SCIENCE CHINA
Chemistry

• ARTICLES •
• SPECIAL TOPIC • Bio-based and Biodegradable Polymers

November 2016 Vol.59 No.11: 1397–1404
doi: 10.1007/s11426-016-0230-9

Polymeric prodrug for bio-controllable gene and drug co-delivery

Huizhen Jia[1,2], Si Chen[1], Renxi Zhuo[1], Jun Feng[1*] & Xianzheng Zhang[1*]

[1]*Key Laboratory of Biomedical Polymers of Ministry of Education; Department of Chemistry, Wuhan University, Wuhan 430072, China*
[2]*School of Materials Science and Engineering, Tianjin Key Laboratory of Composite and Functional Materials, Tianjin University, Tianjin 300072, China*

Received May 16, 2016; accepted July 21, 2016; published online September 23, 2016

A polymeric polyethylenimine (PEI)-based prodrug of anticancer doxorubicin (DOX) (PEI-hyd-DOX) was designed by attaching DOX to PEI via an acid-labile hydrazone bond, for the achievement of biocontrollable gene and drug co-delivery in response to the intracellular acid microenvironments in the late endosome/lysosome compartments. The cytotoxicity of PEI-hyd-DOX was evaluated by the MTT assay and the cellular uptake was monitored using confocal laser scanning microscopy. The polymeric prodrug can respond with a high sensitivity to the specific acid condition inside cells, thus permitting the precise biocontrol over intracellular drug liberation with high drug efficacy. The chemical attachment of drug molecules also led to the relatively reduced toxicity and the enhanced transfection efficiency compared with parent PEI. The resulting data adumbrated the potential of PEI-hyd-DOX to co-deliver DOX and therapeutic gene for the combination of chemotherapy and gene therapy.

gene/drug co-delivery, hydrazone bond, pH-sensitivity, polymeric prodrug, transfection efficiency

Citation: Jia H, Chen S, Zhuo R, Feng J, Zhang X. Polymeric prodrug for bio-controllable gene and drug co-delivery. *Sci China Chem*, 2016, 59: 1397−1404, doi: 10.1007/s11426-016-0230-9

1 Introduction

Gene therapy has shown great promise in cancer treatments and the therapy performance relies on the accomplishment of effective and safe gene delivery [1–3]. Therefore, it is critical to develop proper gene delivery systems that can meet the requirements for desired gene delivery, such as high transfection efficiency, low cytotoxicity and targeting specificity to cancer cells. Over the past decades, non-viral gene vectors have attracted increasing attention for their superior advantages, such as non-immunogenicity, structure diversity, and ease of production as compared to viral vectors [4,5]. Polyethylenimine (PEI) with the molecular weight of 25k (PEI25k) is known as the gold standard of gene vectors for its high transfection efficacy detected in a variety of cell lines due to its strong affinity with DNA, easy intracellular internalization and effective endosomal escape. However, high-molecular-weight PEI always exhibits significant cytotoxicity that impedes its application. To address this issue, an approach of hydrophobic modification toward PEI was reported recently [6–8], leading to both the improved safety profile and the increased transfection efficiency.

Cancer progression is very complex and involves multiple signaling pathways [9]. Studies have shown that the single inhibition of one signaling pathway by a therapy modality may be insufficient for the sustained and complete tumor response. The concept of combinational therapy has emerged in turn and displayed vast potentials to revolutionize the future of cancer treatments [10]. To this end, nucleic acid-based anticancer therapy has been currently under intense investigation in combination with the chemotherapy

*Corresponding authors (email: fengjun@whu.edu.cn; xz-zhang@whu.edu.cn)

using small molecule drugs, with the goal of improving the therapeutic outcome [11,12]. For the drug-nucleic acid combination therapy, nanocarriers play an important role and are necessarily needed. They ought to deliver drug-gene combinations to cancer cells in a synchronized fashion to achieve synergistic antitumor effects [13–15]. Till now, the nanoparticle-based drug delivery still faces great challenges due to the various physiological barriers *in vivo*, which make them far from satisfactory [16,17]. It is preferable to not only effectively deliver but also controllably release the drug cargoes in the cancer cells. Because the nanoparticles are always internalized through endocytic routes, they are inevitably entrapped in acid endosomes and eventually reach lysosomes with a much lower pH of 4–5 [18]. This special biological microenvironment can be used for the bio-controllable release of drug cargoes loaded in the nanosystems.

Taking these into account, a pH-sensitive polymeric prodrug of doxorubicin (DOX) modified PEI was designed to co-deliver DOX and gene. By attaching hydrophobic DOX to PEI5000 (M_w=5 kDa), the cytotoxicity was expected to be decreased while the gene transfection efficiency ought to be further enhanced [6–8]. Of note, DOX was coupled to PEI through an acid-labile hydrazone bond, which was stable in the physiologically normal condition and thus prevented the premature liberation of DOX during the blood circulation (Scheme 1). Upon the endocytosis into the lysosome, the pH decline would induce the drug liberation away from polymeric prodrug, favoring the synchronization of two therapeutic mechanisms [16,19].

2 Experimental

2.1 Materials

Polyethyleneimine with a high molecular weight of 5000 (PEI5k) was purchased from Aladdin (USA). Doxorubicin hydrochloride (DOX·HCl) was purchase from Zhejiang Hisun Pharmaceutical Co. (China). Hydrazine hydrate, bromoacetyl bromide, di-*tert*-butyl dicarbonate ($(Boc)_2O$) were purchased from Sinopharm (China) and used as received. Hoechst 33342, Dulbecco's Modified Eagle's Medium (DMEM), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), fetal bovine serum (FBS) and Dulbecco's phosphate buffered saline (PBS) were purchased from Invitrogen Corp. (USA). Bax was purchased from Sigma (USA). *t*-Butyl carbazate and *N*-boc-2-bromoacetohydrazide were synthesized according to the literature [20,21].

2.2 Instruments

The dynamic light scattering (DLS) was used to determine the size potential of the nanoparticles at 25 °C by Nano-ZS ZEN3600 (Malvern instruments, UK).

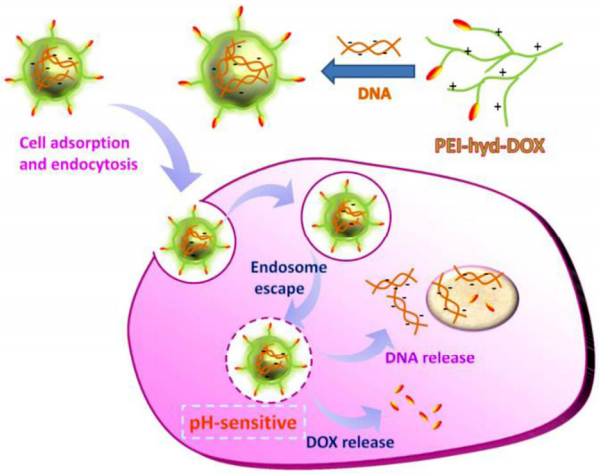
2.3 Synthesis of hydrazone bond modified DOX (DOX-Br)

DOX-Br was synthesized according to the amended method described in the literature through the route as shown in Figure S1 (Supporting Information online). Briefly, the synthesized *N*-boc-2-azideacetohydrazide (80 mg) was dissolved in 3 mL of dichloromethane (DCM) containing 3 mL of trifluoroacetic acid (TFA) and the mixture solution was stirred at room temperature for 30 min. After that, the solvent was removed by rotary evaporation and the residue was co-evaporated three times with toluene to remove the excess TFA. The obtained yellowish oil was dried over P_2O_5 and NaOH under vacuum.

2.4 Synthesis of DOX modified PEI (PEI-hyd-DOX)

The above yellowish oil and doxorubicin hydrochloride (DOX·HCl) (180 mg) were dissolved in 15 mL of anhydrous menthol and then introduced with a drop of TFA. After refluxed for 48 h under dark, the solvent was removed by rotary evaporation after the mixture was cooled down. The residue was precipitated in excess ethyl acetate. Then the precipitate was collected by centrifugation and washed with ether. The crude product was obtained as a dark red solid, which was used in the next step without further purification. Yeild: 100 mg (38.5%).

A typical preparation procedure of PEI-hyd-$DOX_{7.72\%}$ was described as follow: the above product (15 mg) and PEI5k (200 mg) were dissolved in 13 mL of anhydrous menthol. After reaction at 70 °C for 24 h, the solution was poured into 300 mL of ether to isolate the insoluble product.



Scheme 1 Illustration of bio-controllable PEI-hyd-DOX prodrug for tumor-specific drug/gene co-delivery. The prodrug design enables both the low liberation of drug cargo in the physiologically normal conditions and intracellularly the lysosome-induced drug liberation *in situ* and enhanced gene transfection (color online).

The precipitates were collected and dried in vacuum. Using the same method, PEI-hyd-$DOX_{7.72\%}$, PEI-hyd-$DOX_{5.83\%}$, PEI-hyd-$DOX_{2.51\%}$ and PEI-hyd-$DOX_{0.95\%}$ were prepared with different grafting degree (wt%). The grafting degree of DOX was determined on the basis of the fluorescence absorbance intensity at 583 nm.

2.5 Cell culture

Human cervix carcinoma (HeLa) cells and human embryonic kidney transformed 293 (293T) cells were incubated in DMEM containing 10% FBS and 1% antibiotics (penicillin-streptomycin, 10000 U/mL) at 37 °C in a humidified atmosphere containing 5% CO_2.

2.6 Amplification and purification of plasmid DNA

pGL-3 plasmid as the luciferase reporter gene was transformed in *Escherichia coli* (*E. coli*) JM 109. The plasmid was amplified in Terrific Broth medium at 37 °C overnight. The amplified plasmids were purified by a NucleoBand Xtra Maxi Plus EF kit (MACHEREY-NAGEL GmbH & Co. KG, Germany). Then the plasmids were dissolved in TE buffer solution and stored at –20 °C. The integrity of the plasmids was confirmed by agarose gel electrophoresis, and the purity and concentration of the plasmids were determined using a UV spectrophotometer (UV-2401, Shimadzu, Japan) at the wavelengths of 260 and 280 nm.

2.7 Agarose gel retardation assay

The PEI-hyd-DOX/pGL-3 polyplexes were prepared with different N/P ratios of PEI-hyd-DOX vs. pGL-3. 0.1 μg of pGL-3 was added to an appropriate volume of PEI-hyd-DOX solution containing 150 mM NaCl. The obtained solutions were diluted by 150 mM NaCl solution to 8 μL and vortexed for 30 s, then the mixture solution were incubated at 37 °C for 30 min. After that, 1 μL of GelRed^{TM} was added to the complexes solution respectively prior to electrophoresis on 0.7% (*W*/*V*) agarose gel, which was put in Tri-acetate (TAE) running buffer at 80 V for 1 h. Finally the location of the pDNA bands was visualized with a UV lamp using a Vilber Lourmat imaging system (France).

2.8 *In vitro* transfection study by luciferase assay

Transfection of the pGL-3 plasmid mediated by PEI-hyd-DOX was studied in HeLa and 293T cells. The transfection of PEI25k was used as a positive control. The cells were seeded in 24-well plates with a density of 6×10^4 cells per well and incubated for 24 h at 37 °C. The polyplexes were prepared by adding 1 μg pGL-3 to an appropriate volume of a PEI-hyd-DOX solution containing 150 mM NaCl with various N/P ratio of PEI-hyd-DOX vs. pGL-3 ranging from 5 to 25. After that, the polyplex solution were diluted to 1 mL by serum-containing DMEM and added to the cell wells after they were vortexed for 30 s and incubated for 30 min at 37 °C. After incubation with cells for 4 h at 37 °C, the medium was removed and the cells were washed with PBS. Then the cells were fed again for 44 h by 1 mL of fresh DMEM containing 10% FBS at 37 °C. The luciferase assay was performed according to the manufacturer's protocol. Relative light units (RLU) were measured by a chemiluminometer (Lumat LB9507, EG&G Berthold, Germany). The total protein was determined according to the BCA protein assay kit (Pierce, USA). Luciferase activity was expressed as RLU per mg protein. Data were shown as mean±standard deviation (SD) based on three independent measurements.

2.9 Determination of drug loading capacity

A known amount of dried PEI-hyd-DOX was dissolved in 1 mL of methanol, the amount of loaded DOX was determined on the basis of the fluorescence absorbance intensity at 583 nm, using a standard calibration curve that was experimentally obtained. Each value was averaged from three independent experiments. The drug loading efficiency (DLE) was defined as:

DLE=(mass of loaded drug)/(mass of drug loaded polymers)×100%

2.10 *In vitro* drug release profile

The release of DOX from PEI-hyd-DOX conjugates and PEI-DOX was assayed in buffer solution of pH 7.4 (NaH_2PO_4-Na_2HPO_4) (pH of blood plasma) and pH 5.0 (HAc-NaAc) (pH tumor interstitial space and endosome content). The ionic strength of all the buffer solution was fixed at 0.02 M. The equation of $I=1/2(\Sigma C_i Z_i^2)$ was used to calculate the ionic strength and the needed amount of sodium chloride, where C_i is the ionic concentration (mol/L) and Z_i is the amount of electric charge. 6.2 mg of dried PEI-hyd-DOX/ PEI-DOX conjugates in 1 mL of buffer solution (pH 7.4 and 5.0) was sealed in a dialysis bag (MWCO 3500). The dialysis bag was immersed in 20 mL of corresponding buffer, and was incubated at 37 °C. At desired time intervals, 3 mL of release media was taken out and the same volume of fresh buffer solution was added. The amount of released drug was determined on the basis of the fluorescence absorbance intensity at 560 nm for DOX, using a standard calibration curves running with DOX in corresponding buffer solutions at different pH. The amount of unreleased drug that remained in the tube was obtained by the same method as that afore-mentioned for the drug loading determination.

2.11 *In vitro* cytotoxicity assay

Cell cytotoxicity assay of PEI-hyd-DOX conjugates before

and after DNA loading in pH 7.4 and 5.0 were performed by MTT assay in HeLa cells. The HeLa cells were seeded in 96-well plates at a density of 6.0×10^3 cells per well and incubated in 100 μL DMEM containing 10% FBS for 24 h prior to PEI-hyd-DOX conjugates and PEI-hyd-DOX/DNA polyplexes addition. After the cells were incubated with the samples for 4 h, the medium was replaced and the cells were further incubated for 44 h with 200 μL of fresh DMEM containing 10% FBS. After that, the medium was replaced with 200 μL of fresh DMEM containing 10% FBS and 20 μL of MTT (5 mg/mL in PBS buffer) solution was added to each well and further incubated for 4 h at 37 °C. Subsequently, the medium was removed and 150 μL of DMSO was added. The absorbance intensity at 570 nm was measured using a microplate reader (Bio-Red, Model 550, USA). The relative cell viability was calculated as:

Cell viability (%)=$(OD_{570\ (sample)})/OD_{570\ (control)})\times100\%$

where $OD_{570\ (control)}$ and $OD_{570\ (sample)}$ were obtained in the absence/presence of the sample, respectively. Data were shown as mean±standard deviation based on three independent measurements.

2.12 Dynamic light scattering (DLS) analysis

The mean particle size and size distribution of PEI-hyd-DOX/DNA complexes at different pH value were determined by DLS technique at 25 °C with Nano-ZS ZEN3600 instrument (Malvern instruments, UK). The polyplexes with N/P ratio 5 was prepared by adding 1 μg of pGL-3 to an appropriate volume of PEI-hyd-DOX solution containing 150 mM NaCl. The polyplex solution was vortexed for 30 s and incubated for 30 min at 37 °C. Then the polyplex solution was diluted to 1 mL by a 150 mM NaCl solution prior to the measurements. Data were shown as mean±standard deviation based on three independent measurements.

2.13 Confocal laser scanning microcopy (CLSM)

HeLa cells were cultured in a single disc to ~70% confluence (8×10^4 cells/disc) for 24 h, the medium was replaced with 1 mL of fresh medium containing PEI-hyd-DOX/pGL-3 nanocomplexes (N/P=5). After incubation for 3, 6 and 25 h, the culture medium was removed and the cells were rinsed thrice with 500 μL of PBS. Subsequently, 1 mL of fresh DMEM containing 10 μL Hoechst 33342 was added and the cells were further incubated for 15 min at 37 °C. Prior to observation, the cells were washed thrice with 500 μL PBS. The fluorescence was observed using a confocal laser scanning microscope (C1-Si, Nikon, Japan) equipped with 405 and 543 nm argon laser for blue light and red light respectively. The micrographs of cells were recorded using EZ-C1Free Viewer 3.70 version software. The whole preparation process should be performed without exposure to strong light in order to protect the fluorescent dyes.

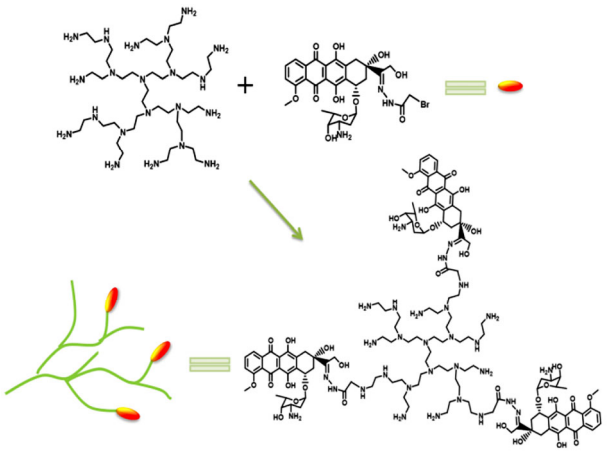
3 Results and discussion

3.1 Synthesis and DNA affinity of PEI-hyd-DOX

The PEI-hyd-DOX conjugates were synthesized through the condensation reaction between PEI5k (M_w=5 kDa) and DOX by forming hydrazone bonds. As shown in Scheme 2. PEI-hyd-$DOX_{7.72\%}$, PEI-hyd-$DOX_{5.83\%}$, PEI-hyd-$DOX_{2.51\%}$ and PEI-hyd-$DOX_{0.95\%}$ were prepared with varying grafting degrees (wt%). As shown in Figure S2, the binding ability of PEI-hyd-DOXs to pGL-3 was indicated by the retarding N/P ratio where the mobility of DNAs could be entirely retarded. It was found that all the PEI-hyd-DOX polymers were able to bind plasmid pGL-3 at the N/P ratio of around 4, almost the same as that of unmodified PEI5k. The result indicated the hydrophobic modification with DOX made few influence on the DNA affinity though the amine amount was reduced, which agreed well with the finding previous reported [6–8].

3.2 *In vitro* cytotoxicity

Biocompatibility is one of the critical properties for gene vectors with regard to *in vivo* application. Studies have revealed that for polycation/DNA transfer systems, free polycations are more possibly the major concern of toxicity issue rather than DNA-combined polycations [8]. To preliminarily evaluate the safety profile of PEI-hyd-DOXs, *in vitro* cytotoxicity tests were conducted in HeLa cells by MTT assay, and free PEI5k was used as the control. As shown in Figure 1, PEI5k showed the highest toxicity in HeLa cells. Relatively, PEI-hyd-DOXs exhibited substantially enhanced biocompatibility. A small grafting degree of 0.95% can even cause a marked reduction of cell cytotoxicity. However, with increasing grafting degree, the cell toxicity was further increased. This is mainly due to the larger availability of free DOX that was liberated from the PEI-hyd-DOX prodrug when entering acid lysosome in cells, which had



Scheme 2 Illustration of the preparation and structure of PEI-hyd-DOX (color online).

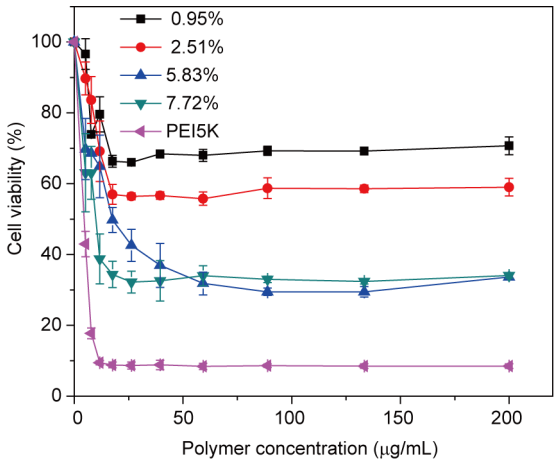


Figure 1 Cytotoxicity profiles of PEI-hyd-DOXs and PEI5k in HeLa cells (data shown as mean±SD (n=3)) (color online).

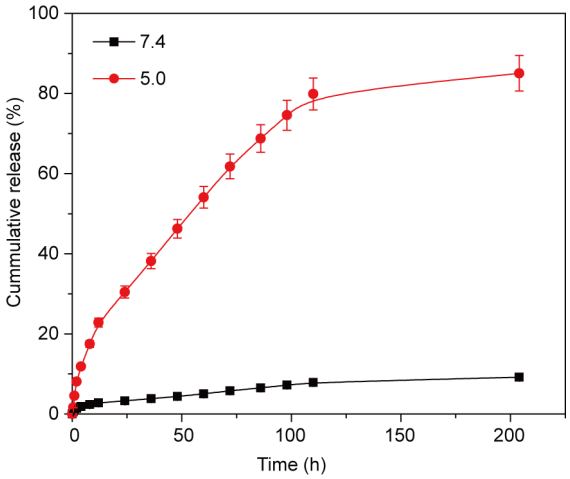


Figure 2 Time-dependent cumulative release of DOX from PEI-hyd-DOX conjugate at different pH (color online).

been proved in the next section. To simplify the research, the PEI-hyd-DOX$_{2.51\%}$ with a moderate grafting degree was used for further study.

3.3 pH-dependent *in vitro* drug release

For tumor-specific drug delivery, the delivery nanosystems should preferably maintain their structural stability during the circulating period. After reaching the target sites and being intracellularly internalized, the drug should be timely released. The site-specific selectivity of drug release behavior is highly preferred so as to improve the drug efficacy and minimize the side effects [5,22,23]. In comparison with the physiologically normal condition with the pH value of 7.4, the late endo-/lysosome organelles are typical acidic environments with the pH of 4.0–5.5 [24]. In our study, DOX was conjugated to the PEI by a hydrazone bond, which is known to be readily cleavable under mildly acidic conditions. To demonstrate this, the conjugates were incubated at different pH values and the *in vitro* drug release of PEI-hyd-DOX was monitored. The pH of 5.0 and 7.4 were selected to simulate the physiological conditions and the late endo-/lysosomal microenvironments, respectively. As shown in Figure 2, no burst release took place at pH 7.4 and less than 10% DOX was leaked out during a 200 h period. The result indicated that the majority of DOX drugs were still chemically attached on PEI surface, consistent with the high stability of hydrazone bond under the normal condition. However, when the pH was adjusted to 5.0, more than 80% of DOX were rapidly released after 100 h incubation, implying the sensitivity of PEI-hyd-DOX to endo-/lysosomal pH. The poor liberation of the drugs at neutral condition was advantageous since the premature drug leakage would be minimized during the circulation. In contrast, after reaching tumor cells, the drug release pattern would be changed to "ON". This controllable transition would enhance the drug bioavailability at the tumor sites while reducing the systematic toxicity, consequently benefiting the cancer treatment.

3.4 pH-conditioned stability

The stability of PEI-hyd-DOX in the buffer solutions with different pH (7.4 and 5.0) was investigated respectively by DLS technique. As shown in Figure 3, the particle size and size distribution of PEI-hyd-DOX/pGL-3 complexes prepared at N/P ratio of 5 always remained steady at pH 7.4. At pH 5.0, however, the size was sharply increased from 250 nm to 2 μm with an apparently broadened size distribution from 0.189 to 0.578. The DLS data manifested the high stability of PEI-hyd-DOX at the normal condition from another perspective. Furthermore, it was suggested that the acidity-induced DOX detachment would weaken the DNA affinity of PEI5k. As documented, this would make it easy for the intracellular DNA liberation and thus favor the following protein expression [25].

3.5 Intracellular sensitivity of PEI-hyd-DOX/pGL-3 complexes

To demonstrate whether the covalently conjugated DOX can be efficiently liberated from PEI-hyd-DOX prodrug inside cells, as triggered by the endo-/lysosomal acidity, PEI-hyd-DOX/pGL-3 complexes were incubated with the HeLa cells at pH 7.4. After a predetermined period, the medium was removed, and the cells were washed with PBS for several times and then subjected to CLSM observation. As shown in Figure 4, after 3 h incubation with HeLa cells, the red signals representing DOX were almost localized in cytoplasm domain. Nevertheless, at 6 to 25 h, the amount of DOX resided in the cell nuclei was apparently increased, suggesting the detachment of DOX away from PEI-hyd-DOX conjugate. To further demonstrate the close relation-

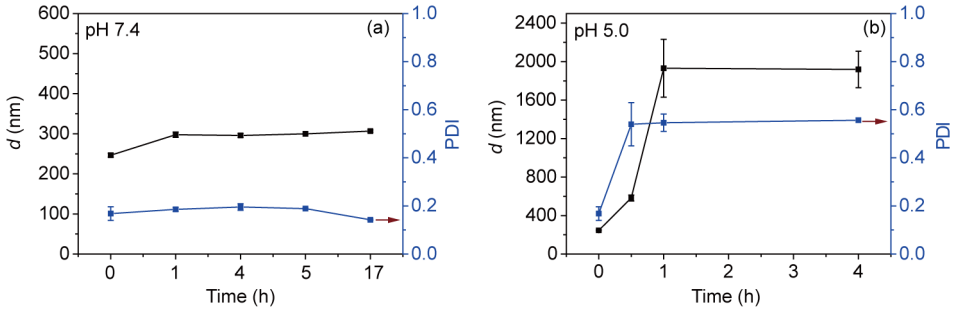


Figure 3 DLS data of PEI-hyd-DOX/pGL-3 complexes prepared at N/P ratio of 5 in the buffer solutions with different pH of 7.4 (a) and 5.0 (b) (color online).

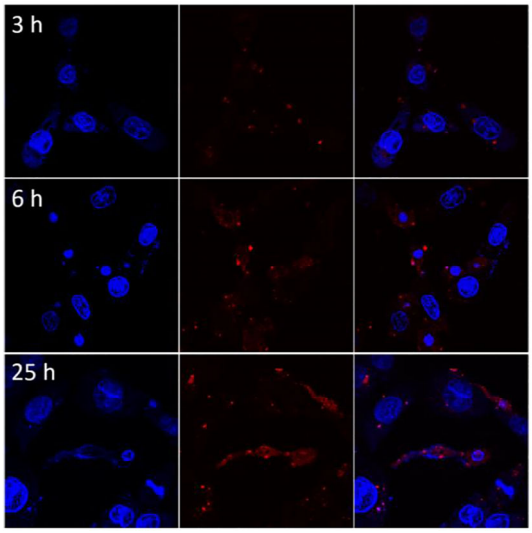


Figure 4 CLSM images of HeLa cells after different time incubation with PEI-hyd-DOX/pGL-3 (red). Hoechst 33342 was used to stain cell nuclei (blue) (color online).

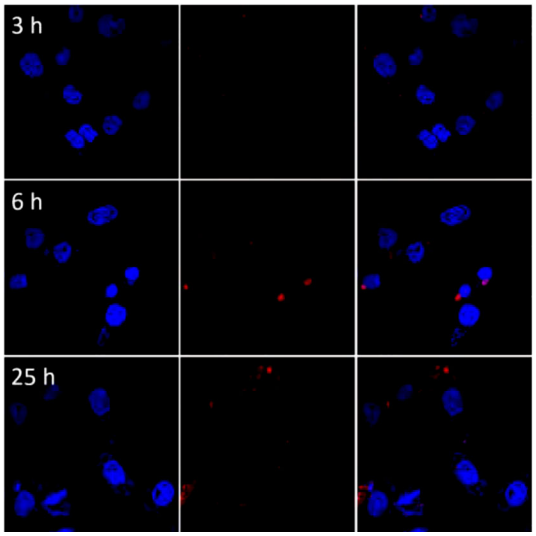


Figure 5 CLSM images of HeLa cells after different time incubation with PEI-DOX/pGL-3 (red). Hoechst 33342 was used to stain cell nuclei (blue) (color online).

ship between the DOX release and the PH-sensitivity of the hydrazone linkage, PEI-DOX with a stable amide bond instead of hydrazone linkage was used as the control for comparison. Following incubation with HeLa cells under identical conditions, the red signal of DOX was barely observed in the nuclei even after 25 h incubation (Figure 5), indicating the nuclear internalization at a very low level owing to the difficult liberation of DOX from PEI-DOX. The marked deviation in the intracellular distributions of DOX signal between two cases confirmed the endo-/lysosomal acidity-triggered DOX release from PEI-hyd-DOX in cellular levels.

A quantitative comparison was conducted in HeLa cells between the cytotoxicity profiles mediated by PEI-hyd-DOX/pGL-3 and PEI/pGL-3 complexes after coincubation for different times (from 1 to 48 h) (Figure 6). PEI-hyd-DOX/pGL-3 afforded much lower cell viabilities than PEI-DOX/pGL-3. This structure-associated difference was represented more evidently with time. The data further demonstrated the hydrazone linkage between DOX and PEI5k can respond with high efficiency to the intracellular bio-stimulus of lysosomal acidity, which resulted in the effective liberation of initially locked drugs and the enhanced drug efficacy in turn.

3.6 *In vitro* transfection assay

A desirable polycation gene vectors should not only possess the lower cytotoxicity, but also transport the gene cargoes to the targeted site and maintain their activities at high levels. Therefore, *in vitro* transfection of the PEI-hyd-DOX/pGL-3 complexes were examined in the 10% serum containing medium. The cells were co-incubated with the PEI-hyd-DOX/pGL-3 complexes for 4 h prior to the 48 h culture in fresh culture medium. As expected, the transfection efficiency was found to be moderately enhanced resulting from DOX modification to PEI25k either in HeLa cells or 293T cells (Figure 7). The improved transfection was supposed to relate to the easy liberation of gene payloads after the DOX liberation, as mentioned above [26]. Based on the results of *in vitro* gene transfection assay, it was suggested that the PEI25k still exhibited good gene transfection efficiency

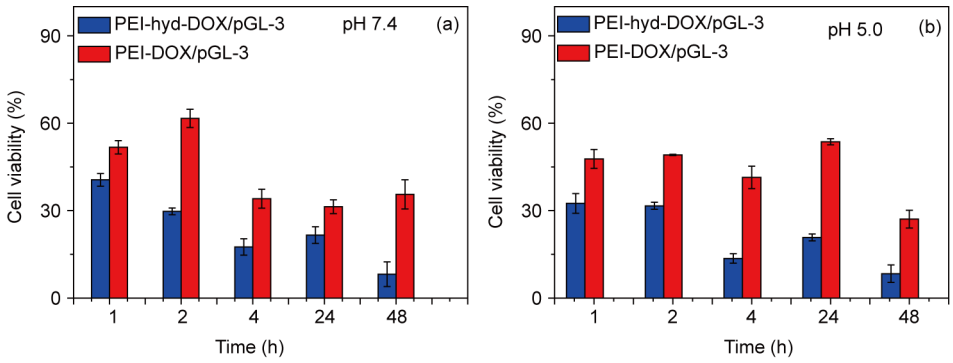


Figure 6 Cytotoxicity of PEI-hyd-DOX/pGL-3 in different buffer solution (pH 7.4 (a) and 5.0 (b)) in comparison with PEI-DOX/pGL-3. The concentration of pGL-3 was fixed at 1 μg/mL (data shown as mean±SD (n=3)) (color online).

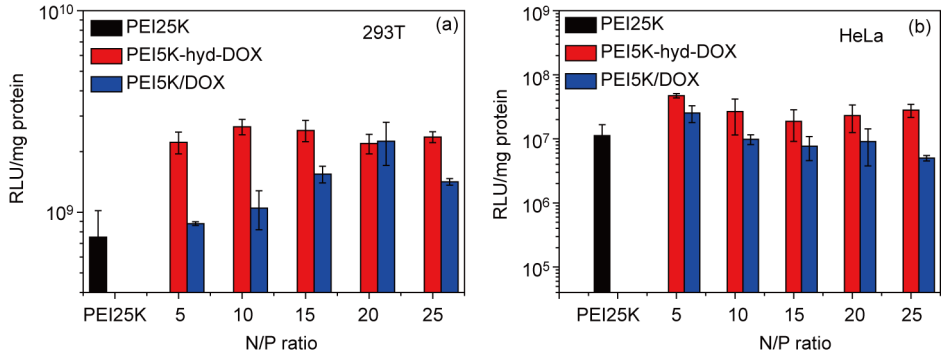


Figure 7 Transfection efficiency of PEI-hyd-DOX/pGL-3 and PEI5k/pGL-3 polyplexes at different N/P ratios in 293T (a) and HeLa (b) cells in the 10% serum containing medium. PEI25k polyplex prepared at *w*/*w* 1.3 was used as the control. The concentration of pGL-3 was fixed at 1 μg/mL (data shown as mean±SD (n=3)) (color online).

after the hydrophobic modification with DOX. In addition, the DOX has proven to be bio-controllably released inside the cells with an enhanced drug efficacy. The results adumbrated the promise of PEI-hyd-DOX to be applicable for the combination of chemotherapy and gene therapy [27].

4 Conclusions

In summary, this work has established an efficient gene/drug co-delivery system based on a polymeric prodrug. The prodrug of pH-sensitive PEI-hyd-drug conjugate can efficiently respond to intracellular acid environments, thus permitting the precise bio-control over intracellular drug liberation and consequently affording the high drug efficacy. The chemical attachment of hydrophobic DOX also led to the relatively reduced toxicity and the enhanced transfection efficiency compared with the parent PEI. The resulting data adumbrated the potential of PEI-hyd-DOX to co-deliver DOX and therapeutic genes for the combination of chemotherapy and gene therapy. Furthermore, this study of prodrug vectors provides useful information in favor of the rational design of vectors for the biocontrollable gene/drug co-delivery.

Acknowledgments This work was supported by the National Natural Science Foundation of China (21374085, 21174110, 51233003), the Natural Science Foundation of Hubei Province of China (2014CFB697) and the Fundamental Research Funds for the Central Universities (2042014kf-0193).

Conflict of interest The authors declare that they have no conflict of interest.

Supporting information The supporting information is available online at http://chem.scichina.com and http://link.springer.com/journal/11426. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

1 Naldini L. *Nature*, 2015, 526: 351–360
2 Wu MY, Meng QS, Chen Y, Du YY, Zhang LX, Li YP, Zhang, LL. Shi JL. *Adv Mater*, 2015, 27: 215–222
3 Yan H, Oommen OP, Yu D, Hilborn J, Qian H, Varghese OP. *Adv Funct Mater*, 2015, 25: 3907–3915
4 Wu ZY, Zhan SY, Fan W, Ding XY, Wu X, Zhang W, Fu YH, Huang YY, Huang X, Chen RB, Li MJ, Xu NY, Zheng YX, Ding BY. *Nanoscale Res Lett*, 2016, 11: 1–13
5 Klauber TCB, Søndergaard RV, Sawant RR, Torchilin VP, Andresen TL. *Acta Biomaterialia*, 2016, 35: 248–259
6 Wang RR, Hu H, Cai Q, Zhao NN, Zhu Y, Xu FJ. *Sci China Chem*, 2015, 58: 1461–1470
7 Liu C, Liu F, Feng L, Li M, Zhang J, Zhang N. *Biomaterials*, 2013, 34: 2547–2564
8 Jia HZ, Luo XH, Cheng H, Yang J, Li C, Liu CW, Feng J, Zhang XZ,

Zhuo RX. *J Mater Chem*, 2012, 22: 24092–24101
9 Li J, Wang Y, Zhu Y, Oupický D. *J Control Release*, 2013, 172: 589–600
10 Lei C, Cui Y, Zheng L, Kah-Hoe Chow P, Wang CH. *Biomaterials*, 2013, 34: 7483–7494
11 Chen S, Lei Q, Li SY, Qin SY, Jia HZ, Cheng YJ, Zhang XZ. *Biomaterials*, 2016, 92: 25–35
12 Ren Y, Wang R, Gao L, Li K, Zhou X, Guo H, Liu CY, Han DL, Tian JG, Ye Q, Ye TH, Sun DX, Yuan XB, Zhang N. *J Control Release*, 2016, 228: 74–86
13 Jia HZ, Zhang W, Zhu JY, Yang B, Chen S, Chen G, Zhao YF, Feng J, Zhang XZ. *J Control Release*, 2015, 216: 9–17
14 Jia HZ, Wang W, Zheng DW, Wang X, Yu WY, Li SY, Zhuo RX, Zhao YF, Feng J, Zhang XZ. *ACS Appl Mater Interf*, 2016, 8: 6784–6789
15 Wang K, Hu Q, Zhu W, Zhao M, Ping Y, Tang G. *Adv Funct Mater*, 2015, 25: 3380–3392
16 Song N, Zhou L, Li J, Pan Z, He X, Tan H, Wan XY, Li JS, Ran R, Fu Q. *Nanoscale*, 2016, 8: 7711–7722
17 Wang T, Wang D, Yu H, Wang M, Liu J, Feng B, Zhou FY, Yin Q, Zhang ZW, Huang YZ, Li YP. *ACS Nano*, 2016, 10: 3496–3508
18 Yang H, Wang Q, Huang S, Xiao A, Li F, Gan L, Yang XL. *ACS Appl Mater Interf*, 2016, 8: 7729–7738
19 Fan Y, Li C, Li F, Chen D. *Biomaterials*, 2016, 85: 30–39
20 Melendez RE, Lubell WD. *J Am Chem Soc*, 2004, 126: 6759–6764
21 Yuan ZF, Que ZY, Cheng SX, Zhuo RX, Li F. *Chem Commun*, 2012, 48: 8129–8131
22 Bao XL, Wang W, Wang C, Wang Y, Zhou JP, Ding Y, Wang XY, Jin YT. *Biomaterials*, 2014, 35: 8450–8466
23 Wang S, Yang W, Cui J, Li X, Dou Y, Su L, Chang J, Wang HJ, Li XD, Zhang BB. *Biomater Sci*, 2016, 4: 338–345
24 Kanamala M, Wilson WR, Yang M, Palmer BD, Wu Z. *Biomaterials*, 2016, 85: 152–167
25 Dai Z, Gjetting T, Mattebjerg MA, Wu C, Andresen TL. *Biomaterials*, 2011, 32: 8626–8634
26 Zheng M, Yang C, Meng F, Peng R, Zhong Z. *Macromol Res*, 2012, 20: 327–334
27 Luo XH, Huang FW, Qin SY, Wang HF, Feng J, Zhang XZ, Zhuo RX. *Biomaterials*, 2011, 32: 9925–9939