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Polymeric Nanoparticles with Precise Ratiometric Control over Drug Loading for Combination Therapy

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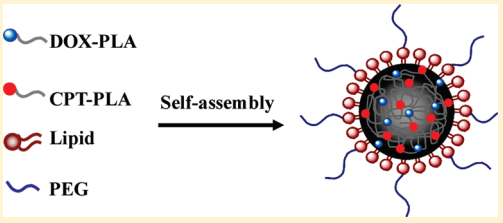
ABSTRACT: We report a novel approach for nanoparticle-based combination chemotherapy by concurrently incorporating two different types of drugs into a single polymeric nanoparticle with ratiometric control over the loading of the two drugs. By adapting metal alkoxide chemistry, we synthesize highly hydrophobic drug−poly-L-lactide (drug−PLA) conjugates, of which the polymer has the same chain length while the drug may differ. These drug−polymer conjugates are then encapsulated into lipid-coated polymeric nanoparticles through a single-step nanoprecipitation method. Using doxorubicin (DOX) and camptothecin (CPT) as two model chemotherapy drugs, various ratios of DOX−PLA and CPT−PLA conjugates are loaded into the nanoparticles with over 90% loading efficiency. The resulting nanoparticles are uniform in size, size distribution and surface charge. The loading yield of DOX and CPT in the particles can be precisely controlled by simply adjusting the DOX−PLA:CPT−PLA molar ratio. Cellular cytotoxicity results show that the dual-drug loaded nanoparticles are superior to the corresponding cocktail mixtures of single-drug loaded nanoparticles. This dual-drug delivery approach offers a solution to the long-standing challenge in ratiometric control over the loading of different types of drugs onto the same drug delivery vehicle. We expect that this approach can be exploited for many types of chemotherapeutic agents containing hydroxyl groups and thus enable codelivery of various drug combinations for combinatorial treatments of diseases.



KEYWORDS: dual-drug delivery, codelivery, polymer−drug conjugate, ratiometric drug loading, hybrid nanoparticles

INTRODUCTION

Nanoparticulate drug delivery systems have become increasingly attractive in systemic cancer drug delivery because of their ability to prolong drug circulation half-life, reduce nonspecific uptake, and better accumulate at the tumors through enhanced permeation and retention (EPR) effect or active targeting.[1−6] As a result, several therapeutic nanoparticles such as Doxil and Abraxane are used as the frontline therapies in clinics. But despite the advancement in nanoparticle drug delivery, most research efforts focus on single drug encapsulation whereas delivering multiple drugs with a single vesicle remains largely unexplored. Combination chemotherapy has shown superior clinical therapeutic efficacy compared to single-drug therapy, particularly in retarding the development of cancer chemoresistance.[7,8] It has been frequently observed that the cancer cells acquire defense mechanisms by overexpressing drug efflux pumps, increasing drug metabolism, enhancing self-repairing ability or expressing altered drug targets,[9,10] resulting in diminishing efficacy and ultimately treatment failures. Many drugs have been routinely administered in combination to improve the treatment effectiveness.[11−13] However, current combination regimens are limited by the distinct pharmacokinetics and biodistribution of different drug molecules and offer little flexibility for treatment optimization.

In a recent review, we summarized the latest progress in concurrently delivering multiple types of drugs for combination chemotherapy using a single vehicle such as liposomes, polymeric nanoparticles, and other nanocarriers.[14] One of the biggest motivations behind nanoparticle-based combination therapies is their ability to unify the pharmacokinetics and biodistribution of different drug molecules, thereby enabling dosage optimization *in vivo*. By delivering multiple therapeutic agents to the target cells simultaneously, the multidrug delivery nanoparticles can promote drug synergism and pave the way to precision design and tailoring in cancer chemotherapeutics. Several strategies have been employed to coencapsulate multiple drugs into a single nanocarrier, including physical loading into the particle core,[15−17] chemical conjugation to the particle surface,[18] and covalent linkage to the polymer backbone prior to nanoparticle synthesis.[19−22] However, controlling the ratios of different types of drugs in the same nanoparticles remains a major challenge because of factors such as steric hindrance between the different drug molecules and the polymer backbones, batch-to-batch heterogeneity in conjugation chemistry, and variability in drug-to-drug and drug-to-polymer interactions. One approach to address these issues is to covalently conjugate dual drugs using a hydrolyzable linker and then encapsulate the drug−drug conjugates into nanoparticles for codelivery.[23] Despite the success in maintaining the ratio between two drugs with drastically different properties, this linker approach is constrained to small drug-to-drug molar ratio such as 1:1 and thus has limited clinical applicability. Herein, we present a versatile dual-drug encapsulation

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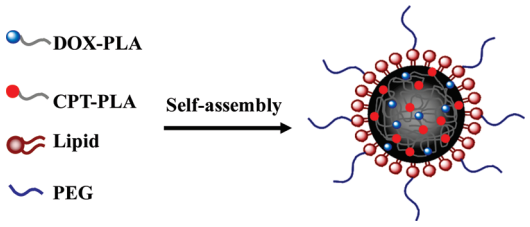

Figure 1. Schematic illustration of a dual-drug loaded lipid−polymer hybrid nanoparticle, of which the polymeric core consists of two distinct drug−polymer conjugates with ratiometric control over drug loading.

scheme in which each different drug molecule is linked to an individual polymer backbone that has the same physicochemical properties and nearly the same chain length. These drug−polymer conjugates are subsequently mixed at predetermined ratios for nanoparticle synthesis. The long and sharply distributed polymer chain gives each drug molecule a predominant and uniform hydrophobic property, yielding nearly 100% drug encapsulation efficiency upon nanoparticle formation.

In this study, we demonstrated the synthesis of drug−polymer conjugates with two different chemotherapeutics, doxorubicin (DOX) and camptothecin (CPT).[24] Utilizing ring-opening polymerization of L-lactide, we synthesized DOX and CPT polymer conjugates using metal-amido catalyst, which reacts selectively with hydroxyl groups of the drug molecules to initiate polymerization.[24−26] Using a nanoprecipitation technique (Figure 1), the drug−polymer conjugates were quantitatively loaded into lipid−polymer hybrid nanoparticles at high loading yield and precisely controlled drug ratios. The combinatorial treatment proposed here showed superior efficacy to cocktail therapy *in vitro* and offers a solution to the aforementioned limitations in multidrug encapsulation into the same nanoparticles. Since this drug−polymer conjugation approach can be generalized to a variety of therapeutic agents, we expect this combinatorial drug delivery system would set a new paradigm in nanomedicine for different combination therapies.

EXPERIMENTAL SECTION

Materials. L-Lactide was purchased from Sigma-Aldrich Co. (Milwaukee, WI), recrystallized three times in ethyl acetate and dried under vacuum. L-Lactide crystals were further dried inside a glovebox, sealed into a glass vial under dry argon and then stored at −20 °C prior to use. 2,6-Di-isopropylaniline (Sigma-Aldrich Co.) and 2,4-pentanedione (Alfa Aesar Co., Ward Hill, MA) were used as received. All other chemicals and anhydrous solvents were purchased from Sigma-Aldrich Co. unless otherwise specified. Anhydrous etrahydrofuran (THF) and toluene were prepared by distillation under sodium benzophenone and were kept anhydrous by using molecular sieves. The 2-((2,6-diisopropylphenyl)amino)-4-((2,6-diisopropylphenyl)imino)-2-pentene (BDI) ligand and the corresponding metal catalysts (BDI)ZnN($SiMe_3$)$_2$ were prepared inside a glovebox following a published protocol and stored at −20 °C prior to use. DOX·HCl was purchased from Jinan Wedo Co., Ltd. (Jinan, China) and used as received. Removal of HCl from DOX·HCl was achieved by neutralizing DOX·HCl solution in water with triethyleamine, after which the solution color changed from red to purple. The free base form of DOX was subsequently extracted with dichloromethane. The organic extract was filtered through anhydrous Na_2SO_4 and dried under vacuum to collect DOX crystals. (*S*)-(+)-Camptothecin (CPT) was purchased from TCI America and used as received.

Synthesis of 2-((2,6-Diisopropylphenyl)amino)-4-((2,6-diisopropylphenyl)imino)-2-pentene (BDI). Ligand BDI was prepared following a previously published protocol with minor modification.[27] Briefly, 2,6-di-*n*-propylaniline (13.0 mmol) and 2,4-pentanedione (6.5 mmol) in the ratio of 2:1 were dissolved in absolute ethanol (20 mL). The mixture solution was acidified with concentrated HCl (0.6 mL) and heated at reflux for 48 h, which resulted in white precipitates. After being cooled to room temperature, the white precipitates were dissolved with dichloromethane and saturated aqueous bicarbonate solution. The orange colored solution was then extracted, washed with brine three times, and filtered through anhydrous Na_2SO_4, followed by being concentrated and precipitated in hexane. The resulting precipitates were collected by filtration, suspended in diethyl ether (20 mL), and washed with saturated aqueous bicarbonate followed by brine. The organic layer was then separated through filtration in the presence of Na_2SO_4 to absorb moisture and then precipitated in hexane as a light brown powder (yield ∼60%). ^{1}H NMR (JEOL, $CDCl_3$, 500 MHz): δ 12.20 (br, ^{1}H, NH), 7.12 (m, 6H, ArH), 4.83 (s, ^{1}H, Hβ), 3.10 (m, 4H, $CHMe_2$), 1.72 (s, 6H, α-Me), 1.22 (d, 12H, CHMeMe′), 1.12 (d, 12H, CHMeMe′) ppm. ESI-MS (positive): m/z = 419.43 $[M + H]^+$.

Synthesis of (BDI)ZnN($SiMe_3$)$_2$ Catalyst. Zinc bis(trimethylsilyl)amide (463 mg, 1.19 mmol) in toluene (20 mL) was added into a solution of BDI (500 mg, 1.19 mmol) in toluene (20 mL). The mixture solution was stirred for 18 h at 80 °C, and then the solvent was removed under vacuum to form (BDI)ZnN($SiMe_3$)$_2$ as a light yellow solid, which was recrystallized from toluene at −30 °C to yield colorless blocks (yield ∼70%). ^{1}H NMR (JEOL, C6D6, 500 MHz): δ (br, ^{1}H, NH), 6.9−7.13 (m, 6H, ArH), 4.85 (s, ^{1}H,Hβ), 3.25 (m, 4H, $CHMe_2$), 1.67 (s, 6H, α-Me), 1.1−1.25 (d, 12H + 12H = 24H, CHMeMe′), 0.08−0.1 (18H, s, $SiCH_3$) ppm.

Ring-Opening Polymerization of L-Lactide. Following previously published protocols, DOX−PLA and CPT−PLA polymers were synthesized through ring-opening polymerization of L-lactide initiated by alkoxy complex of (BDI)ZnN($SiMe_3$)$_2$ in a glovebox under argon environment at room temperature. For the synthesis of DOX−PLA, (BDI)ZnN($SiMe_3$)$_2$ (6.4 mg, 0.01 mmol) and DOX (5.4 mg, 0.01 mmol) were mixed in 0.5 mL of anhydrous THF. L-Lactide (101.0 mg, 0.7 mmol) dissolved in 2 mL of anhydrous THF was added dropwise. After the L-lactide was completely consumed, the crude product was precipitated in cold diethyl ether, yielding DOX−PLA conjugates. The CPT−PLA conjugates were synthesized in the same procedures as the DOX−PLA. These drug−polymer conjugates had a molecular weight of about 10,000 g/mol determined by gel permeation chromatography.

Synthesis of Lipid-Coated Drug−Polymer Conjugate Nanoparticles. Lipid−polymer hybrid nanoparticles with polymeric cores consisting of the synthesized drug−polymer conjugates were prepared through a nanoprecipitation method.[28,29] In detail, 200 μg of egg PC (Avanti Polar Lipids Inc.) and 260 μg of 1,2- distearoyl-*sn*-glycero-3- phosphoethanolamine-*N*-carboxy-(polyethyleneglycol)-2000 (DSPE-PEG-COOH) (Avanti Polar Lipids Inc.) were dissolved in 4% ethanol and stirred for 3 min. A total of 500 μg of DOX−PLA and CPT−PLA was dissolved in acetonitrile and added dropwise to the lipid solution while stirring. The solution was then vortexed for 3 min followed by the addition of deionized water (1 mL). Then the diluted solution was stirred at room temperature for 2 h, washed with

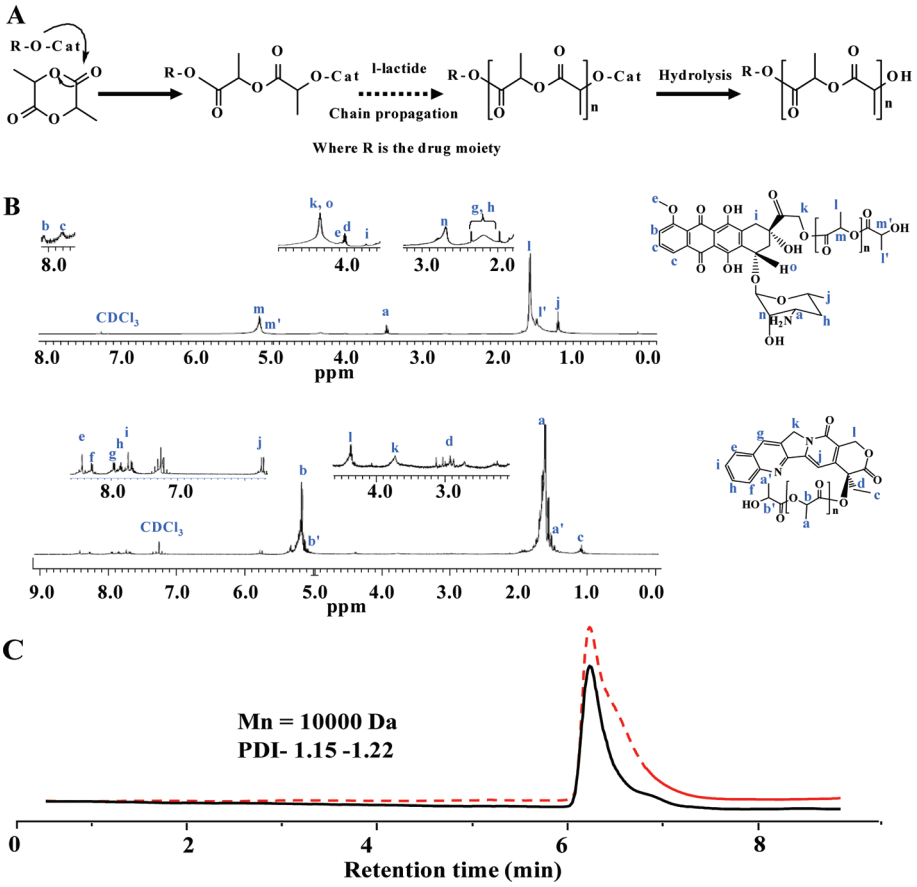

Figure 2. Chemical characterization of the drug−polymer conjugates. (A) Schematic description of the living ring-opening polymerization of L-lactide catalyzed by an activated metal alkoxide complex. (B) Qualitative ^{1}H NMR spectra showing the characteristic proton resonance peaks of DOX−PLA (upper panel) and CPT−PLA (lower panel). (C) Gel permeation chromatograms of DOX−PLA (red dashed line) and CPT−PLA (black solid line).

PBS buffer using an Amicon Ultra centrifugal filter with a molecular weight cutoff of 100 kDa (Millipore, Billerica, MA), and resuspended in 1 mL of PBS. Nanoparticles with different DOX/CPT drug ratios were prepared by adjusting the amount of each type of drug−polymer conjugates while keeping the total polymer weight at 500 μg. The nanoparticle size and surface zeta potential were obtained from three repeat measurements by dynamic light scattering (DLS) (Malvern Zetasizer, ZEN 3600) with a backscattering angle of 173°. The morphology of the particles was characterized by scanning electron microscopy (SEM) (Phillips XL30 ESEM). Samples for SEM were prepared by dropping nanoparticle solution (5 μL) onto a polished silicon wafer. After drying the droplet at room temperature overnight, the sample was coated with chromium and then imaged by SEM. The drug loading yield of the synthesized nanoparticles was determined by UV-spectroscopy (TECAN, infinite M200) using the maximum absorbance at 482 nm for DOX and 362 nm for CPT. No shift in the absorbance peak was observed between the free drugs and their polymer conjugates. Standard calibration curves of both DOX and CPT at various concentrations were obtained to quantify drug concentrations in the nanoparticles.

Cellular Colocalization and Cytotoxicity Studies. The MDA-MB-435 cell line was maintained in Dulbecco's modification of Eagle's medium (DMEM, Mediatech, Inc.) supplemented with 10% fetal calf albumin, penicillin/streptomycin (GIBCO), L-glutamine (GIBCO), nonessential amino acids, sodium bicarbonate, and sodium pyruvate (GIBCO). The cells were cultured at 37 °C and 5% CO_2. For the dual-drug colocalization and cellular internalization study, the cells were incubated with dual-drug loaded nanoparticles for 4 h, washed with PBS, and fixed on a chamber slide for fluorescence microscopy imaging. The cytotoxicity of the dual-drug loaded nanoparticles was assessed using the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Promega, Madison, WI). Briefly, the cells were seeded at 25% confluency (~4 × 10^3 cells/well) in 96-well plates and incubated with different concentrations of drug loaded nanoparticles for 24 h. The cells were then washed with PBS three times and incubated in fresh media for an additional 72 h. MTT assay was then applied to the samples to measure the viability of the cells following the manufacturer's instruction.

RESULTS AND DISCUSSION

Synthesis and Characterization of Drug−Polymer Conjugates. In the study, we used (BDI)ZnN($SiMe_3$)$_2$, a metal−amido complex in which BDI refers to 2-((2,6-diisopropylphenyl)-amido)-4-((2,6-diisopropylphenyl)imino)-2-pentene, as a catalyst for the *in situ* formation of metal-alkoxide with the hydroxyl group of DOX and CPT to initiate the living polymerization of L-lactide and form drug−poly-L-lactide (drug−PLA) conjugates (Figure 2A). The formation of the drug−polymer conjugates was verified by the ^{1}H NMR spectroscopy, which exhibits all the characteristic proton resonance peaks corresponding to the parent drug molecules. The appearance of the

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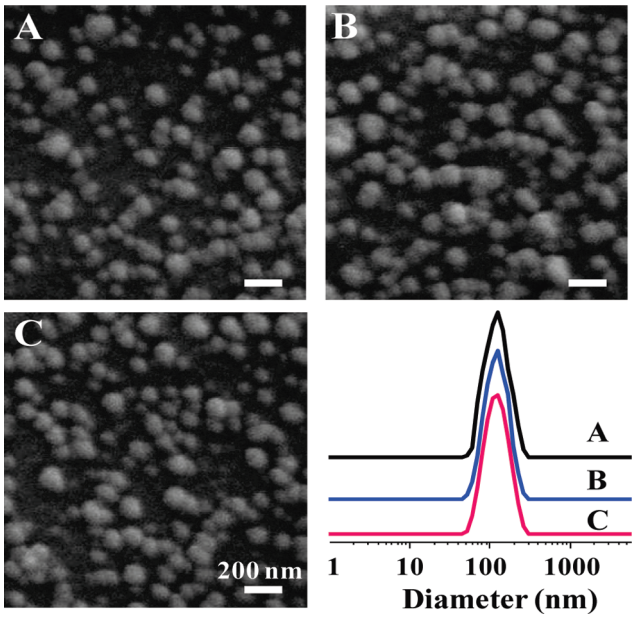

Figure 3. Scanning electron microscopy (SEM) and dynamic light scattering (DLS) measurements showing the morphology and size of lipid−polymer hybrid nanoparticles with the polymer cores consisting of (A) DOX−PLA conjugates, (B) CPT−PLA conjugates, and (C) DOX−PLA and CPT−PLA conjugates with a molar ratio of 1:1.

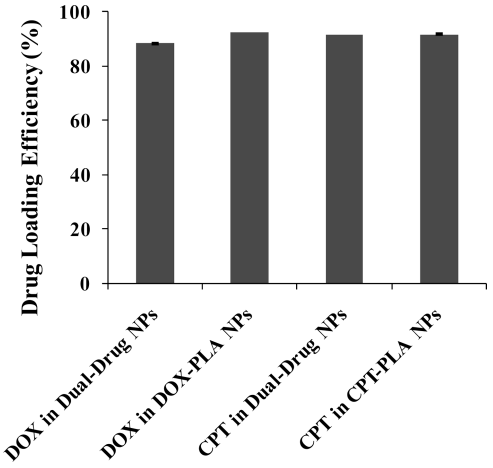

Figure 4. Quantification of DOX and CPT loading efficiency in dual-drug loaded nanoparticles (containing both DOX−PLA and CPT−PLA) and single-drug loaded nanoparticles (containing DOX−PLA or CPT−PLA), respectively. NPs: nanoparticles.

aromatic proton resonance at δ 7.5 to 8.0 ppm in DOX−PLA conjugates (Figure 2B, top panel) and δ 7.5 to 8.5 ppm in CPT−PLA conjugates (Figure 2B, bottom panel) along with the characteristic $-CH_3$ proton of PLA at δ 1.5 ppm and $-CH$ proton at δ 5.2 ppm confirms the formation of the drug−polymer conjugates. The desired drug−polymer conjugation products were further validated by gel permeation chromatography (GPC) which shows the molecular weight as 10,000 Da for both DOX−PLA and CPT−PLA conjugates (Figure 2C). The molecular weight is in accord with the monomer-to-initiator feed ratio which indicates near 100% conversion of the monomers to polymers. Since the formation of metal alkoxide complex is quantitative and the reaction is homogeneous, the reaction proceeded quantitatively such that all monomers were converted into products. Also the molecular weight of the polymer matches that from an earlier study conducted by Tong et al., who used $(BDI)ZnN(SiMe_3)_2$ to catalyze the ring-opening polymerization of both DOX and CPT.[24,26]

Synthesis and Characterization of Dual-Drug Loaded Polymeric Nanoparticles. Upon successful synthesis of the drug−polymer conjugates, we used them to prepare lipid−polymer hybrid nanoparticles for dual-drug delivery. Using DSPE-PEG and phospholipids to coat the polymeric nanoparticle core, the resulting lipid−polymer hybrid nanoparticles are highly stable in water, PBS, and serum and have high drug loading yield as the entire polymeric core consists of the drug−polymer conjugates. Moreover, by simply adjusting the DOX−PLA:CPT−PLA molar ratio, dual-drug loaded nanoparticles with ratiometric drug loading of DOX and CPT were prepared. Keeping the total drug−polymer conjugate weight constant at 500 μg, we varied the DOX−PLA:CPT−PLA ratio to tune the ratiometric drug loading. The resulting drug-loaded nanoparticles exhibit a unimodal size distribution at ~100 nm with low PDI values (Figure 3). In addition, the particles possess negative surface zeta potential, which is consistent with the DSPE-PEG-COOH coating and serves to prevent the particles from aggregation. The particle size measured by DLS was consistent with the SEM images of the particles (Figure 3).

Ratiometric Control Over Dual-Drug Loading. Following the physicochemical characterization of the particles, we next examined the drug loading efficiency in these drug−polymer conjugate nanoparticle systems. We prepared various formulations of the nanoparticles with different ratios of drug−polymer conjugates and found that, in all cases, over 90% of the conjugates were encapsulated into the nanoparticles (Figure 4). No change in loading efficiency was observed when DOX−PLA and CPT−PLA conjugates were loaded in combination or separately, presumably due to the fact that the long and sharply distributed PLA polymer chain gives each drug molecule a predominant and uniform hydrophobic property. Therefore, they were completely encapsulated and stabilized by the lipid and the lipid-PEG layers in the lipid−polymer hybrid nanoparticle system. Furthermore, we varied the DOX−PLA:CPT−PLA molar ratios from 1:1, to 3:1 and to 1:3, while keeping the total drug−polymer conjugate mass constant. It was found that the final loading yields of DOX and CPT in the dual-drug loaded nanoparticles were highly consistent with the initial DOX−PLA:CPT−PLA molar ratios (Table 1). These results further confirm that this approach enables one to encapsulate different types of drugs to the same nanoparticles with ratiometric control over drug loading.

Dual-Drug Colocalization and Cellular Internalization. Upon verifying the excellent drug loading efficiency in the present system, we then examined whether the different drug−polymer conjugates are loaded into the same nanoparticles as opposed to forming two different particle populations. To this end, we studied the colocalization of the two drug molecules and their internalization into cells through fluorescence microscopy. Since DOX is also a highly fluorescent molecule, the DOX−PLA conjugates can be identified from DOX's characteristic fluorescence wavelength (excitation/emission = 540 nm/600 nm). To visualize CPT−PLA, we attached a fluorescent probe, 6-((7-amino-4-mehylcoumarin-3-acetyl)amino)hexanoic acid succinimidyl ester (excitation/emission = 353 nm/442 nm), to the hydroxyl end of the CPT−PLA. The nanoparticle size and

Table 1. Characteristic Features of the Lipid-Coated Drug−Polymer Conjugate Nanoparticles[a]

DOX−PLA:CPT−PLA molar ratios	1:0	0:1	1:1	3:1	1:3
DOX loading (μM)	47.8 ± 0.2	0	24.0 ± 0.1	35.8 ± 0.2	12.0 ± 0.8
CPT loading (μM)	0	48.2 ± 0.1	24.4 ± 0.1	12.3 ± 0.1	36.2 ± 0.2

[a] In all cases, particle size (nm) = 100 ± 2, particle PDI = 0.17−0.22, and particle zeta potential (mV) = −47 ± 2.

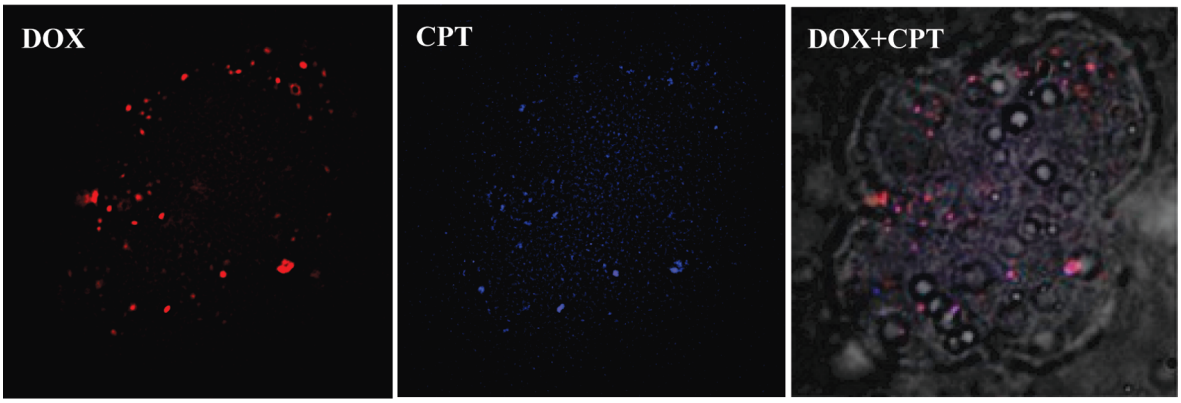

Figure 5. Cellular colocalization studies of the DOX−PLA and CPT−PLA loaded dual-drug nanoparticles. Fluorescence microscopy images showing the colocalization of DOX and CPT in the cellular compartment of MDA-MB-435 breast cancer cells.

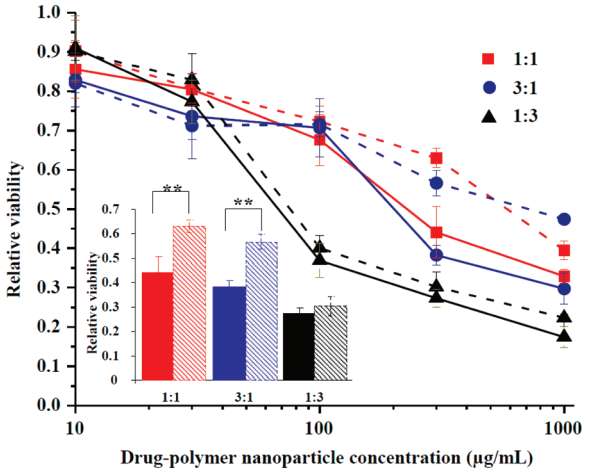

Figure 6. A comparative study of cellular cytotoxicity of the DOX−PLA and CPT−PLA loaded dual-drug nanoparticles against the MDA-MB-435 breast cancer cells. The ratios shown in the figure legend are the molar ratios of DOX−PLA to CPT−PLA. Solid lines represent the dual-drug loaded nanoparticles, and dashed lines represent the cocktail mixture of DOX−PLA loaded and CPT−PLA loaded single-drug nanoparticles. All samples were incubated with cells for 24 h, and the cells were subsequently washed and incubated in media for a total of 72 h prior to MTT assay (n = 4). The inset highlights the cytotoxicity comparison of dual-drug loaded nanoparticles and cocktail mixtures of single-drug loaded nanoparticles at 300 μg/mL of nanoparticle concentration. A paired Student's t-test was performed to determine the significance of the difference. (**$p < 0.01$, n = 4).

size distribution were consistent before and after the conjugation of a fluorophore to CPT−PLA, which further confirms the stability and reproducibility of the lipid−polymer hybrid nanoparticle platform. Figure 5 shows the fluorescence microscopy images that exhibit the colocalization of the DOX−PLA and the CPT−PLA-probe. The colocalization study indicates that no segregation between the two types of drug−polymer conjugates occurs and each particle contains both DOX and CPT.

***In Vitro* Cytotoxicity of Dual-Drug Delivery Nanoparticles.** After having confirmed that the nanoparticles contain a mixture of DOX and CPT, we next examined the cytotoxicity of these dual-drug loaded nanoparticles in comparison to the cocktail mixtures of the corresponding single-drug loaded nanoparticles against MDA-MB-435 breast cancer cells in vitro. The cocktail system was prepared by mixing DOX−PLA loaded nanoparticles and CPT−PLA loaded nanoparticles at a ratio that is equivalent to the DOX−PLA:CPT−PLA molar ratio in the dual-drug nanoparticles. Figure 6 shows the results of IC50 measurements of the dual-drug loaded nanoparticles and cocktail combination of single-drug loaded nanoparticles. It was found that the dual-drug loaded nanoparticles consistently showed higher potency as compared to the cocktail systems for the 3 different drug ratios. In the 3:1, 1:1, and 1:3 DOX−PLA:CPT−PLA combinations, the dual-drug loaded nanoparticles showed an enhancement in efficacy by 3.5, 2.5, and 1.1 times, respectively, compared to the cocktail particle mixtures. This enhanced cytotoxicity of the dual-drug delivery system can be explained, at least partially, by the fact that dual-drug loaded nanoparticles can deliver more consistent combination drug payloads when compared to cocktail nanoparticle systems and hence maximize their combinatorial effect. In the cocktail, variations in the nanoparticle uptake and the random drug distribution in cells likely compromised the efficacy of the drug combinations. Figure 5 suggests that the dual-drug loaded nanoparticles enable concurrent combination drug delivery through particle endocytosis. Once engulfed by the plasma membrane, nanoparticles are transported by endosomal vesicles before unloading their drug payloads. This endocytic uptake mechanism is particularly favorable to the drug−polymer conjugate system used in the present combinatorial drug delivery scheme. The pH drop associated with endosome maturation subjects the nanoparticles to an acidic environment and enzymatic digestions,[30,31] which facilitate the cleavage of the ester

linkage between the drugs and the polymers. In addition, the degradation of the polymer PLA releases lactic acid to further lower the pH surrounding the nanoparticles, thereby further accelerating the drug release.

CONCLUSIONS

In conclusion, a new and robust approach for combination chemotherapy was presented by incorporating two different types of drugs with ratiometric control over drug loading into a single polymeric nanoparticle. By adapting metal alkoxide chemistry, drug conjugated polymers were synthesized in quantitative yield with 100% monomer conversion, resulting in the formation of highly hydrophobic drug−polymer conjugates. These drug−polymer conjugates were successfully encapsulated into lipid-coated polymeric nanoparticles with over 90% loading efficiency. Using DOX and CPT as two model chemotherapy drugs, various ratios of DOX−PLA and CPT−PLA were loaded into the nanoparticles, yielding particles that are uniform in size, size distribution and surface charge. The cytotoxicity of these dual-drug carrying nanoparticles was compared with their cocktail mixtures of single-drug loaded nanoparticles and showed superior therapeutic effect. This strategy can also be exploited for various other chemotherapeutic agents containing hydroxyl groups as well as different types of combinations for combinatorial treatments of various diseases. While only two drugs (DOX and CPT) were used to demonstrate the concept of this combinatorial drug delivery approach, this method can be generalized to incorporate three or more different types of drugs into the same nanoparticles with ratiometric control over drug loading.

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