Schiff-linked prodrugs of pH-responsive nanoparticles to facilitate drug delivery

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Abstract. Endosomal pH-responsive micelle nanoparticles were prepared by self-assembly of amphiphilic polyethylene glycol Schiff-doxorubicin (PEG-Schiff-DOX) precursor drugs. The free DOX could be encapsulated in the hydrophobic core of the nanoparticles. These nanoparticles exhibited decompose rapidly in weakly acidic environments, but can keep excellent storage stability under normal conditions. Due to the differences in drug release mechanisms and rates between encapsulated DOX and linked DOX, programmed drug release behavior was observed, which may result in higher intracellular drug concentrations and longer duration of action.CCK-8 analysis showed that the nanoparticles showed better antitumor activity than free DOX. These nanomedicines based on precursor drugs have great potential for the development of transformational DOX agents for cancer therapy.

Keywords: Prodrug; Schiff base; drug delivery; pH response; nanoparticle.

1. Introduction

Polymer-based nanomaterials have been intensively studied for can cancer treatment over the past few decades, considering their potential to increase drug solubility, decrease side effects and enhance therapeutic efficacy. [1–5] Nanomedicines have shown many advantages over small molecule anticancer drugs, including prolonged circulation time through evading glomerular filtration, improved pharmacokinetic properties, and improved tumor accumulation by enhancing osmotic and retention (EPR) effects. With the deepening research on polymer, there were many types of polymerization monomer in synthesis to choose and they can modify and transform the initiator in the composition freely, as well connect bioactive compounds or drugs and impart specific -targeting and stimuli- responsiveness to the polymer so that it can make the function polymer with a variety of properties (such as to temperature, magnetic field, pH, ionic strength, chemicals) [6-10]. pH sensitive polymer nano drug carrier research is received great attention, because the normal tissue and blood pH is stable at around 7.4, but due to the abnormal reproduction of tumor cells in tumor tissue, its pH is about 6.8-7.2, which is significantly lower than the normal tissue.[11-14] With the difference of pH in the tumor tissue and intracellular, a specific organ environment pH responsive polymer rug delivery carrier can be designed to realize the function to target control release the drugs, proteins and gene. [15-22] One of the most important applications of pH-sensitive polymer nanocarriers is the targeted delivery of anticancer drugs using the slightly acidic environment of tumor tissues and cells.

Doxorubicin (DOX) is a commonly used clinical anthracycline anti-tumor drug, which enters tumor cells through the cell membrane and acts on DNA, to achieve the purpose of anti-tumor. Adriamycin has a wide anti-tumor spectrum and good curative effect. It is mainly used in the treatment of acute and chronic leukemia, malignant lymphoma, and a variety of solid tumors. Due to poor oral absorption, doxorubicin is usually administered intravenously or arterially in clinic. However, but such drugs have relatively high toxicity, and long-term application may lead to dose-dependent irreversible cardiomyopathy, myelosuppression, alopecia, digestive tract symptoms. At the same time, the existence of multi-drug resistance also limits its clinical application. In order to solve the above problems, clinical has been looking for the method to reduce the toxic side effects of doxorubicin. Through many studies, it has been found that changing the dosage form, reducing the leakage of doxorubicin in the delivery process, and making the targeted release of doxorubicin in the tumor site is the most effective way at present, and it has made rapid development in clinical research.

At present, the main targeted drug delivery systems for DOX include liposomes, microspheres, vesicles, nanoparticles, and polymeric micelles. Among them, DOX liposome can reduce the toxicity of Adriamycin to the heart because of its characteristics such as significantly reduced plasma clearance rat and significantly prolonged the half-life. Currently, conventional DOX liposome was Myocet® and polyethylene glycol modified long-circulating liposome Doxil®. Although adriamycin liposomes reduce the toxicity of common formulations, they also introduce new toxicity, such as cutaneous toxicity, and increase the toxicity to bone marrow. Kataoka and Younsoo Bae linked DOX with polymer using pH-sensitive hydrazone bond to make a series of pH-responsive nanoparticles [25-28]. By endocytosis, the low pH environment in the cell makes the hydrazone bond very easy to break, and it is easy to achieve rapid intracellular release.

Diamond-like composite and drug release kinetics are very important for drug delivery system. Higher diamond-like composites not only mean lower costs because less carrier material is required, but also lower risk of toxic side effects. For this reason, a lot of efforts have been made to explore new nanocarriers with high diamond-like composite sheets [22-24]. Drug release kinetics is related to intracellular drug concentration and drug action time. To minimize tumor growth, an effective drug concentration must be maintained for as long as possible. It has been reported that the addition of encapsulated DOX and conjugated DOX into a drug delivery system will achieve programmed drug release, which means that the rapid release of encapsulated DOX can enhance intracellular drug concentration in a short period of time, and the subsequent release of conjugated DOX can continue for a longer period, thus improving therapeutic efficacy [17]

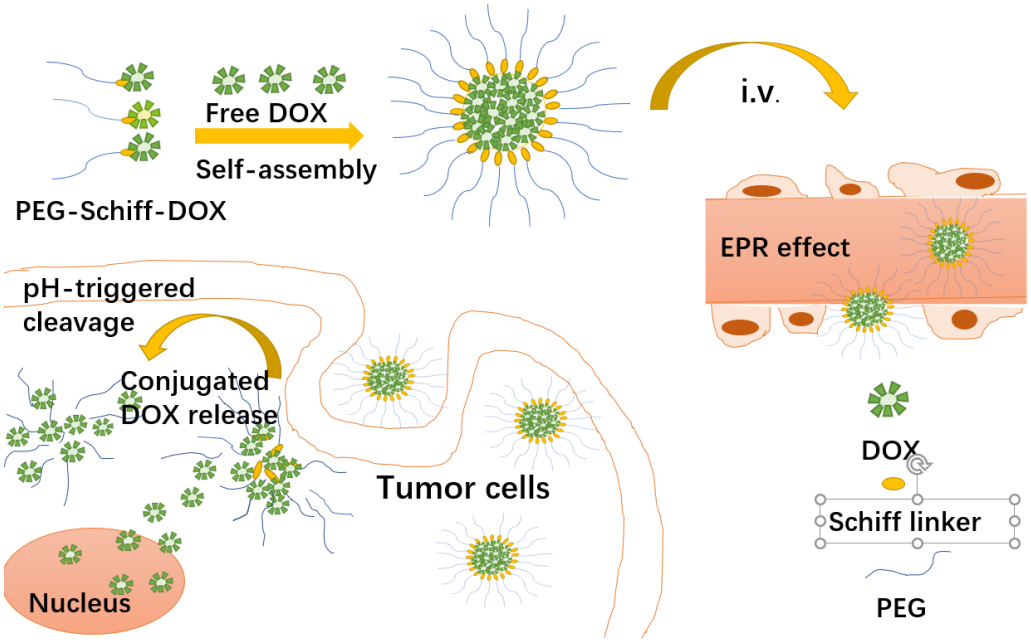

Fig 1. Schematic illustration of formation and delivery of the PEG-Schiff-DOX nanoparticles

In this study, we designed and synthesized an amphiphilic polymer drug conjugate, poly (ethylene glycol)-Schiff-paclitaxel (PEG-Schiff-DOX), which could self-assemble into acid-labile micellar nanoparticles and such a resulted nanoparticle could be used as drug carriers to encapsulate free DOX. Because of the combination of physical encapsulation and chemical conjugation, the nanoparticles not only achieved high DOX concentration, but also exhibited a programmed drug release behavior. As showed in Fig.1, the nanoparticles penetrated tumor tissues trough EPR effect then they were internalized by tumor cells though endocytosis. Because of being in the acidic environment of intracellular endosomal/lysosomal compartments, pH-triggered cleavage of Schiff bonds led to disassembly of encapsulated DOX.

2. Materials and methods

2.1. Materials

Para-carboxyl benzaldehyde, 4-dimethylaminopyridine, doxorubicin hydrochloride, anhydrous N, N-dimethylformamide (DMF), and anhydrous dimethyl sulfoxide (DMSO), purchased directly from Anagen Chemicals. The water used in the experiment is ultra-pure water, and the other chemical reagents used are purchased from Beijing Chemical Plant and used directly.

2.2. Characterizations

Hydrogen Nuclear Magnetic Resonance (1H NMR): Avance Hocky 400 (400 MHz) spectrometer. The deuterium reagents were deuterium chloroform (CDCl3), deuterium acetone (Actone-D6) and deuterium dimethyl sulfoxide (DMSO-D6). TMS was the internal standard and measured at 25 ℃

Transmission electron microscopy (TEM) was tested on a JEM-2200FS (JEOL, Japan) electron microscope with an acceleration voltage of 100 kV.3 μL sample was added to the copper mesh (300 mesh) covered with carbon film, and the excess liquid was absorbed with a filter paper. After standing for natural drying, observation was made. Electron microscope photos were recorded by Gatan Multiscan CCD and processed by Digital Micrograph.

Dynamic Light Scattering (DLS): Malvern Zetasizer Nano ZS dynamic light scattering particle size analyzer. Equip with 633 nm He-Ne laser, detection Angle 173°, particle size test sample pool is quartz colorimetric dish.

UV-Vis (UV-Vis): Shimazu TU1901 UV-Vis spectrophotometer.

2.3. Synthesis of pH-responsive prodrugs

The composition of the pH-responsive prodrug was performed in two steps, as shown in Fig. 2.

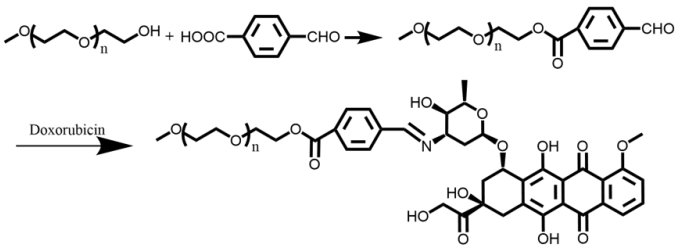

Fig 2. Synthetic route of the PEG-DOX

2.3.1 The synthesis of PEG-CHO

Carboxylic benzaldehyde (150 mg, 1 mmol), EDCI (191.7 mg, 1 mmol) and DMAP (61 mg, 0.5 mmol) were dissolved in ultra-dry DCM, and then PEG-OH (375 mg, 0.5 mmol) was added under the protection of nitrogen. The whole system was stirred at 37 ℃ for 24 h, and then washed for three times with HCl, saturated NaHCO3 and saturated salt solution respectively. Collect organic fruit and dry with anhydrous MgSO4, filter, rotary steam and dry. The final yield was 86%.

2.3.2 The synthesis of PEG-Schiff-DOX

PEG-CHO (100 mg, 110 μmol), doxorubicin (50 mg, 90 μmol) and TEA (70 μL, 500 μmol) were dissolved and oscillated overnight in 3 mL anhydrous DMF. After the reaction solvent was removed by rotary steam, it was dissolved with a large amount of DCM, extracted with saturated salt water for three times, and precipitated in cold ethyl ether. The final yield was 78%.

2.4. Stability and pH responsiveness of nanomedicines

Nanoparticles were dissolved in PBS buffer solution with pH=7.4 (0.5 mg/mL), and their particle size distribution was measured by dynamic light scattering. After two weeks of placement, they were tested again to compare the changes. Nanoparticles were dissolved (0.5 mg/mL) in PBS buffer with

pH=5.0 and placed in 37 ℃ constant temperature water bath for 2 h, then their particle size distribution was measured by dynamic light scattering.

2.5. In vitro drug release of the nanoparticles

The release profile of DOX from the nanoparticles were studied using a dialysis tube (MWCO 3500 Da) under shaking (100 rpm) at 37 °C in phosphate buffer with a pH of 7.4 and acetate buffer with a pH of 5.0, respectively. Typically, 2mL of nanoparticle solution was dialyzed against 30 mL of release media. At pre-determined intervals, 10 mL of release medium was withdrawn and renewed with an equal volume of fresh medium. Then the concentration of DOX in the medium was determined by HPLC measurements. The cumulative release ratio of DOX was calculated according to the following formula:

$$\begin{cases} R_i = \frac{V_0 C_i}{m_{drug}} \times 100\% & (i = 1) \\ R_i = \frac{V_0 C_i + V_e \sum_1^{i-1} C_{i-1}}{m_{drug}} & i \geq 2 \end{cases} \quad (1)$$

Ri is the cumulative release ratio of DOX (%), mdrug is the total mass of DOX in the nanoparticles (μg), V0 andVe are the volume of the total release medium and the exchanged medium respectively (mL), and Ci is the DOX concentration in the release medium.

3. Result and Discussion

3.1. The synthesis of PEG-CHO

The 1H NMR spectra of PEG-CHO are shown in Figure 3. It can be seen from the analysis that the ratio of benzene hydrogen in the synthesized PEG-CHO corresponds to the methyl peak at the PEG end, which can be inferred that the successful synthesis of PEG-CHO is achieved.

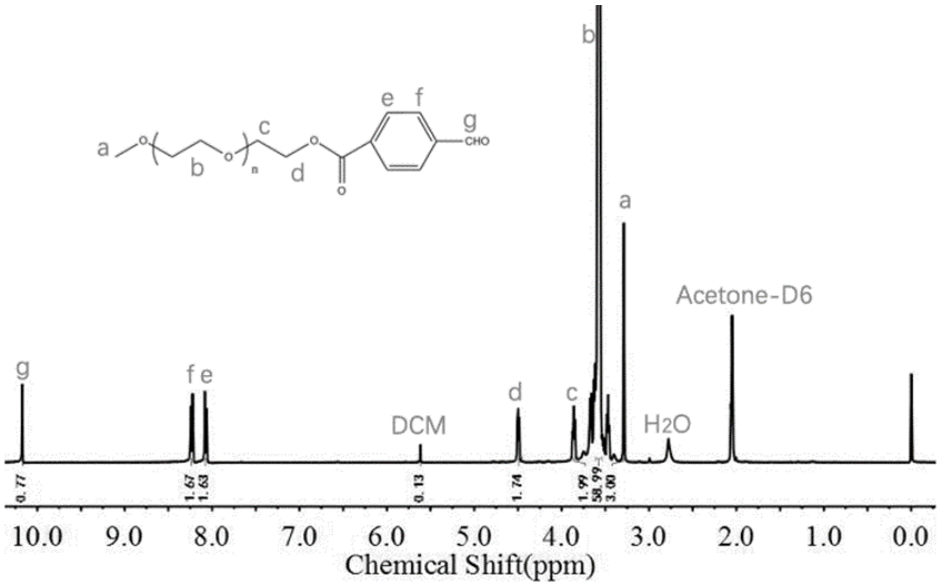

Fig 3. [1]H NMR spectra of PEG-CHO

3.2. The synthesis of PEG-CHO

In [1]H NMR spectra (figure 4), due to the drug molecule peak shape messier and shorter, methyl at end of PEG as a starting point was used to analysis basic corresponding methyl peak area and the main chain of PEG-Schiff-DOX, at the same time think low field area of the peak from the benzene ring and a total of 8 H, Schiff base key points after the basic corresponding area, showed that PEG-Schiff-the successful preparation of DOX.

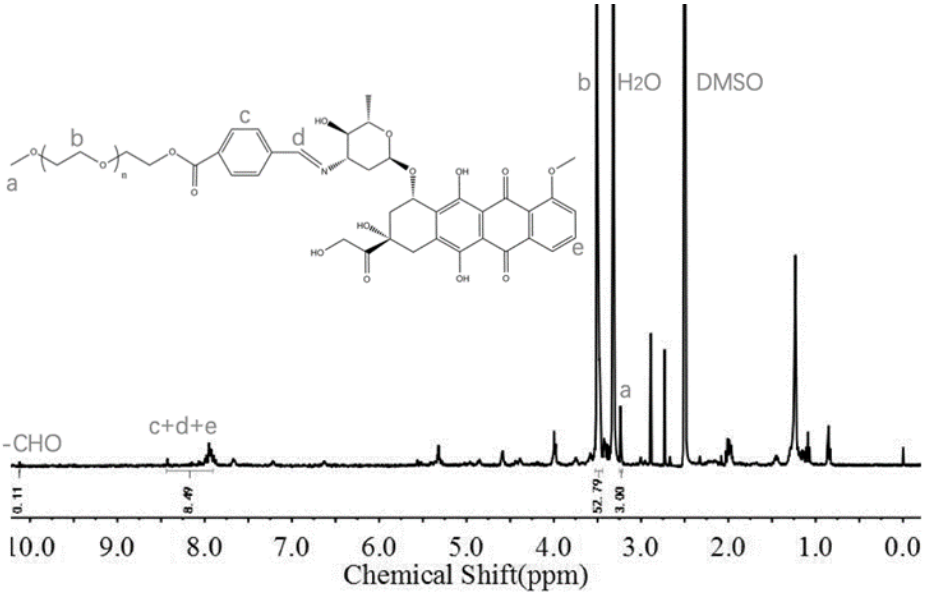

Fig 4. ^{1}H NMR spectra of PEG-Schiff-DOX

3.3. Stability and pH-responsive degradation of the nanoparticles

PEG-Schiff-Dox can be assembled into a spherical micelle with a size below 200 nm in an aqueous solution at pH=7.4 (Fig. 5A). When the nanoparticles are placed in an aqueous solution at pH 5.0, the morphology and structure of the nanoparticles are obviously destroyed, showing an amorphous structure. The pH responsiveness of nanomaterials was given by the particle size change after being oscillated at 37 ℃ for 2 hours in a buffer of pH=5.0.

Fig.5B shows that the particle size of PEG-Schiff-Dox nanoparticles has significant changes before and after acid treatment, and some of the nanoparticles have completely disintegrated. The narrow particle size distribution indicates that the heterogeneous nanoparticles disintegrate under the action of acid and release the drug.

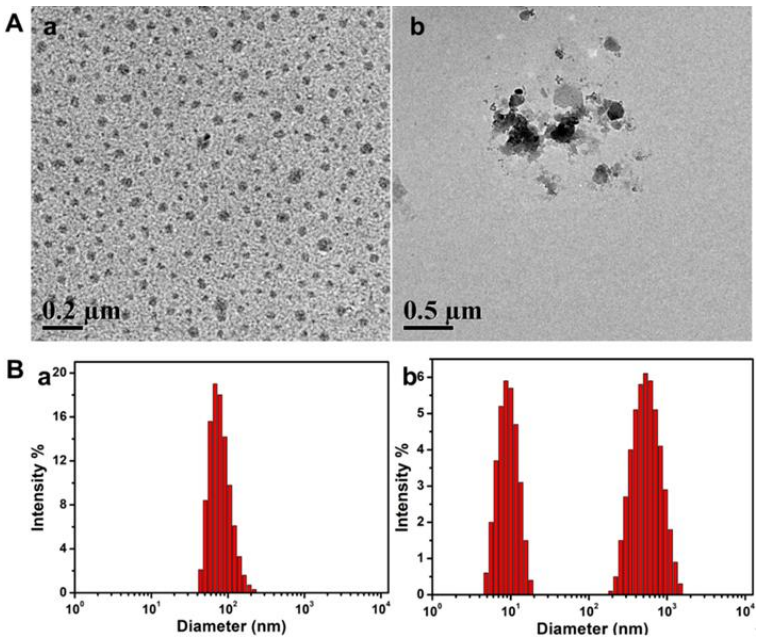

Fig 5. Morphology and size of PEG-Schiff-DOX nanoparticles at (A) pH=7.4 and (B) pH=5.0

The results of the drug release behavior test are more intuitive, as can be seen from the UV test results. As shown in Fig. 6, PEG-Schiff-Dox nanoparticles loaded at pH=7.4 released almost no drug, while the release behavior was significantly enhanced at pH=5.0.

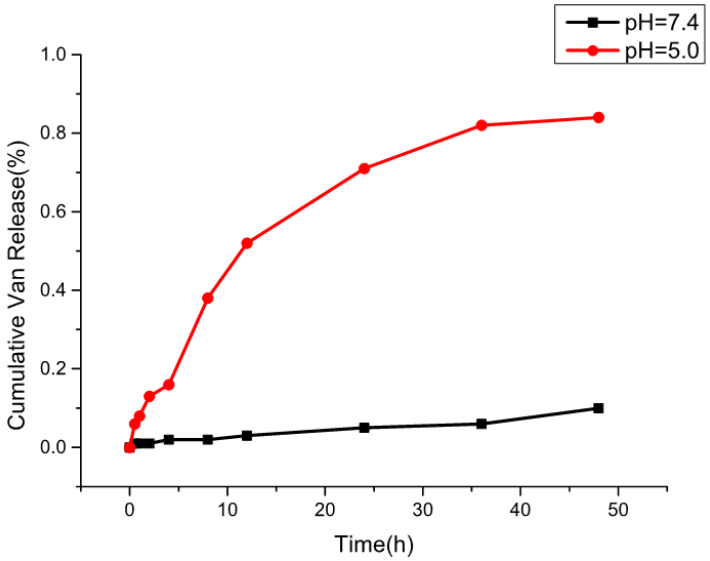

Fig 6. In vitro release curve of PEG-Schiff-DOX nanoparticles

4. Conclusion

In summary, we designed and synthesized an amphiphilic PEG-Schiff-DOX prodrug, and developed novel pH-responsive micellar nanoparticles through self-assemble of the prodrug and free DOX. Notably, these prodrug-based nanoparticles possess several advantages:(1) clear and well-defined structures and simple preparation process. (2) high drug concertation with an good drug loading capacity. (3) good storage stability; (4) negligible or few drug leakages in neutral circulation and endosomal pH-triggered release of pristine DOX retaining potent therapeutic activity of DOX and reducing side effects; (5) two-phase programmed drug release, achieving higher intracellular drug concentration and longer action time; and (6) superior tumor inhibition than free DOX. These prodrug-based nanoparticles provide a promising option for developing translational DOX formulations.

References

[1] K. Ulbrich, K. Hola, V. Subr, A. Bakandritsos, J. Tucek, R. Zboril, Chem. Rev. 116.(2016) 5338–5431.

[2] Z. Ge, S. Liu, Chem. Soc. Rev. 42 (2013) 7289–7325.

[3] H. Wei, R.X. Zhuo, X.Z. Zhang, Prog. Polym. Sci. 38 (2013) 503–535.

[4] J.A. Kemp, M.S. Shim, C.Y. Heo, Y.J. Kwon, Adv. Drug Deliv. Rev. 98 (2016) 3–18.

[5] J.L. Xian, J.D. Barrio, P.P.C. Toh, T.C. Lee, D. Jiao, U. Rauwald, E.A. Appel, .O.A. Scherman, Biomacromolecules 13 (2012) 84–91.

[6] R.B. Greenwald, J. Controlled, Release 74 (2001) 159–171.

[7] T. Luo, C. Loira-Pastoriza, H.P. Patil, B. Ucakar, G.G. Muccioli, C. Bosquillon, R. Vanbever, J. Control. Release 239 (2016) 62–71.

[8] J. Lu, X. Chuan, H. Zhang, W. Dai, X. Wang, X. Wang, Q. Zhang, Int. J. Pharm. 471 .(2014) 525–535.

[9] L. Paz-Ares, H. Ross, M. O'Brien, A. Riviere, U. Gatzemeier, J. Von Pawel, E. Kaukel, L. Freitag, W. Digel, H. Bischoffff, R. Garcia-Campelo,N. Iannotti, P. Reiterer, I. Bover, J. Prendiville, A.J. Eisenfeld, F.B. Oldham, B. Bandstra, J.W. Singer, P. Bonomi, Brit. J. Cancer 98 (2008) 1608–1613.

[10] Y. Wang, J. Liu, J. Zhang, L. Wang, J. Chan, H. Wang, Y. Jin, L. Yu, D.W. Grainger, W. Ying, Int. J. Med. Sci. 11 (2014) 479–487.

[11] C. Li, D.F. Yu, R.A. Newman, F. Cabral, L.C. Stephens, N. Hunter, L. Milas, S. Wallace, Cancer Res. 58 (1998) 2404–2409.

[12] N. Larson, J. Yang, A. Ray, D.L. Cheney, H. Ghandehari, J. Kopecek, Int. J. Pharm. 454 (2013) 435–443.

[13] J.M. Harris, R.B. Chess, Nat. Rev. Drug Discov. 2 (2003) 214–221.

[14] S. Lv, Z. Tang, D. Zhang, W. Song, M. Li, J. Lin, H. Liu, X. Chen, J. Controlled. Release 194 (2014) 220–227.

[15] T. Yin, Q. Wu, L. Wang, L. Yin, J. Zhou, M. Huo, Mol. Pharmaceutics 12 (2015) 3020–3031.

[16] D. Huang, F. Yang, X. Wang, H. Shen, Y. You, D. Wu, Polym. Chem. 7 (2016) 6154–6158.

[17] X. Chuan, Q. Song, J. Lin, X. Chen, H. Zhang, W. Dai, B. He, X. Wang, Q. Zhang, Mol. Pharmaceutics 11 (2014) 3656–3670.

[18] K. Nam, H.Y. Nam, P.H. Kim, S.W. Kim, Biomaterials 33 (2012) 8122–8130.

[19] Y. Gu, Y. Zhong, F. Meng, R. Cheng, C. Deng, Z. Zhong, Biomacromolecules 14.(2013) 2772–2780.

[20] S.K. Cho, A. Pedram, E.R. Levin, Y.J. Kwon, Biomaterials 34 (2013) 10228–10237.

[21] Y. Bao, Y. Guo, X. Zhuang, D. Li, B. Cheng, S. Tan, Z. Zhang, Mol. Pharmaceutics 11. (2014) 3196–3209.

[22] P. Huang, D. Wang, Y. Su, W. Huang, Y. Zhou, D. Cui, X. Zhu, D. Yan, J. Am. Chem. Soc. 136 (2014) 11748–11756.

[23] S. Yang, F. Zhu, Q. Wang, F. Liang, X. Qu, Z. Gan, Z. Yang, J. Mater. Chem. B 3.(2015) 4043–4051.

[24] Y. Shen, E. Jin, B. Zhang, C.J. Murphy, M. Sui, J. Zhao, J. Wang, J. Tang, M. Fan, E. Van Kirk, W.J. Murdoch, J. Am. Chem. Soc. 132.(2010) 4259–4265.

[25] C. Lee, S.T. Lo, J. Lim, V.C. da Costa, S. Ramezani, O.K. Oz, G.M. Pavan, O. Annunziata, X. Sun, E.E. Simanek, Mol. Pharmaceutics 10.(2013) 4452–4461.

[26] Y. Yu, C.K. Chen, W.C. Law, J. Mok, J. Zou, P.N. Prasad, C. Cheng, Mol. Pharmaceutics 10 (2013) 867–874.

[27] M.C. Jones, J.C. Leroux, Eur. J. Pharm. Biopharm. 48 (1999) 101–111.

[28] J.N. Xu, X.L. Zhang, Y.C. Chen, Y.X. Huang, P.C. Wang, Y. Wei, X.C. Ma, S. Li, Mol. Pharmaceutics 14 (2017) 31–41.