Vitamin E-Oligo(methyl diglycol L-glutamate) as a Biocompatible and Functional Surfactant for Facile Preparation of Active Tumor-Targeting PLGA Nanoparticles

Jintian Wu, Jian Zhang,* Chao Deng,* Fenghua Meng, and Zhiyuan Zhong*

Biomedical Polymers Laboratory, and Jiangsu Key Laboratory of Advanced Functional Polymer Design and Application, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, People’s Republic of China

ABSTRACT: Poly(d,l-lactide-co-glycolide) (PLGA) nanoparticles have attracted an enormous interest for controlled drug delivery. Their clinical applications are, however, partly hindered by lack of biocompatible, biodegradable and functional surfactants. Here, we designed and developed a novel biocompatible surfactant based on amphiphilic vitamin E-oligo(methyl diglycol L-glutamate) (VEOEG) for facile fabrication of robust and tumor-targeting PLGA-based nanomedicines. VEOEG was prepared with controlled Mₙ of 1.7–2.6 kg/mol and low molecular weight distribution (D = 1.04–1.16) via polymerization of methyl diglycol L-glutamate N-carboxyanhydride using vitamin E-ethylenediamine derivative (VE-NH₂) as an initiator. VEOEG had a hydrophilic−lipophilic balance data of 13.8–16.1 and critical micellar concentration of 189.3–203.8 mg/L depending on lengths of oligopeptide. Using VEOEG as a surfactant, PLGA nanoparticles could be obtained via nanoprecipitation method with a small and uniform hydrodynamic size of 13.5 nm and positive surface charge of +26.6 mV, in accordance with presence of amino groups at the surface. The resulting PLGA nanoparticles could be readily coated with hyaluronic acid (HA) to form highly stable, small-sized (143 nm), monodisperse, and negatively charged nanoparticles (HA-PLGA NPs). Notably, paclitaxel-loaded HA-PLGA NPs (PTX-HA-PLGA NPs) exhibited better antitumor effects in CD44-positive MCF-7 breast tumor cells than Taxol (a clinical paclitaxel formulation). The in vivo pharmacokinetics assay in nude mice displayed that PTX-HA-PLGA NPs possessed a long plasma half-life of 3.14 h. The in vivo biodistribution studies revealed that PTX-HA-PLGA NPs had a high tumor PTX level of 8.4% ID/g, about 6 times better than that of Taxol. Interestingly, therapeutic studies showed that PTX-HA-PLGA NPs caused significantly more effective tumor growth inhibition, better survival rate and lower adverse effects than Taxol. VEOEG has emerged as a versatile and functional surfactant for the fabrication of advanced anticancer nanomedicines.

1. INTRODUCTION

Biodegradable nanoparticles and microparticles based on poly(d,l-lactide-co-glycolide) (PLGA) are undoubtedly one of the most important platforms to achieve targeted or sustained delivery of varying therapeutic agents.¹⁻⁶ For example, several protein and peptide-loaded PLGA microparticles like Lupron Depot, Decapeptyl, Somatuline LA, and Nutropin Depot have come to the market for the treatment of prostate cancer, acromegaly, and growth hormone deficiency.⁷⁻¹⁰ PLGA nano-microparticles are typically prepared by emulsion solvent evaporation technique and nanoprecipitation method, which require surfactants to stabilize the dispersed droplets, reduce their surface tension, and inhibit coalescence. Because of their high viscosity in aqueous solution and strong adsorption around the suspension droplets, poly(vinyl alcohol), poloxamer, and poly(vinylpyrrolidone) are the most commonly applied to fabricate uniform PLGA microspheres and nanoparticles for controlled delivery of various proteins and drugs.¹⁰,¹¹ These macromolecular surfactants are, however, associated with several drawbacks such as nonbiodegradability, potential toxicity, and lack of functional groups.¹²

Vitamin E polyethylene glycol-succinate (TPGS) has recently appeared as a versatile and biocompatible surfactant.¹³ Feng and Mu reported that TPGS could be applied for the preparation of 300–800 nm PLGA nanoparticles (PLGA NPs), and exhibited higher emulsification effects and improved loading efficiency compared with PVA.¹⁴,¹⁵ TPGS has shown to inhibit drug efflux, thus enhancing the cytotoxicities of anticancer drugs like doxorubicin, docetaxel, and PTX in multidrug resistant cancer cells.¹⁶⁻¹⁸ Moreover, TPGS showed intrinsic anticancer activities and could inhibit the growth of human lung tumor in nude mice.¹⁹ PLGA micro/nanoparticles stabilized by TPGS have, however, generally low stability and difficulty in surface functionalization with bioactive molecules. Here, we report on a novel class of biocompatible and functional surfactants based on vitamin E-oligo(methyl diglycol L-glutamate) and their application for facile preparation of multifunctional PLGA NPs for active tumor-targeting paclitaxel

Received: March 13, 2016
Revised: June 12, 2016
Published: June 15, 2016
E-ethylenediamine derivative (VE-NH$_2$) as a initiator. The following is a typical example on synthesis of VEOEG5 with pyrene (0.6 μM) as a fluorescent probe. VEOEG had varying fluorescence intensity at high and low concentration regions. The critical micelle concentration (CMC) of VEOEG was measured by using the fluorescence method. The CMC of VEOEG was determined as the cross-point when extrapolating the intensity rate at high and low concentration regions. The amount of amino groups at the chain end of VEOEG was determined by TNBSA assay.

2. EXPERIMENTAL METHODS

2.1. Synthesis of Vitamin E-NH$_2$. Briefly, 4-nitrophenyl chloroformate (4-NC) (1.98 g, 9.8 mmol) in DCM (30 mL) was dropwise added to DCM (10 mL) solution of vitamin E (VE) (2.12 g, 4.9 mmol) and pyridine (1.98 mL, 24.5 mmol) under nitrogen atmosphere at 0 °C. The reaction proceeded at 30 °C overnight. The mixture was filtered to remove the pyridine hydrochloride, and then evaporated to remove DCM to obtain raw product. The product was purified by dissolving in petroleum ether (b.p: 60−90 °C) and removing the insolubles by centrifugation at −5 °C. The evaporation of the solvent yielded Vitamin E-NH$_2$ as a yellowish oil. Yield: 93.4%. 1H NMR (400 MHz, CDCl$_3$, Figure S1): δ 4.35 (t, J = 6.0 Hz, CH$_3$); 3.67 (s, −NH$_2$); 2.90 (t, J = 7.0 Hz, −CH$_2$−); 2.03 (m, 3 × −CH$_2$−); 1.26 (m, 2 × −CH$_2$−); 0.86 (d, 3 × −CH$_3$−). To a DCM solution of ethylenediamine (1.35 mL, 20 mmol) and pyridine (1.6 mL, 20 mmol) in DCM (4 mL), VE-NH$_2$ (0.6 g, 1.0 mmol) solution in DCM (14 mL) was added dropwise under a N$_2$ atmosphere. The reaction proceeded overnight at room temperature (r.t.). The reaction mixture was washed with distilled water until the water phase became colorless. The DCM solution was dried with anhydrous magnesium sulfate at −24 °C for 24 h. The solution was filtered and the filtrate was evaporated. Yield: 78.1%. 1H NMR (400 MHz, CDCl$_3$, Figure S1B): δ 3.54 (s, −NH$_2$); 2.90 (t, J = 6.0 Hz, −CH$_2$−); 2.03 (m, 3 × −CH$_2$−); 1.35 (m, 2 × −CH$_2$−); 1.07 (m, 3 × −CH$_2$−); 0.83 (d, 3 × −CH$_3$−).

2.2. Synthesis of Vitamin E-O(EG2-Glu) (VEOEG). VEOEG was synthesized by polymerization of EG$_2$-Glu-NCA with VE-NH$_2$ as an initiator. The following is a typical example on synthesis of VEOEG$_3$ (5 denoted as the degree of polymerization of EG$_2$-Glu). Under a N$_2$ atmosphere, a DCM solution (37 mL) of VE-NH$_2$ (1.16 g, 2.25 mmol) was quickly added into a DCM solution (37 mL) of EG$_2$-Glu-NCA (3.71 g, 13.50 mmol). The reaction mixture was stirred for 12 h at 25 °C, condensed to about 18 mL, and then precipitated in diethyl ether. The product was dried at r.t. in vacuum for 24 h. Yield: 55.8%. 1H NMR (600 MHz, CDCl$_3$, Figure 1A): δ 4.24 (m, −NHCOCH$_2$−; t, −CONH−; 3.88 (s, −CH$_2$−); 1.94 (m, −CH$_2$−); 1.67 (m, −CH$_2$−). The critical micelle concentration (CMC) of VEOEG was measured with pyrene (0.6 μM) as a fluorescent probe. VEOEG had varying fluorescence intensity at high and low concentration regions. The CMC of VEOEG was determined by TNBSA assay.

The critical micelle concentration (CMC) of VEOEG was measured with pyrene (0.6 μM) as a fluorescent probe. VEOEG had varying concentrations from 0.2 to 2000 μg/mL. The fluorescence spectra were obtained using Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, excitation wavelength: 330 nm). The CMC was determined as the cross-point when extrapolating the intensity rate I$_{372}$/I$_{383}$ at high and low concentration regions. DOI: 10.1021/acs.biomac.6b00380

Biomacromolecules 2016, 17, 2367−2374
2.3. Fabrication of PLGA NPs. PLGA NPs were prepared using VEOEG as a surfactant by nanoprecipitation method.\textsuperscript{56} Briefly, 0.9 mL of PLGA solution in acetone (10.0 mg/mL) was added dropwise to 9.0 mL of 0.45 mol/L VEOEG aqueous solution (0.45 mg/mL) under stirring at rt. After stirring for 6 h, the acetone was evaporated. The resulting sample was collected by centrifugation (12 000 rpm, 10 min, 4 °C; Savant Biofuge Stratos, Thermo Scientific) and washed once with deionized water. The amount of VEOEG located on the surface of PLGA NPs was determined by comparing the integrals of OEG methylene protons at δ 3.54–3.68 with methine proton of PLGA at δ 5.21 (Figure S2).

2.4. Preparation of Hyaluronic Acid Coated PLGA NPs (HA-PLGA NPs). HA-PLGA NPs were formed through coating positively charged PLGA NPs with negatively charged HA followed by coupling reaction using EDC/NHS as coupling agents. Briefly, HA (45.5 mg, 1.3 μmol, 120.0 μmol carboxyl groups) was dissolved in 4.0 mL of acetate buffer (0.1 M, pH 5.0) and preactivated using EDC (6.9 mg, 36.0 μmol) and NHS (2.1 mg, 18.0 μmol) for 0.5 h at rt. The activated HA was added to the PLGA NPs suspension (VEOEG/activated carboxyl = 3:1 mol/mol) in NaCO\textsubscript{3}/NaHCO\textsubscript{3} buffer (pH 9.0) followed by incubation overnight at 37 °C to form HA-PLGA NPs. Excess reactive agents and byproducts were removed by extensive dialysis (MWCO 350 000 Da) against deionized water. The amount of VEOEG located on the surface of HA-PLGA NPs was determined by comparing the integrals of OEG methylene protons at δ 3.54–3.68 with methine proton of PLGA at δ 5.21 (Figure S2).

2.5. Drug Loading. PTX was loaded into nanoparticles through dropwise addition of acetone solution of PTX and PLGA (theoretical drug loading content: 8 wt %) to water phase containing a predetermined amount of VEOEG under stirring at room temperature. PTX-loaded PLGA NPs (PTX–PLGA NPs) were coated with HA as described above.

Drug loading content (DLC) and drug loading efficiency (DLE) were measured by high performance liquid chromatography (HPLC, Agilent Technologies 1260 Infinity) equipped with a reverse-phase HPLC column (Sephax GP-C18, 4.6 × 150 mm, 5 μm). Briefly, 200 μL of PTX-loaded HA-PLGA NPs (PTX–HA-PLGA NPs) were freeze-dried, dissolved in acetonitrile/water (1:1, v/v) solution, and filtered through 0.45 μm filter. The drug was detected with UV at 227 nm. The acetonitrile/water solvent mixture was used as a mobile phase, the flow rate was fixed at 1.0 mL/min, and the standard curve was obtained at different PTX concentrations (0.05–100 μg/mL). DLC and DLE were calculated according to the following formula:

\[
\text{DLC (wt %)} = \frac{\text{weight of loaded drug}}{\text{total weight of loaded drug and polymer}} \times 100
\]

\[
\text{DLE (％)} = \frac{\text{weight of loaded drug}}{\text{weight of drug in feed}} \times 100
\]

2.6. Pharmacokinetics Studies. The mice were handled under protocols approved by Soochow University Laboratory Animal Center and the Animal Care and Use Committee of Soochow University. Pharmacokinetics studies of PTX-HA-PLGA NPs were performed in nude mice (5 mg PTX/kg, n = 3). Taxol was used as a control. At 0.05, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h post injection for PTX-HA-PLGA NPs while 0.0333, 0.0833, 0.1667, 0.5, 1, 2, and 4 h post injection for the Taxol group, 20 μL of blood samples were collected in heparinized tubes. Each blood sample was dissolved in 1.0 mL of acetonitrile/water (1/1, v/v) and prefiltered with a 0.45 μm filter. The amount of PTX was measured by HPLC.

2.7. NIRF Imaging and Biodistribution. To determine the in vivo biodistribution of HA-PLGA NPs, a near-infrared fluorophore Cy5 labeled HA (HA-Cy5) was employed to coat onto PLGA NPs as described above for fluorescence imaging. HA-Cy5 was prepared by the reaction of Cy5-NHS with amino groups of HA-Lys-NH\textsubscript{2} which was obtained as a previous report.\textsuperscript{51} Human breast tumor xenografts were established by subcutaneous inoculation of 5 × 10\textsuperscript{6} MCF-7 cells in 50 μL of PBS into the right hind flank of each mouse. When the tumor size reached about 200 mm\textsuperscript{3}, 150 μL of Cy5-labeled HA-PLGA NPs in PBS was intravenously administrated at a concentration of 20 μg/mL. Noninvasive fluorescent imaging at various time intervals from 2 to 48 h after the injection was carried out using the IVIS Lumina II imaging system (Caliper Life Sciences). For ex vivo fluorescence imaging, MCF-7 tumor-bearing mice were sacrificed following 8 h iv injection with Cy5-labeled HA-PLGA NPs. The tumor block and several major organs were collected and imaged using the IVIS Lumina II imaging system.

The in vivo biodistribution of PTX-HA-PLGA NPs was studied in MCF-7 tumor-bearing mice. Eighteen mice with tumor size of ca. 200 mm\textsuperscript{3} were randomly assigned to two groups followed by intravenously injection of PTX-HA-PLGA NPs and Taxol, respectively (5 mg PTX/kg). Three mice were sacrificed at 4, 8, and 12 h post injection and the tumor block and several major organs were collected. The samples were homogenized in methanol (2 volumes of tissue) to extract PTX using a homogenizer (IKA T25) at 20 000 rpm for 1 min, 500 μL of acetonitrile was added, and the mixture was placed at −24 °C for 24 h followed by centrifugation. The supernatant was taken, dried, and redissolved in acetonitrile (500 μL) for HPLC measurement.

2.8. In Vivo Antitumor Efficacy. The in vivo antitumor efficacy of PTX–PLGA NPs was assessed using MCF-7 human breast cancer xenografts. Treatments were initiated when tumor size reached 50–80 mm\textsuperscript{3}. The mice were randomly divided into four groups (n = 6): (i) PTX-HA-PLGA NPs, (ii) Taxol, (iii) bare HA-PLGA NPs, and (iv) PBS (blank control). Different PTX formulations in 0.15 mL of PBS (5 mg PTX/kg) was intravenously administrated on day 0, 3, 6, 9, and 12. Tumor volume was calculated by the formula: \(V = 0.5 \times L \times W \times H\) where \(L\), \(W\), and \(H\) are the tumor dimension at the longest, widest, and highest points, respectively. Relative tumor volumes were calculated as \(V/V_0\) (\(V_0\) is the tumor volume at day 0). Mice were weighed with the relative body weights normalized to their initial weights. Mice were deemed to be dead once the tumor volume reaching 1000 mm\textsuperscript{3}.

3. RESULTS AND DISCUSSION

3.1. Synthesis of Vitamin E-O(EG\textsubscript{2}-Glu). Vitamin E-O(EG\textsubscript{2}-Glu) (VEOEG) was obtained by ROP of EG\textsubscript{2}-Gluc using vitamin E-NH\textsubscript{2} as an initiator (Scheme 2). Vitamin E-NH\textsubscript{2} was readily synthesized with a yield of 72.9% by coupling ethylenediamine to vitamin E (Scheme 2). \textsuperscript{1}H NMR demonstrated clear signals owing to both VE (δ 0.83–0.87, 1.07–1.37, 1.52, 1.77, 2.02, 2.08, 2.58) and ethylenediamine moieties (δ 2.90 and 3.33) (Figure S1B). The signals at δ 2.58

Scheme 2. Synthetic Route for Vitamin E-O(EG\textsubscript{2}-Glu) (VEOEG)\textsuperscript{a}

\textsuperscript{a}Conditions: (i) 4-nitrophenyl chloroformate, pyridine, DCM, 30 °C, 24 h; (ii) ethylenediamine, pyridine, DCM, 30 °C, 24 h; (iii) EG\textsubscript{2}-Gluc-NCA, DCM, 25 °C, 12 h.
positive surface charge of around +25.5 mV, which arises from Table 2). Mei et al. recently reported that PLGA NPs (S3A). The size of NPs decreased from 157 to 135 nm with Poisson distribution centered at the exact molecular weight 1B). Moreover, the mass spectrum of VEOEG displayed a morphology (Figure S3B). As expected, PLGA NPs displayed a micrograph revealed that PLGA NPs possessed a spherical morphology (Figure S5B). The in vitro drug release was

and 2.90 had an integral ratio close to 1:1, supporting equivalent coupling of VE and ethylenediamine. The polymerization of EG2-Glu-NCA was conducted in DCM at 25 °C, similar to previous reports. H NMR showed characteristic signals of O(EG2-Glu) block (δ 4.24, 3.68–3.54, 3.40–3.36, 1.99–1.88) and vitamin E end-group (δ 2.07, 2.03, 1.78, 1.53, 1.37–1.07, 0.87–0.83) (Figure 1A). The degree of polymerization (DP) of O(EG2-Glu) was estimated, through comparing the signals at δ 3.68–3.54 (methylene protons of EG2, n) and δ 0.87–0.83 (vitamin E methyl protons, a), to be 5.0, 8.4 and 9.2, which were proportional to the design (Table 1). GPC measurements showed that VEOEG had a narrow molecular weight distribution with major peaks corresponding to δ 0.83. (pyroglutamate) as a result of backbiting reaction and 

Table 1. Characteristics of Vitamin E-O(EG2-Glu)

<table>
<thead>
<tr>
<th>entry</th>
<th>VEOEG Conc (mg/mL)</th>
<th>PLGA NPs</th>
<th>HA-PLGA NPs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a)</td>
<td>size (nm)</td>
<td>DP (%)</td>
</tr>
<tr>
<td>1</td>
<td>0.15</td>
<td>157</td>
<td>0.07</td>
</tr>
<tr>
<td>2</td>
<td>0.30</td>
<td>148</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>0.45</td>
<td>135</td>
<td>0.08</td>
</tr>
</tbody>
</table>

and 2.90 had an integral ratio close to 1:1, supporting equivalent coupling of VE and ethylenediamine. The polymerization of EG2-Glu-NCA was conducted in DCM at 25 °C, similar to previous reports. H NMR showed characteristic signals of O(EG2-Glu) block (δ 4.24, 3.68–3.54, 3.40–3.36, 1.99–1.88) and vitamin E end-group (δ 2.07, 2.03, 1.78, 1.53, 1.37–1.07, 0.87–0.83) (Figure 1A). The degree of polymerization (DP) of O(EG2-Glu) was estimated, through comparing the signals at δ 3.68–3.54 (methylene protons of EG2, n) and δ 0.87–0.83 (vitamin E methyl protons, a), to be 5.0, 8.4 and 9.2, which were proportional to the design (Table 1). GPC measurements showed that VEOEG had a narrow molecular weight distribution with major peaks corresponding to δ 0.83. (pyroglutamate) as a result of backbiting reaction and 

Table 2. Characteristics of PLGA NPs and HA-PLGA NPs

<table>
<thead>
<tr>
<th>entry</th>
<th>VEOEG Conc (mg/mL)</th>
<th>PLGA NPs</th>
<th>HA-PLGA NPs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a)</td>
<td>size (nm)</td>
<td>DP (%)</td>
</tr>
<tr>
<td>1</td>
<td>0.15</td>
<td>157</td>
<td>0.07</td>
</tr>
<tr>
<td>2</td>
<td>0.30</td>
<td>148</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>0.45</td>
<td>135</td>
<td>0.08</td>
</tr>
</tbody>
</table>
proceeded in the medium containing Tween 80 (0.1%, v/v) to increase the solubility of PTX and maintain the sink conditions for PTX release from HA-PLGA NPs. The results showed that ca. 54.0%, 69.2%, and 79.3% of PTX was released from HA-PLGA NPs in 7 d at pH 7.4, 5.0, and 4.0, respectively (Figure 2). Remarkably, burst PTX release that is often reported for PLGA NPs\textsuperscript{44} was absent, confirming that PLGA NPs following HA coating have an excellent stability.

3.4. CD44-Targetability and Antitumor Activity of PTX-HA-PLGA NPs. MTT assays demonstrated that blank HA-PLGA NPs were nontoxic to L929 cells (normal cells) at 350 μg/mL, while displayed moderate cytotoxicity to MCF-7 and U87MG cells (cancerous cells) (Figure 3A). It has been reported that VE derivatives like vitamin E succinate (TOS) and TPGS display selective toxicity to malignant tumor cells.\textsuperscript{19,28} Remarkably, PTX-HA-PLGA NPs exhibited slightly higher antitumor effects to CD44 positive MCF-7 cells than Taxol at drug concentrations of 0.01\textsuperscript{−}10 μg/mL (Figure 3B). The IC\textsubscript{50} of PTX-HA-PLGA NPs in MCF-7 cells was determined to be ca. 0.37 μg PTX equiv./mL, 2-fold lower than Taxol. The pretreatment of MCF-7 cells with free HA resulted in a marked reduction of antitumor activity for PTX-HA-PLGA NPs (Figure 3B), confirming receptor-mediated internalization of HA-PLGA NPs by MCF-7 cells. In contrast, PTX-HA-PLGA NPs showed slightly lower antitumor effect in U87MG cells expressing low level of CD44 receptors than Taxol (Figure 3C). It is clear that PTX-HA-PLGA NPs can actively target and efficiently release PTX into CD44 overexpressing cancer cells leading to superb therapeutic activity.

3.5. In Vivo Pharmacokinetics and Biodistribution Studies in MCF-7 Tumor-Bearing Mice. The in vivo pharmacokinetic studies were performed by measuring the plasma PTX levels in mice at predetermined time points after injection of PTX-HA-PLGA NPs (5 mg PTX equiv./kg) using HPLC. Notably, PTX-HA-PLGA NPs revealed a long elimination half-life of 3.14 h, which is significantly improved as compared to Taxol (0.32 h) (Figure 4A).

The in vivo biodistribution of Cy5-labeled HA-PLGA NPs in human MCF-7 tumor-bearing mice was monitored using an IVIS Lumina II imaging system (Caliper Life Sciences). Interestingly, substantial tumor accumulation of HA-PLGA NPs was observed at 2 h post injection and the accumulation in tumor tissue reached the maximum at 8 h (Figure 4B). Strong fluorescence was detected even at 48 h post injection. The ex vivo fluorescence images confirmed that tumor Cy5 fluorescence was stronger than in all the healthy organs (Figure 4C). It should be noted that strong fluorescence was also observed in the liver, which has been reported for most HA nanoparticles due to cellular uptake of phagocytic cells and endothelial cells.\textsuperscript{31,45}

Moreover, PTX levels in the healthy organs and tumors following 4, 8, and 12 h injection of PTX-HA-PLGA NPs (5 mg PTX/kg) were quantified by HPLC measurements. The results displayed that PTX-HA-PLGA NPs at 4 h post injection gave a high tumor PTX level of 8.4%ID/g, which was about 6 times higher than in the healthy organs. The biodistribution data indicated that HA-PLGA NPs can accumulate in tumor tissue and efficiently release PTX into CD44 positive cancer cells leading to superb therapeutic activity.

Table 3. Characteristics of PTX-HA-PLGA NPs (Theoretical DLC = 8 wt %)

<table>
<thead>
<tr>
<th>entry</th>
<th>VEOEG Conc (mg/mL)</th>
<th>size (nm)</th>
<th>PDI\textsuperscript{a}</th>
<th>zeta (mV)</th>
<th>DLC (%)</th>
<th>DLE (%)\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.15</td>
<td>183</td>
<td>0.23</td>
<td>−26.9</td>
<td>6.1</td>
<td>74.7</td>
</tr>
<tr>
<td>2</td>
<td>0.30</td>
<td>174</td>
<td>0.21</td>
<td>−27.3</td>
<td>6.3</td>
<td>78.2</td>
</tr>
<tr>
<td>3</td>
<td>0.45</td>
<td>164</td>
<td>0.16</td>
<td>−27.9</td>
<td>6.4</td>
<td>80.1</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Determined by DLS. \textsuperscript{b}Measured by electrophoresis at 25 °C in water. \textsuperscript{c}Determined by HPLC.
higher than Taxol (1.4%ID/g). Tumor-to-normal tissue (T/N) distribution ratios showed that PTX-HA-PLGA NPs significantly enhanced tumor selectivity as compared to Taxol (Table S1). All the above results conclude that HA-PLGA NPs can selectively deliver PTX to the breast tumor.

3.6. In Vivo Antitumor Efficacy. The therapeutic performance of PTX-HA-PLGA NPs was investigated using MCF-7 tumor bearing nude mice. The mice were injected with 5 mg PTX equiv./kg on days 0, 3, 6, 9 and 12. Remarkably, tumor growth was almost completely suppressed by PTX-HA-PLGA NPs (Figure 5A). In comparison, continuous tumor progression was witnessed for mice received Taxol, blank HA-PLGA NPs, and PBS, in which the relative tumor volume on day 21 reached 7.7, 12.3, and 16.3, respectively. Figure 5B confirmed that mice following 21 day treatment with PTX-HA-PLGA NPs had the smallest tumor size. Notably, treatment with PTX-HA-PLGA NPs had no influence on mice body weight (Figure 5C), indicating that HA-PLGA NPs had low systemic toxicity. Moreover, survival data revealed that treatment with PTX-HA-PLGA NPs markedly improved the survival time of tumor-bearing mice, in which no death took place even after an experimental period of 38 days (Figure 5D). The histological analyses demonstrated that PTX-HA-PLGA NPs generated extensive tumor necrosis without causing significant side effects to the liver and kidney (Figure 6). The low liver damage is surprising as PTX-HA-PLGA NPs were shown to accumulate in the liver. In comparison, Taxol caused significant damage to the liver and kidney. It is evident that robust PTX-HA-PLGA NPs mediate efficient and targeted delivery of PTX to human breast tumor, resulting in superior therapeutic efficacy with little systemic side effects.

4. CONCLUSIONS

We have shown that amphiphilic vitamin E-oligo(methyl diglycol l-glutamate) (VEOEG) can be easily obtained and applied as a novel biocompatible and functional surfactant for the facile preparation of multifunctional and robust PLGA NPs to achieve targeted, safe, and efficient chemotherapy in vivo. To elaborate our concept, we have coated PLGA NPs with HA (HA-PLGA NPs) and studied their systemic delivery of PTX to CD44 positive breast tumor subcutaneously implanted in the nude mice. Thus, obtained PTX-HA-PLGA NPs exhibit several interesting merits: (i) they show a great stability without burst drug release and with prolonged blood circulation time in vivo; (ii) they show a superb tumor accumulation of 8.4%ID/g; (iii)
they demonstrate effective tumor inhibition with little adverse effects; and (iv) VEOEG is biocompatible and biodegradable, and shows selective cytotoxicity to cancerous cells. We are convinced that HA-PLGA NPs with straightforward fabrication, great safety, and excellent targetability to CD44 positive tumor have a great potential for translation to the clinics. This study has shown that, through rational design of functional surfactant, we can obtain multifunctional and robust PLGA nanoparticles for safe and significantly enhanced cancer therapy.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bio- macromol.6b00380.

Detailed information on materials and characterization, in vitro drug release, and histological analysis; 1H NMR spectra of vitamin E-4-NC, vitamin E-NH2, PLGA and VEOEG stabilized PLGA NPs; size distributions of PTX-PLGA NPs and PTX-HA-PLGA NPs; T/N distribution ratios of PTX (PDF)

**AUTHOR INFORMATION**

* Corresponding Authors

**AUTHOR INFORMATION**

**Corresponding Authors**

Tel./Fax: +86-512-65884933. E-mail: cdeng@suda.edu.cn (C.D.).

Tel./Fax: +86-512-65880098. E-mail: yzyzhong@suda.edu.cn (Z.Z.).

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was supported by the National Natural Science Foundation of China (NSFC 51273137, 51473110, and 51403147) and the National Science Fund for Distinguished Young Scholars (NSFC 51225302).

**REFERENCES**


(33) Zhao, X.; Tan, S.; Feng, S.-S. Biomaterials 2013, 34, 6058–6067.

(34) Zhao, X.; Tan, S.; Feng, S.-S. Biomaterials 2013, 34, 6058–6067.


(36) Zhao, X.; Tan, S.; Feng, S.-S. Biomaterials 2013, 34, 6058–6067.

(37) Zhao, X.; Tan, S.; Feng, S.-S. Biomaterials 2013, 34, 6058–6067.

(38) Zhao, X.; Tan, S.; Feng, S.-S. Biomaterials 2013, 34, 6058–6067.