Enzymatically and Reductively Degradable α-Amino Acid-Based Poly(ester amide)s: Synthesis, Cell Compatibility, and Intracellular Anticancer Drug Delivery

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Supporting Information

ABSTRACT: A novel and versatile family of enzymatically and reductively degradable α-amino acid-based poly(ester amide)s (SS-PEAs) were developed from solution polycondensation of disulfide-containing di-p-toluenesulfonic acid salts of bis-α-phenylalanine diesters (SS-Phe-2TsOH) with di-p-nitrophenyl adipate (NA) in N,N-dimethylformamide (DMF). SS-PEAs with $M_n$ ranging from 16.6 to 23.6 kg/mol were obtained, depending on NA/SS-Phe-2TsOH molar ratios. The chemical structures of SS-PEAs were confirmed by $^1$H NMR and FTIR spectra. Thermal analyses showed that the obtained SS-PEAs were amorphous with a glass transition temperature ($T_g$) in the range of 35.2–39.5 °C. The in vitro degradation studies of SS-PEA films revealed that SS-PEAs underwent surface erosion in the presence of 0.1 mg/mL α-chymotrypsin and bulk degradation under a reductive environment containing 10 mM dithiothreitol (DTT). The preliminary cell culture studies displayed that SS-PEA films could well support adhesion and proliferation of L929 fibroblast cells, indicating that SS-PEAs have excellent cell compatibility. The nanoparticles prepared from SS-PEA with PVA as a surfactant had an average size of 167 nm in phosphate buffer (PB, 10 mM, pH 7.4). SS-PEA nanoparticles while stable under physiological environment underwent rapid disintegration under an enzymatic or reductive condition. The in vitro drug release studies showed that DOX release was accelerated in the presence of 0.1 mg/mL α-chymotrypsin and was stable under physiological environment. Confocal microscopy observation displayed that SS-PEA nanoparticles effectively transported DOX into both drug-sensitive and drug-resistant MCF-7 cells. MTT assays revealed that DOX-loaded SS-PEA nanoparticles had a high antitumor activity approaching that of free DOX in drug-sensitive MCF-7 cells, while more than 10 times higher than free DOX in drug-resistant MCF-7/ADR cells. These enzymatically and reductively degradable α-amino acid-based poly(ester amide)s have provided an appealing platform for biomedical technology in particular controlled drug delivery applications.

INTRODUCTION

Synthetic biodegradable polymers have received continuous attention due to their wide range of applications, particularly in biomedical fields such as controlled drug release, gene transfer, and tissue engineering.1–5 Biodegradable aliphatic polyesters and polycarbonates due to their favorable biocompatibility and approved use in biomedical devices by the U.S. Food and Drug Administration (FDA) have become the most important synthetic biomaterials.6–10 However, in practice, these classical biomedical polymers often cannot meet the requirements of a particular application due to their high hydrophobicity, lack of reactive centers, and improper degradation rates.

In the past decades, α-amino acid-based poly(ester amide)s (PEAs), which possess the favorable properties of both polyesters and polypeptides, such as enzymatic degradability and bioactivity, have been developed as a versatile class of biodegradable polymers.11–15 A series of α-amino acid-based PEAs, which contain functional groups at the side chain (such as amine, carboxyl, hydroxyl, dithiopyridyl, carbon–carbon double bond, etc.)16–22 or in the main chain (such as carbon–carbon double bond, oligo(ethylene glycol), poly(ε-caprolactone), etc.)23–26 have been synthesized via solution polycondensation or interfacial polymerization and studied for various biomedical applications. For example, Chu et al. stated...
Scheme 1. Illustration of Enzymatically and Reductively Degradable SS-PEA Polymer for Cell Culture and Active Intracellular Anticancer Drug Delivery

In this paper, we report on the synthesis of novel enzymatically and reductively degradable L-phenylalanine-based PEs (SS-PEAs) and their applications for cell culture and anticancer drug delivery (Scheme 1). Notably, SS-PEAs exhibited excellent cell compatibility and efficient intracellular drug release resulting in effective reversal of drug resistance (ADR) in cancer cells. Herein, synthesis of SS-PEAs, cell compatibility, in vitro and intracellular release behaviors of doxorubicin (DOX)-loaded SS-PEA nanoparticles, and their antitumor activity in drug-sensitive and -resistant MCF-7 cells were investigated.

**EXPERIMENTAL SECTION**

**Materials.** SS-Phe-2TxsOH was synthesized via reaction of L-phenylalanine with bis(2-hydroxyethyl)disulfide (HES) in the presence of TxsOH·H2O, and the detailed procedure is described in the Supporting Information. NA was synthesized according to Chu's work and used as dicarboxylic acid segment to afford SS-PEA. The monomer was obtained as light yellow acicular crystal with Tm at 124.1–124.5 °C, which was in accordance with the previous report.

**Synthesis of Enzymatically and Reductively Degradable L-Phenylalanine-Based Poly(ester amide)s (SS-PEAs).** SS-PEAs bearing repeated disulfide bonds were synthesized via solution synthesized PEAs bearing pendant or embedding carbon–carbon double bonds, which could provide additional functional PEA derivatives via conjugation of thiol containing molecules or bioactive agents. Chen et al. reported that electroactive tetraaniline grafted PEA exhibited good electroactivity, mechanical properties as well as favorable cell adhesion and growth behavior of mouse preosteoblastic MC3T3-E1 cells. It should be noted that, despite their advantageous features and facile synthesis, there are little studies of α-amino acid-based PEs for drug delivery applications.

In recent years, tremendous efforts have been directed to the development of reduction-sensitive biodegradable polymers containing disulfide bonds for efficient intracellular drug and gene delivery. The disulfide bonds, while stable during the circulation and in the extracellular environment, would be cleaved rapidly in the cytosol due to the presence of 2–3 orders higher level of glutathione tripeptide (GSH; about 2–10 μM) than in the extracellular fluids (about 2–20 μM). The fast intracellular drug release triggered by cytoplasmic GSH has marked enhancement of the therapeutic effects of anticancer drugs in vitro and in vivo.

In this paper, we report on the synthesis of novel enzymatically and reductively degradable L-phenylalanine-based PEs (SS-PEAs) and their applications for cell culture and anticancer drug delivery. Herein, synthesis of SS-PEAs, cell compatibility, in vitro and intracellular release behaviors of doxorubicin (DOX)-loaded SS-PEA nanoparticles, and their antitumor activity in drug-sensitive and -resistant MCF-7 cells were investigated.
polycondensation of SS-Phe-2TsOH and NA using Et3N as the acid receptor for TsOH (Scheme 2). Take the synthesis of SS-PEA at a

**Scheme 2. Synthesis of Reduction-Sensitive L-Phenylalanine-Based Poly(ester amide)s (SS-PEA)**

\[
\begin{align*}
\text{SS-Phe-2TsOH} & \quad \text{DMF, Et3N} \\
70 \degree \text{C} & \quad 48 \text{ h}
\end{align*}
\]

enzymatically and reductively degradable poly(ester amide) (SS-PEA)

NA/SS-Phe-2TsOH molar ratio of 1:1 as an example. To a Schlenk bottle equipped with a magnetic stir bar were charged SS-Phe-2TsOH (0.710 g, 0.895 mmol), NA (0.347 g, 0.895 mmol), Et3N (0.275 mL, 1.969 mmol), and 0.471 mL of DMF. After 20 min of degassing with a nitrogen flow, the reaction vessel was sealed and immersed in an oil bath thermostated at 70 °C. The polymerization was allowed to proceed for 48 h. The resulting polymer was isolated by dialysis with DMF, precipitation in ethyl acetate two times to remove p-nitrophenol, precipitation in water once to remove Et3N, Na2SO4, and freeze-drying for 2 d. Yield: 82.5%, \( M_\text{w}/M_\text{n} = 2.4 \).

**Reductive Degradation of SS-PEAs.** Under a nitrogen atmosphere, SS-PEA (100 mg), DTT (61 mg, 0.4 mmol), and 2 mL of DMF were charged into a Schlenk bottle equipped with a magnetic stir bar. After stirring at 30 °C for predetermined intervals, aliquots of polymer solutions (e.g., 0.4 mL) were taken to obtain the degraded products via precipitation in ultrapure water, filtration, washing several times with water under a N2 atmosphere, SS-PEA (100 mg), DTT (61 mg, 0.4 mmol), and 2 mL of DMF. The collection of samples was then dried under vacuum to a constant weight. The collected samples were then dried in vacuo to a constant weight. The degree of degradation was estimated from the weight loss (%)

\[
\text{weight loss(%) = (W_0 - W) / W_0 \times 100%}
\]

in which \( W_0 \) and \( W \) represent initial weight of film and weight of film at time \( t \). In addition, the molecular weight and PDI of the SS-PEA films after degradation were determined by GPC.

**In Vitro Enzymatic and Reductive Degradation of SS-PEA Films.** SS-PEA (NA/SS-Phe-2TsOH = 1:1) films were cast from a 40 mg/mL chloroform solution onto glass microscope slides (1 cm × 1 cm), which was allowed to thoroughly dry by evaporation overnight at room temperature (r.t.) followed by drying in vacuo for 2 d. The films (each in duplicate) were immersed in 1 mL of PBS buffer (pH 7.4, containing 0.05 w/v% sodium azide to inhibit bacterial growth) with \( \alpha \)-chymotrypsin (0.1 mg/mL) or 10 mM DTT in a 24-well cell culture plate and incubated at 37 °C and 120 rpm. SS-PEA films incubated in pure PBS buffer under otherwise the same conditions were used as a control. The degradation medium was refreshed every 24 h. At predetermined intervals, the remaining polymer samples (on slides) were collected via aspiration of the incubation medium followed by rinsing of the wells three times for 5 min with ultrapure water. The collected samples were then dried in vacuo to a constant weight. The degree of the degradation was estimated from the weight loss of the SS-PEA films based on the following formula:

**Enzyme or DTT.** The size change of nanoparticles in response to reductive or enzymatic conditions was followed by DLS measurements. Briefly, to a SS-PEA nanoparticle dispersion (0.5 mg/mL) was added a predetermined amount of \( \alpha \)-chymotrypsin or DTT to yield a final enzyme concentration of 0.1 mg/mL or a final DTT concentration of 10 mM. The solution was placed in a shaking bed at 37 °C with a rotation speed of 200 rpm. At different time intervals, the size of the nanoparticles was determined using DLS.

**Encapsulation of DOX into Nanoparticles.** DOX was loaded into SS-PEA nanoparticles by dropwise addition of 0.4 mL of water containing 2 w/v% PVA to 0.2 mL of SS-PEA (NA/SS-Phe-2TsOH = 0.96:1) solution (2 mg/mL) in DMSO at r.t., followed by extensive dialysis (MWCO 350 kDa) against PB (10 mM, pH 7.4) for 24 h with at least 5 times change of media. The final nanoparticle concentration was about 0.5 mg/mL. The size and size distribution of the nanoparticles were determined via DLS.

**Size Change of SS-PEA Nanoparticles in Response to Enzyme or DTT.** The size change of nanoparticles in response to reductive or enzymatic conditions was followed by DLS measurements. Briefly, to a SS-PEA nanoparticle dispersion (0.5 mg/mL) was added a predetermined amount of \( \alpha \)-chymotrypsin or DTT to yield a final enzyme concentration of 0.1 mg/mL or a final DTT concentration of 10 mM. The solution was placed in a shaking bed at 37 °C with a rotation speed of 200 rpm. At different time intervals, the size of the nanoparticles was determined using DLS.

**Preparation of SS-PEA Nanoparticles.** SS-PEA nanoparticles were prepared under stirring by dropwise addition of 0.4 mL water containing 2 w/v% PVA to 0.2 mL of SS-PEA (NA/SS-Phe-2TsOH = 0.96:1) solution (2 mg/mL) in DMSO at r.t., followed by extensive dialysis (MWCO 350 kDa) against PB (10 mM, pH 7.4) for 24 h with at least 5 times change of media. The final nanoparticle concentration was about 0.5 mg/mL. The size and size distribution of the nanoparticles were determined via DLS.

**Determination of DLC and DLE.** The size and size distribution of the nanoparticles were determined via DLS.

**Drug Loading Content (DLC) and Drug Loading Efficiency (DLE).** The size and size distribution of the nanoparticles were determined via DLS.

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**Encapsulation of DOX into Nanoparticles.** DOX was loaded into SS-PEA nanoparticles by dropwise addition of 0.4 mL of water containing 2 w/v% PVA to a mixture of 0.2 mL of SS-PEA solution (2 mg/mL) and DOX solution (5 mg/mL) in DMSO at varying drug/polymer weight ratios (1:5–30 wt %) under stirring at r.t., followed by dialysis against PB (10 mM, pH 7.4) for 8 h (MWCO 350 kDa) in the dark. The dialysis media were changed five times. For determination of drug loading content (DLC) and drug loading efficiency (DLE), lyophilized drug-loaded nanoparticles were dissolved in DMSO. The amount of DOX was determined using fluorescence (FLS920) measurement (excitation at 480 nm and emission at 560 nm). The DLC and DLE were calculated according to the following formula:

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

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

**Enzyme and Reduction-Triggered DOX Release.** The in vitro release profiles of DOX-loaded SS-PEA nanoparticles were studied using a dialysis tube (MWCO 12000–14000) at 37 °C in three different media, that is, PB (10 mM, pH 7.4) only, PB with 0.1 mg/mL \( \alpha \)-chymotrypsin, or PB with 10 mM DTT. In order to acquire sink conditions, drug release studies were performed at a nanoparticle concentration of 0.5 mg/mL and with a 0.6 mL of nanoparticle dispersion dialysis against 20 mL of the same media. At desired time intervals, 6 mL of release media was taken out and replenished with an equal volume of fresh media. The amount of DOX released was determined using fluorescence (FLS920) measurement (excitation at 570 nm of each well was measured using a microplate reader. Data are presented as average ± standard deviation (n = 3).

**Preparation of SS-PEA Nanoparticles.** SS-PEA nanoparticles were prepared under stirring by dropwise addition of 0.4 mL water containing 2 w/v% PVA to 0.2 mL of SS-PEA (NA/SS-Phe-2TsOH = 0.96:1) solution (2 mg/mL) in DMSO at r.t., followed by extensive dialysis (MWCO 350 kDa) against PB (10 mM, pH 7.4) for 24 h with at least 5 times change of media. The final nanoparticle concentration was about 0.5 mg/mL. The size and size distribution of the nanoparticles were determined via DLS.
Table 1. Characteristics of Enzymatically and Reductively Degradable SS-PEA Polymers

<table>
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<th>entry</th>
<th>NA/SS-Phe-2TsOH (mol/mol)</th>
<th>Mn,GPC (kg/mol)</th>
<th>Mw,GPC (kg/mol)</th>
<th>PDI</th>
<th>Tg (°C)</th>
<th>yield (%)</th>
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<td>1</td>
<td>1.0:1.0</td>
<td>23.6</td>
<td>56.6</td>
<td>2.40</td>
<td>39.5</td>
<td>82.5</td>
</tr>
<tr>
<td>2</td>
<td>0.98:1.0</td>
<td>22.3</td>
<td>44.1</td>
<td>1.97</td>
<td>35.2</td>
<td>65.7</td>
</tr>
<tr>
<td>3</td>
<td>0.96:1.0</td>
<td>21.8</td>
<td>36.6</td>
<td>1.68</td>
<td>37.0</td>
<td>63.0</td>
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<td>16.6</td>
<td>24.0</td>
<td>1.45</td>
<td>37.5</td>
<td>62.9</td>
</tr>
</tbody>
</table>

at 480 nm). The release experiments were conducted in triplicate and the results presented are the average data with standard deviations.

**Cytotoxicity Assays.** The anticancer activity of DOX-loaded SS-PEA nanoparticles was evaluated in human breast adenocarcinoma MCF-7 cells and P-gp overexpressing human breast adenocarcinoma cells (DOX-resistant MCF-7 cells, MCF-7/ADR) via MTT assays. The MCF-7 and MCF-7/ADR cells were seeded in a 96-well plate at a density of 1 × 10^4 cells/well in 90 μL of DMEM supplemented with 10% FBS, 1% l-glutamine, and antibiotics penicillin (100 IU/mL) and streptomycin (100 μg/mL) for 24 h. A total of 10 μL of DOX-loaded SS-PEA nanoparticles or free DOX in PBS (10 mM, pH 7.4) was added to give varying drug dosages from 0.0001 to 100 μg/mL. The cells were cultured for another 72 h, and 10 μL of MTT solution in PBS (5 mg/mL) was added. The cells were incubated for 4 h. The medium was aspirated, the MTT-formazan generated by live cells was dissolved in 150 μL of DMSO, and the absorbance at a wavelength of 570 nm of each well was measured using BioTek microplate reader. The relative cell viability (%) was determined by comparing the absorbance at 570 nm with control wells containing only cell culture medium. Data are presented as average ± standard deviation (n = 4). The cytotoxicity of blank SS-PEA nanoparticles following 48 h incubation was determined in a similar way using MCF-7 and MCF-7/ADR cells with various nanoparticle concentrations of 0.1–1.0 mg/mL.

**Confocal Laser Scanning Microscopy (CLSM) Studies.** MCF-7 and MCF-7/ADR cells were plated on microscope slides in a 24-well plate (5 × 10^4 cells/well) under 5% CO2 atmosphere at 37 °C using DMEM medium supplemented with 10% FBS, 1% l-glutamine, antibiotics penicillin (100 IU/mL) and streptomycin (100 μg/mL) for 24 h. A total of 50 μL of DOX-loaded SS-PEA nanoparticles or free DOX (dosage: 20 μg/mL) was added. After incubation for 3 h, the culture medium was removed and the cells were washed 3 times with PBS. The cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, blue) for 15 min and washed 4 times with PBS. The images of cells were obtained using a confocal laser scanning microscope (TCS SP2).

**RESULTS AND DISCUSSION**

**Synthesis of Enzymatically and Reductively Degradable SS-PEAs.** SS-PEAs were readily synthesized via solution polycondensation of NA and SS-Phe-2TsOH in the presence of Et3N with L-phenylalanine in the presence of TsOH (Scheme 2). SS-Phe-2TsOH was obtained as white crystals by reacting disulde-containing diol (HES) with L-phenylalanine in the presence of TsOH. SS-Phe-2TsOH was then recrystallized from methanol/water (1:1 v/v). The reductive degradation of SS-PEAs. The reductive cleavage of disulfide bonds in the repeating units of SS-PEA was investigated in DMF at 30 °C using DTT as a reducing agent. 1H NMR and GPC measurements confirmed successful cleavage of disulfide bonds to afford small molecules after 23 h. The resonances at δ 4.24 and 2.86 attributable to the thiol protons were clearly visible in the 1H NMR spectra of (A) SS-PEA and (B) SS-PEA after treatment with DTT for 23 h. The lower Tg of SS-PEAs is most likely related to their more flexible main chain resulting from the presence of multiple disulfide bonds along the backbone.
100% following 2.5, 5, 8, 11, and 23 h treatment with DTT, respectively. GPC measurements revealed no eluent peak for SS-PEA after 23 h of treatment with DTT, supporting complete reductive degradation of polymer.

**In Vitro Enzymatic and Reductive Degradation of SS-PEA Films.** The enzymatic and reductive degradation kinetics of SS-PEA films were investigated in α-chymotrypsin (0.1 mg/mL or DTT solution (10 mM) in PBS at pH 7.4. As expected, SS-PEA films exhibited slow hydrolysis in pure PBS buffer with less than 15% weight loss in 30 d (Figure 2), similar to α-amino acid based PEAs reported previously.26,38,39 In contrast, fast weight loss occurred in the presence of α-chymotrypsin, in which 70 and 94% weight loss were observed in 1 and 3 d, respectively. SS-PEA films showed also much faster weight loss (16% in 3 d and 70% in 30 d) in 10 mM DTT than in PBS. It is clear, therefore, that SS-PEAs are prone to both enzymatic and reductive degradation.

To further study the degradation behaviors of SS-PEAs, molecular weights of SS-PEA film residues following enzymatic or reductive degradation were determined by GPC. The results showed that though α-chymotrypsin caused significant weight loss (70%) of SS-PEA film following 1 d incubation, little change in the molecular weight and molecular weight distribution was observed for SS-PEA film residues (Table S1), which is consistent with a surface-erosion degradation mechanism.40,41 In contrast, no high molecular weight polymer was observed for SS-PEA film residues following 1 d incubation in 10 mM DTT, although the weight loss was only 12.5%. These results indicated that SS-PEAs are likely subject to bulk degradation under a reductive condition. The low weight loss could be due to the fact that reductive degradation products are water insoluble. Notably, SS-PEA film following 12 d hydrolytic degradation (14% weight loss) resulted in a slight decrease in molecular weight.

The surface morphology of SS-PEA films following degradation in different media was studied using SEM (Figure S3). After 1 d incubation in pure PBS buffer, SS-PEA film showed negligible surface erosion. However, severe surface erosion was observed following treatment with α-chymotrypsin or DTT, further supporting fast enzymatic and reductive degradation. Notably, α-chymotrypsin resulted in the most significant surface erosion, in line with the weight loss data.

**Formation and Triggered Disruption of SS-PEA Nanoparticles.** SS-PEA nanoparticles were prepared by the solvent exchange method using PVA as a stabilizer. DLS measurements revealed that SS-PEA polymer formed nanoparticles with average size of about 167 nm, low polydispersity of 0.08 (Figure 3 and Table S2). TEM showed that these nanoparticles had a spherical morphology (Figure 3A). Zeta potential measurements revealed a negative zeta potential of −8.9 mV.

The change of SS-PEA nanoparticle sizes in response to 0.1 mg/mL α-chymotrypsin or 10 mM DTT was studied in PB buffer (10 mM, pH 7.4) using DLS. Notably, α-chymotrypsin caused fast disintegration of SS-PEA nanoparticles, in which nanoparticle size decreased quickly with time yielding 8.7 nm unimers after 5 h (Figure 3A). Similarly, fast disruption of nanoparticles was also detected under a reductive condition containing 10 mM DTT, wherein nanoparticle size decreased to 14 nm in 6.5 h (Figure 3B). In contrast, little change in nanoparticle sizes was discerned in 26 h in the absence of DTT and α-chymotrypsin under otherwise the same conditions.

**Cell Compatibility Studies.** The cell compatibility of SS-PEAs was studied by culturing L929 fibroblast cells on SS-PEA films for 4 d as well as incubating MCF-7 and MCF-7/ADR cells with SS-PEA nanoparticles for 2 d. The results showed that SS-PEA film, similar to the tissue culture plate control, well supported the adhesion and growth of L929 cells (Figure 4), indicating that SS-PEA film was nontoxic, as reported for other α-amino acid-based poly(ester amide)s.42–45 The proliferation of L929 fibroblast cells on SS-PEA film was quantitatively determined by MTT assays. Notably, cells cultured on SS-PEA film exhibited similar proliferation rate to that for tissue culture plate from 1 to 4 d (Figure 5), further confirming that SS-PEAs are compatible to cells. Interestingly, MTT assays of SS-PEA

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**Figure 2.** Percentage of SS-PEA film weight loss as a function of degradation time (d) in PBS containing 0.1 mg/mL α-chymotrypsin or 10 mM DTT at 37 °C and 120 rpm.

**Figure 3.** Change of SS-PEA nanoparticle size in response to (A) 0.1 mg/mL α-chymotrypsin and (B) 10 mM DTT determined by DLS. The inset in A represents the TEM image of SS-PEA nanoparticles.
nanoparticles in MCF-7 and MCF-7/ADR cells also revealed high cell viabilities (ranging from 92.4 to 115.2%) up to a tested nanoparticle concentration of 1.0 mg/mL following 2 d incubation (Figure 6), supporting that SS-PEAs have excellent cell compatibility.

**Loading and Triggered Release of DOX.** DOX was loaded into SS-PEA nanoparticles at theoretical drug loading contents (DLC) of 5, 10, 20, and 30 wt %. The results showed that SS-PEA nanoparticles could achieve a high DOX loading content of 17.3 wt % (Table S2). DLS showed that DOX-loaded SS-PEA nanoparticles had a low PDI of 0.04–0.07 and average sizes ranging from 169 to 182 nm, depending on drug contents. Remarkably, in vitro release studies revealed accelerated DOX release from SS-PEA nanoparticles in the presence of 0.1 mg/mL α-chymotrypsin or 10 mM DTT, in which about 84 and 79% of DOX was released in 24 h, respectively (Figure 7). In comparison, only about 45% of DOX was released in 24 h in PB buffer under otherwise the same conditions. These results point out that drug release from SS-PEA nanoparticles is promoted by enzyme or a reductive condition.

**Intracellular DOX Delivery and Antitumor Activity of DOX-Loaded SS-PEA Nanoparticles.** The cellular uptake and intracellular drug release behaviors of DOX-loaded SS-PEA nanoparticles were investigated using CLSM in both MCF-7 and MCF-7/ADR cells. Interestingly, strong DOX fluorescence was observed in MCF-7 cells following a 3 h incubation with DOX-loaded SS-PEA nanoparticles (Figure 8A), indicating fast internalization of nanoparticles and rapid release of DOX inside the cells. The cellular uptake and intracellular drug release behaviors of DOX-loaded SS-PEA nanoparticles were investigated using CLSM in both MCF-7 and MCF-7/ADR cells. Interestingly, strong DOX fluorescence was observed in MCF-7 cells following a 3 h incubation with DOX-loaded SS-PEA nanoparticles (Figure 8A), indicating fast internalization of nanoparticles and rapid release of DOX inside the cells.
Remarkably, CLSM observations showed that DOX-loaded SS-PEA nanoparticles could also effectively transport and release DOX into the cytosol and nuclei of MCF-7/ADR cells in 3 h (Figure 8B). In comparison, negligible DOX fluorescence was observed in MCF-7/ADR cells treated with free DOX under otherwise the same conditions (Figure S4B). It is evident that enzyme and reduction dual-responsive SS-PEA nanoparticles can markedly enhance the drug concentration in MDR cancer cells, which might effectively overcome drug resistance.

The antitumor activity of DOX-loaded SS-PEA nanoparticles was investigated via MTT assays in MCF-7 and MCF-7/ADR cells. The results revealed that DOX-loaded SS-PEA nanoparticles exhibited a high antitumor effect to MCF-7 cells with a low half-maximal inhibitory concentration (IC_{50}) of 1.47 μg DOX equiv/mL following 72 h incubation, which was approaching to that for free DOX (Figure 9A). It is even more interesting to note that DOX-loaded SS-PEA nanoparticles caused also potent antitumor effect to MCF-7/ADR cells with an IC_{50} of 10.1 μg DOX equiv/mL following 72 h incubation (Figure 9B). In contrast, free DOX exhibited marginal cytotoxicity toward MCF-7/ADR cells under otherwise the same conditions (ca. 70% cell viability at a DOX dosage of 100 μg/mL), supporting strong DOX resistance. The effective reversal of drug resistance observed for DOX-loaded SS-PEA nanoparticles is likely due to their uptake by cells via endocytosis in combination with fast and efficient intracellular drug release.46,47 Notably, several studies showed that DOX-loaded nanoparticles exhibited several times higher antitumor activity toward MCF/ADR cells than free DOX.48−50 The antitumor efficacy of DOX-loaded SS-PEA nanoparticles in MCF/ADR cells was much enhanced (>10 times) as compared to free DOX, which makes it an intriguing system to overcome drug resistance. The occurrence of drug resistance is a major challenge for cancer chemotherapy.51,52 It is worthy to note that functionalization of SS-PEA nanoparticles with a specific ligand such as antibody, peptide, and aptamer might further enhance their antitumor potency.53 We are convinced that SS-PEAs with facile synthesis, excellent cell compatibility, reductive and enzymatic degradability, and efficient intracellular drug release have a tremendous potential in development of multifunctional drug delivery systems.

**CONCLUSIONS**

We have demonstrated that enzymatically and reductively degradable α-amino acid-based poly(ester amide)s (SS-PEAs) can be readily prepared under mild conditions. Notably, SS-PEAs either in the form of films or nanoparticles exhibit excellent cell compatibility, fast surface degradation by α-chymotrypsin, and bulk degradation under an intracellular-mimicking reductive environment. The initial drug loading and
release studies show that SS-PEA nanoparticles have decent loading of DOX and drug release is accelerated by α-chymotrypsin or dithiothreitol. Moreover, DOX-loaded SS-PEA nanoparticles exhibit potent antitumor efficacy toward both drug-sensitive and drug-resistant MCF-7 cells. SS-PEAs with unique properties of facile synthesis, excellent biocompatibility, enzymatic degradability, and reduction-sensitivity are of particular interest in the development of smart nanosystems for targeted cancer chemotherapy.

**ASSOCIATED CONTENT**

- Supporting Information
  - Synthesis of SS-Phe-2TsOH and NA monomers; 1H NMR spectrum of SS-Phe-2TsOH; FTIR spectra; SEM images, weight loss, and GPC data of SS-PEA films prior to and after incubation in different media; CLSM images of MCF-7 and MCF-7/ADR cells incubated with free DOX; and DOX loading results of SS-PEA nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.

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