}$ phosphate buffered saline containing 0.01 mol L$^{-1}$ MgCl$_2$, pH 7.4,) using a dialysis membrane tubing (Spectra/Por$^\text{®}$, Float-A-Lyzer, MWCO=25,000). DOX-loaded nanogel (3 mL, with 9.10% DOX loading in weight) dissolved in the release medium at 1.0 mg mL$^{-1}$ was introduced to the dialysis membrane tubing and the tubing was placed in 22 mL of release medium and incubated at 37 °C. Phosphodiesterase I was mixed with DOX-loaded nanogel in the tubing at a final concentration of 5 unit L$^{-1}$ (to study the effect of enzyme. At predetermined intervals, release medium was collected and the tubing was replaced in an equal volume of fresh medium. The concentration of DOX in the release medium was then measured by HPLC as described above.

**The biocompatibility test of nanogel.** The relative cytotoxicity of the nanogel was assessed with a
methyl tetrazolium (MTT) viability assay against A549 cells. Sodium dodecyl sulfate (SDS) was used a control. The cells were seeded in 96-well plate at 10,000 cells per well in 100 µL of RPMI 1640 culture medium containing 10% fetal bovine serum, and incubated at 37 °C in 5% CO₂ humidified atmosphere for 24 hours. The medium was then replaced with 100 µL of nanogel in complete culture medium at different concentrations from 0 to 10 mg mL⁻¹. The cells were further incubated for 72 h, then 25 µL of MTT stock solution (5 mg/mL in phosphate buffered saline, PBS) was added to each well to achieve a final concentration of 1 mg mL⁻¹, with the exception of the wells as blank, to which 25 µL of PBS was added. After incubation for another 2 h, 100 µL of extraction buffer (20% SDS in 50% DMF, pH 4.7, prepared at 37 °C) was added to the wells and incubated overnight at 37 °C. The absorbance was measured at 570 nm using a Bio-Rad 680 microplate reader. The cell viability was normalized to that of A549 cells cultured in complete culture medium.

A549 cells treated with 10 mg mL⁻¹ of the nanogel as above were also stained with a LIVE/DEAD Viability/Cytotoxicity Kit (Molecular Probes, L-3224) according to the protocol provided by the supplier. Live and dead cells were imaged with a Nikon TE 2000-U fluorescence microscope. Samples were excited with light of 488 nm (green emission) to show viable cells, and excited with light of 532 nm (red emission) to show the dead cells.
Figure S1. Synthesis pathway and $^1$H, $^{13}$C-NMR spectra (in CDCl$_3$, ppm) of TEGDP monomer.

Figure S2. HPLC chromatograms of aliquots from the reaction mixtures.
Figure S3. The size and size distribution of the nanogel in dioxane.

Figure S4. Pyrene fluorescence measurements at 25 °C in the presence of the nanogel at various concentrations.
