SUPPORTING INFORMATION

Temperature Sensitive Poly(phenyleneethynlenes) Nanomedicines for Intracellular Tracking via Fluorescence Resonance Energy Transfer

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1. Materials

All materials were purchased from commercial suppliers and used without further purification, unless otherwise noted. 1,4-Benzenediol, 1,4-Dibromo-butane, potassium carbonate (K$_2$CO$_3$), Iodine (I$_2$), Trimethylamine (Et$_3$N), Cuprous iodide (CuI) and Cuprous chloride (CuCl) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). (Triisopropylsilyl) acetylene, Tetraakis(triphenylphosphine) palladium (Pd(PPh$_3$)$_4$) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl 2H-terazolium bromide (MTT) were purchased from J&K Scientific Ltd. (Beijing, China). [Bis(trifluoroacetoxy) iodo] benzene (Ph[I(CF$_3$COO)$_2$]), Tetrabutylammonium fluride (N$^+$(C$_4$H$_9$)$_4$F$^-$) and N-isopropylacrylamide (NIPAM) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). NIPAM was recrystallized from hexane before use. Tris(2-dimethylaminoethyl)amine (Me$_6$TREN) was purchased from Alfa Aesar Chemical Co.Ltd. (Ward Hill, MA, USA).

Dulbecco’s modified eagle medium (DMEM), fetal bovine serum (FBS), trypsin and penicillin/streptomycin were obtained from Gibco BRL (Gaithersburg, MD, USA). Doxorubicin hydrochloride (DOX $\cdot$ HCl), 4,6-diamidino-2-phenyl-indole (DAPI) and LysoTracker Deep Red fluorescence dye were purchased from Huafeng United Technology Co., Ltd (Beijing, China), Beyotime Co., (Shanghai, China) and Invitrogen (Carlsbad, Canada) respectively. The human hepatocellular carcinoma cell line HepG2 was purchased from China Center for Type Culture Collection (Wuhan, China). Milli-Q (Millipore) deionized (DI) water was used during the whole experiment.

2. Characterization

The molecular composition and structure of PPE and pNE were characterized using a $^1$H-NMR spectrometer (600 MHz, AV400, Bruker, Switzerland), and DMSO-d$_6$ was used as deuterated solvent. Molecular weight and distribution was measured with Gel Permeation Chromatography (GPC, Viscotek 270, Malvern Co. Ltd., England). The samples were dissolved in dimethyl sulfoxide at the concentration of 5.0 mg/mL. Polystyrene (PS 99K, $M_n = 99,152$ Da; $M_w = 97,241$ Da) and dimethyl sulfoxide were used as standards and mobile phase respectively. Flowing rate was 1.0 mL/min, and column temperature was 35 $^o$C. The spectra of UV and fluorescence were recorded on Puxi TU-1901 spectrometer and Hitachi F-4500 fluorescence spectrometer equipped with a xenon lamp respectively. The size and zeta potential of PPE and pNE NPs (50 $\mu$g/mL), which was ultrasonically oscillated for 5.0 min, were determined at room temperature by dynamic light scattering (DLS, Zetasizer Nano ZS90, Malvern Instruments Ltd, UK) equipped with a 4 mW He-Ne laser source ($\lambda = 633$ nm) with a scattering angle of 90°. The morphologies of pNE NPs and Dox-pNE NPs were characterized using a transmission electron microscope (TEM, 200 kV, Tecnai G2 20, FEI Corp., Netherlands). The samples were diluted to 50 $\mu$g/mL with ultrapure water. After ultrasonic oscillating for 5 minutes, 5 $\mu$L of the dilution was dropped onto a TEM copper grid (carbon film coated, 300-mesh), and dried at room temperature for TEM characterization. The AFM image was taken in ScanAsyst mode of Nanoscope Multimode 8 (Bruker, Santa Barbara, USA) to investigate microtopography of pNE NPs. One drop of the dispersions was placed on the surface of freshly cleaved mica and then dried for AFM measure.

3. Synthesis of poly(phenyleneethynylene) (PPE)

As reported before,$^{1,2}$ compound 1-5 were synthesized in term of the synthetic route in Figure S1. As a classical $\pi$-conjugated polymer, PPE was synthesized via so-called Sonogashira coupling between compound 3 and 5, and Pd(PPh$_3$)$_4$ and CuI were used as co-catalysts. In brief, 3 (60 mg, 0.149 mmol) and 5 (113.4 mg, 0.149 mmol) were dissolved in a mixed solution including 10 mL of water and 10 mL of DMF, and then heated to 75 $^o$C under argon atmosphere. In order to initiate sonogashira coupling reaction, 10 mL of mixed solution of DMF and triethylamine (1:1, v/v), in which contained Pd(PPh$_3$)$_4$ (17.2 mg, 0.015 mmol) and CuI (4.3 mg, 0.024 mmol), were rapidly injected into the above solution with a syringe under argon atmosphere. After 24 h of polymerization at 75 $^o$C, the reactive mixture was poured into 100 mL of MeOH/acetone (4:1 v/v) to obtain crude PPE precipitations. After
4. **Synthesis of poly(N-isopropylacrylamide)-grafted poly (phenyleneethynylene) (pNE)**

Using the resultant PPE as macro-initiator, poly(N-isopropylacrylamide)-grafted poly (phenyleneethynylene) (pNE) was synthesized by atom transfer radical polymerization (ATRP) of NIPAM monomers. In brief, PPE (50 mg, ca. 8.3 μmol), NIPAM (0.973 g, 8.6 mmol) and H₂O (4 mL) were added in a Schlenk tube, while stirring. Afterwards, three times of freezing-pumping-thawing process were performed to removing oxygen in the solutions. That is, the solutions were firstly frozen with liquid nitrogen for 5 min, degassed by a pump, and subsequently put at room temperature for thawing. Me₆TREN (46.5 μL, 0.172 mmol) and CuCl (17.0 mg, 0.172 mmol) was added respectively in the first thawing and the second freezing stages of sample solution. After the polymerization was carried out at 50 °C for 72 h, the reaction solution were dialyzed (MWCO 3500) against DI water for 3 days. pNE powders (ca. 590 mg) were obtained via lyophilization of the dialysis solution, and kept in dark for further use.

5. **Assembly of pNE nanoparticles and Doxorubicin loading**

pNE nanoparticles (pNE NPs) were prepared firstly by solvent diffusion method. In brief, 10 mL of THF was used to dissolve pNE powders (20 mg, 0.28 μmol), and then added dropwise to DI water (20 mL) under ultrasound. After rotary evaporation, the resultant pNE NP dispersions were dialyzed (COMW 3500) with DI water for 2 days to remove residual THF. In order to prepare Doxorubicin-loading pNE NPs (Dox-pNE NPs), Dox·HCl were dissolved in pNE NP dispersions, and then triethylamine was slowly added dropwise to the mixtures of Dox·HCl and pNE NPs, while stirring. The unloaded doxorubicins were removed from the mixtures with the method of ultrafiltration (MWCO 3000, 10000 rpm×15 min×3 times), and measured their contents by a fluorescent spectrophotometer with 488 nm of excitation wavelength and 556 nm of emission wavelength. The drug loading content (DL) and entrapment efficacy (EE) were calculated respectively based on Equation (1) and (2):

\[
DL\% = \frac{W_f - C_f V_f}{C_n V_n} \times 100\%
\]

(1)

\[
EE\% = \frac{W_f - C_f V_f}{W_0} \times 100\%
\]

(2)

Here, \(W_0\) is the feeding amount of doxorubicin, \(C_f\) and \(V_f\) are the concentration and volume of free doxorubicin, respectively. \(C_n\) and \(V_n\) are the concentration and volume of Dox-pNE NP dispersions, respectively.

6. **In vitro Drug release**

Intracellular release of Dox from Dox-pNE NPs was performed at 37 °C with phosphate buffers as releasing media. In brief, a dialysis bag (COMW 3500), which contained 1.0 mL of Dox-pNE NP dispersions (0.25 mg of Dox contained therein), was immersed into 25 mL of phosphate buffers (6.7 mmol/L) at 37 °C while constantly shaking (120 rpm). The whole phosphate buffers were taken out at desired time intervals for fluorescence measurement, and an equal volume of fresh buffer media were added again. The concentration of Dox in releasing solutions was measured by a fluorescence spectrophotometer (F4500, Hitachi Co., Japan). The instrument parameters: \(\lambda_{ex} = 485\) nm, \(\lambda_{em} = 554\) nm and PMT Voltage was 700 V. The accumulative releasing amount of Dox (AR%) was calculated according to Equation (3):
Here, \( C_i \) and \( V_i \) are the concentration and volume of the sample solution which was taken out at time \( i \), respectively.

7. **In vitro Cytotoxicity**

*In vitro* cytotoxicity of pNE NPs was measured via MTT assay. In brief, HepG2 cells were pre-cultured with 5% CO\(_2\) atmosphere at 37 °C in DMEM solutions containing 10% fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 μg/mL). Afterwards, the cells were seeded in 96-well plates at 5000 cells per well, and incubated overnight. pNE NP dispersions with various concentrations were used to co-incubate with HepG2 cells, and the cells without treatments were set as blank control. In order to evaluate their cytotoxicity, the cells were washed with phosphate buffers, and then MTT solution (5 mg/mL, 20 μL/well) was added into every well. After an additional 4 h of incubation at 37 °C, the cells were washed again with phosphate buffers to remove extracellular MTT. The formazan crystals in living cells were dissolved with dimethyl sulfoxide (DMSO, 150 μL), and the absorbance was measured at 492 nm using a Labsystems iEMS microplate reader (Helsinki, Finland).

8. **Intracellular Drug release**

HepG2 cells were seeded in 6-well plates at 2×10^5 cells/well, and then incubated for 24 h at 37 °C and 5% CO\(_2\). The cells were treated with 100 μg/mL of Dox-pNE NPs for 4 h. The medium was removed and the cells were washed with phosphate buffered saline (PBS) twice. The cells were further incubated at 37 °C for a certain period of time, and then the images were taken by Olympus FV1000 confocal microscope (Olympus, Tokyo, Japan) with excitation at 405 nm (460-510 nm for pNE channel), excitation at 488 nm (530-580 nm for Dox channel) and excitation at 635 nm (655-755 nm for Lysotracker deep red channel).

9. **Additional Figures**

![Synthetic routes for compound 3 and compound 5.](image)
**Fig. S1.** Dox loading capacity and encapsulating efficiency of Dox-pNE NPs

**10. References**