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PLGA–lecithin–PEG core–shell nanoparticles for controlled drug delivery

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ABSTRACT

Current approaches to encapsulate and deliver therapeutic compounds have focused on developing liposomal and biodegradable polymeric nanoparticles (NPs), resulting in clinically approved therapeutics such as Doxil/Caelyx and Genexol-PM, respectively. Our group recently reported the development of biodegradable core–shell NP systems that combined the beneficial properties of liposomal and polymeric NPs for controlled drug delivery. Herein we report the parameters that alter the biological and physicochemical characteristics, stability, drug release properties and cytotoxicity of these core–shell NPs. We further define scalable processes for the formulation of these NPs in a reproducible manner. These core–shell NPs consist of (i) a poly(D,L-lactide-*co*-glycolide) hydrophobic core, (ii) a soybean lecithin monolayer, and (iii) a poly(ethylene glycol) shell, and were synthesized by a modified nanoprecipitation method combined with self-assembly. Preparation of the NPs showed that various formulation parameters such as the lipid/polymer mass ratio and lipid/lipid–PEG molar ratio controlled NP physical stability and size. We encapsulated a model chemotherapy drug, docetaxel, in the NPs and showed that the amount of lipid coverage affected its drug release kinetics. Next, we demonstrated a potentially scalable process for the formulation, purification, and storage of NPs. Finally, we tested the cytotoxicity using MTT assays on two model human cell lines, HeLa and HepG2, and demonstrated the biocompatibility of these particles *in vitro*. Our data suggest that the PLGA–lecithin–PEG core–shell NPs may be a useful new controlled release drug delivery system.

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1. Introduction

Over the past two decades, there has been a steady rise in the number of commercially available nanoparticle (NP) therapeutics [1–3]. Among these products, liposomal drugs [4] and polymer–drug conjugates [5] are two dominant classes, accounting for the majority of clinically approved products. Concurrently, the development of biodegradable polymeric NPs in the ~100 nm range has become an increasingly exciting field in academic and industrial research [2,3].

Doxil (liposomal doxorubicin) was the first Food and Drug Administration (FDA)-approved liposomal drug formulation for the treatment of AIDS associated with Kaposi's sarcoma in 1995 [6–8]. The benefits of liposomal formulations include their ability to encapsulate hydrophilic therapeutic agents at high loading efficiency, shield encapsulated drugs from external conditions, and also be coated with inert and biocompatible polymers such as polyethylene glycol (PEG) for reduced systemic clearance rates and prolonged circulation half-life *in vivo* [9]. These PEG-end groups may also be functionalized with specific ligands for targeting to specific sites of the cells, tissues and organs of interest [10]. However, liposomes may face some obstacles from their low ability to encapsulate very hydrophobic drugs, burst release of drugs, and having multiple manufacturing steps associated with liposome preparation and purification.

The use of biodegradable polymeric NPs for drug delivery has been gaining momentum and shown significant therapeutic potential [11–13]. Biodegradable polymers such as poly(D,L-lactic acid), poly(D,L-lactic-*co*-glycolic acid) and poly(ε-caprolactone) and their co-polymers diblocked or multiblocked with PEG have been commonly used to form core–shell structured NPs to encapsulate a variety of therapeutic compounds [14–17]. These NPs have a number of appealing features: their hydrophobic core is capable of carrying highly insoluble drugs with high loading capacity, while

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their hydrophilic shell provides steric protection and functional groups for surface modification. Drug release can be manipulated by choosing biodegradable polymers with different surface or bulk erosion rates, and external conditions such as pH and temperature changes may function as a switch to trigger drug release [18]. Worth noting is their ease of manufacturing: amphiphilic co-polymers spontaneously assemble into core–shell structures in aqueous environments [19]. However, to date, polymeric NPs have shown moderate circulation half-lives compared to their liposomal counterparts, despite also being coated with inert and biocompatible polymers such as polyethylene glycol (PEG) [20].

Recently, we developed in our laboratory a protocol for the self-assembly of NPs that combine the properties of liposomes and polymeric NPs [21]. Existing strategies to make such fusion particles (lipopolyplexes) involved multi-step synthesis methods, resulting in inherently inefficient systems which are not easily scalable and show batch-to-batch variation [22,23]. We engineered a simple, scalable, efficient and more controllable system using a well-defined and predictable formulation strategy. The NPs are formed from three biomaterials: (i) poly(D,L-lactide-*co*-glycolide) (PLGA) was selected for the hydrophobic core due to its biodegradable nature and ability to encapsulate high amounts of hydrophobic drugs; (ii) soybean phosphatidylcholine (or lecithin) was chosen for a monolayer around the hydrophobic core; and (iii) 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-carboxy(polyethylene glycol)2000 (DSPE–PEG–COOH) intersperses in the lecithin monolayer to form a PEG shell which provides electrostatic and steric stabilizations, a longer circulation half-life *in vivo* as well as functional-end groups for the attachment of targeting ligands such as antibodies, peptides and aptamers.

Herein we report our studies on the systematic preparation and characterization of these PLGA–lecithin–PEG core–shell NPs. We evaluate parameters that affect the core–shell nanostructure to find an optimal formulation, and subsequently characterize the NPs for physical stability, controlled drug release kinetics, post-formulation purification, storage methods and finally material cytotoxicity. We establish the function of the lipid monolayer ring between the PLGA core and the PEG shell in controlled drug release kinetics. This novel NP system may represent a new way of combining existing lipid and polymer classes of materials for controlled drug delivery applications.

2. Materials and methods

2.1. Materials

PLGA (poly(D,L-lactide-*co*-glycolide)) with a 50:50 monomer ratio, ester-terminated, and viscosity of 0.72–0.92 dl/g was purchased from Durect Corporation (Pelham, AL). Soybean lecithin consisting of 90–95% phosphatidylcholine was obtained from MP Biomedicals (Solon, OH), and DSPE–PEG_{2000}–COOH (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-carboxy(polyethylene glycol)2000) was obtained from Avanti (Alabaster, AL). Docetaxel (Dtxl) was purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Preparation of PLGA–lecithin–PEG NPs

PLGA–lecithin–PEG core–shell NPs were synthesized from PLGA, soybean lecithin and DSPE–PEG–COOH using a modified nanoprecipitation technique combined with self-assembly. PLGA was first dissolved in organic solvent (acetonitrile, unless specified) with concentrations ranging from 1 to 25 mg/mL. Lecithin and DSPE–PEG–COOH (7:3, molar ratio) were dissolved in a 4% ethanol aqueous solution at 20% of the PLGA polymer weight and heated to 65 °C. The PLGA/acetonitrile solution was then added into the preheated lipid aqueous solution drop-wise (1 mL/min) under gentle stirring followed by vortexing for 3 min. The nanoparticles were allowed to self-assemble for 2 h with continuous stirring while the organic solvent was allowed to evaporate. The remaining organic solvent and free molecules were removed by washing the NP solution 3 times using an Amicon Ultra-4 centrifugal filter (Millipore, Billerica, MA) with a molecular weight cut-off of 10 kDa and then re-suspended in water to obtain a final desired concentration. The NPs were used immediately, stored at 4 °C, or freeze dried in liquid nitrogen and lyophilized for storage at −80 °C for later use.

2.3. NP characterization

NP size (diameter, nm), polydispersity index, and surface charge (zeta potential, mV) were determined by Quasi-elastic laser light scattering using a ZetaPALS dynamic light scattering (DLS) detector (15 mW laser, incident beam = 676 nm) (Brookhaven Instruments, Holtsville, NY) at room temperature. Viscosity and refraction indices were set equal to those specific of water. Particle concentration was calculated based on the PLGA polymer concentration in the PLGA–lecithin–PEG NPs.

2.4. Transmission electron microscopy (TEM)

TEM experiments were carried out on a JEOL JEM-200CX instrument at an acceleration voltage of 200 kV. The TEM sample was prepared by administering the NP suspension (4 mg/mL) onto a 300-mesh Formvar-coated copper grid that had been previously hydrophilized under UV light (Electron Microscopy Sciences, Hatfield, PA). Samples were blotted away after 30 min incubation and grids were negatively stained for 10 min at room temperature with freshly prepared and sterile-filtered 2% (w/v) uranyl acetate aqueous solution. The grids were then washed twice with distilled water and air dried prior to imaging.

2.5. Release of docetaxel from PLGA–lecithin–PEG NPs

To prepare drug-encapsulated NPs, Dtxl at an initial dosage of 10% (wt/wt) of the polymer was dissolved into the PLGA/acetonitrile solution before nanoprecipitation. To measure the drug loading yield and release profile of Dtxl from each type of NP, 3 mL NP solutions at a concentration of 0.5 mg/mL were split equally into 30 Slide-A-Lyzer MINI dialysis microtubes with a molecular weight cut-off of 10 kDa (Pierce, Rockford, IL) and subject to dialysis against 3 L distilled water with gentle stirring at room temperature. Distilled water was changed periodically during the whole dialysis process. At the indicated times, 0.1 mL of solution from three microtubes was removed and mixed with an equal volume of acetonitrile to dissolve the NPs. Dtxl content was subjected to quantitative analysis using an Agilent 1100 HPLC (Paolo Alto, CA) equipped with a pentafluorophenyl column (Curosil-PFP, 250 × 4.6 mm, 5 μm; Phenomenex, Torrance, CA). Dtxl absorbance was measured by an UV–vis detector at 227 nm and a retention time of 12 min in 1 mL/min 50:50 acetonitrile/water non-gradient mobile phase.

2.6. Cytotoxicity assays for PLGA–lecithin–PEG NPs

The cytotoxicity of the NPs was evaluated using an MTT assay. HeLa and HepG2 cells were cultured in DMEM supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, 100 μg/mL streptomycin under 5% CO_2 at 37 °C. They were seeded in a 96-well plate in 200 μL medium per well at a density of 10,000 cells/well for 24 h. The medium was then replaced with 200 μL of medium-containing NPs at different concentrations and incubated for 24 h in triplicate. The NP-containing media were then removed to avoid interference in the assays. 0.5 mg/mL MTT solution in medium was added and cells were incubated for another 4 h. The MTT-containing media were removed and cells were rinsed 3 times with PBS, 200 μL of isopropanol/DMSO was added at a 1:1 ratio to lyse the cells and was incubated at 37 °C for 5 min. MTT absorbance was measured at 570 nm and background at 660 nm using a SpectraMax Plus^{384} microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). A higher MTT absorbance with background value subtracted indicates higher relative cell viability. The results were plotted on a graph and fitted using the OriginPro 8 software (OriginLab Corp, Northampton, MA).

3. Results and discussion

3.1. Formulation of PLGA–lecithin–PEG NPs

As shown schematically in Fig. 1A, a modified nanoprecipitation technique was used to prepare the NPs. The aqueous phase consisted of soybean phosphatidylcholine (sPC) and pegylated phospholipids (DSPE–PEG_{2000}–COOH) in the appropriate molar ratio to form the monolayer around the PLGA polymeric core. The lipids were heated at 65 °C before adding the Dtxl/polymer mixture and subsequently vortexed for 3 min. This increase in thermal and mechanical energy allows for the lipids to disperse and self-assemble, forming a monolayer around the PLGA/organic solvent core to shield their hydrophobic fatty acid tails. Stirring at 2 h allowed for NP formation by self-assembly combined with organic solvent evaporation (Fig. 1A). The NPs were purified by either ultracentrifugation or dialysis before further characterization. We chose sPC for use as a lipid monolayer because sPC is a natural component of soybean. Compared with its synthetic alternatives,

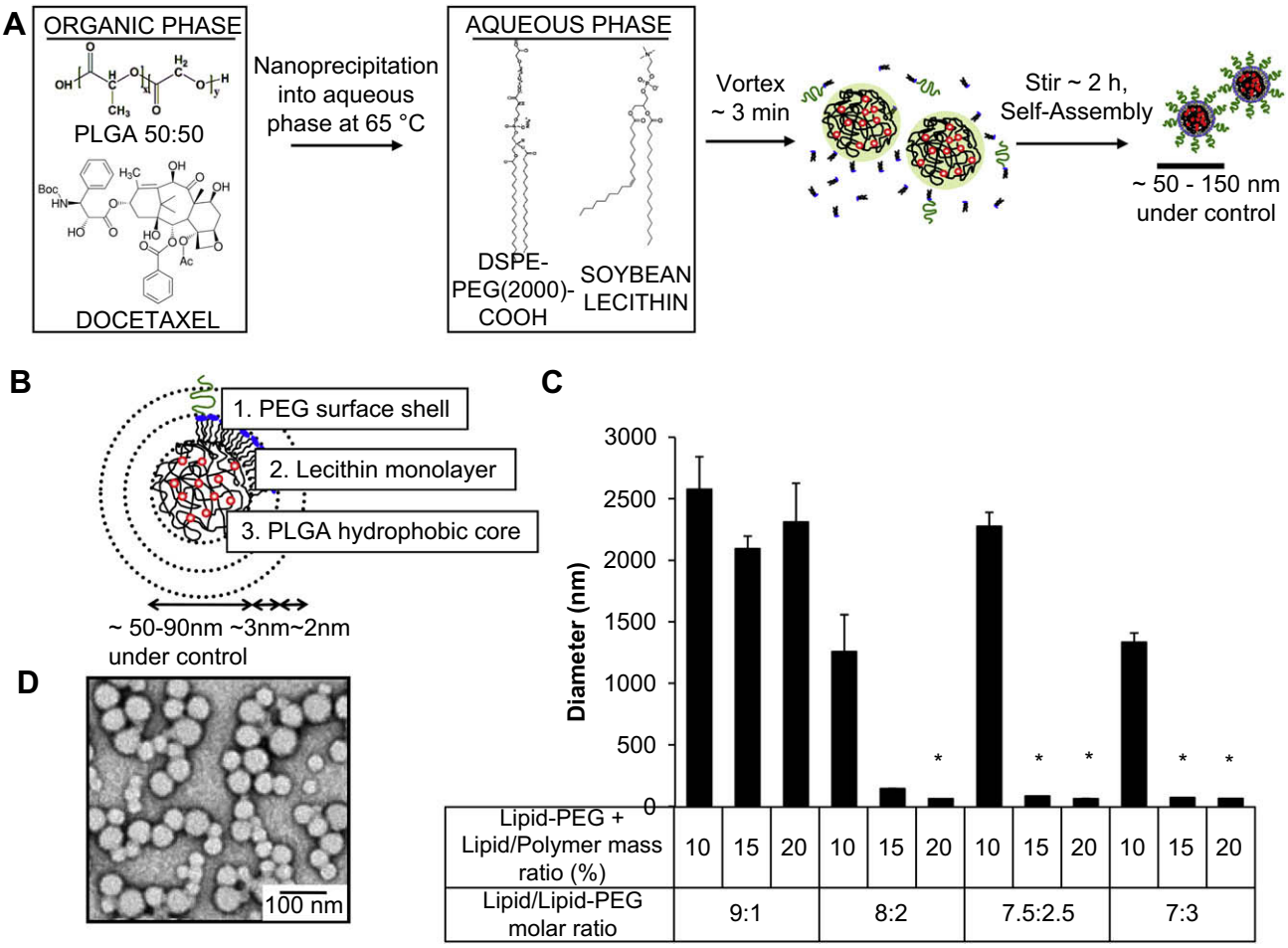

Fig. 1. Synthesis of PLGA–lecithin–PEG core–shell NPs. (A) A schematic illustration shows the processes of preparing PLGA–lecithin–PEG NPs by a modified nanoprecipitation method. (B) The NPs are comprised of a hydrophobic poly (lactic-*co*-glycolic acid) (PLGA) core, a hydrophilic poly(ethylene glycol) (PEG) shell, and a lecithin monolayer at the interface of the hydrophobic core and the hydrophilic shell. (C) By varying the parameters of total lipid/polymer mass ratio and lipid/lipid–PEG molar ratio, we can tune NP size in a physiological environment. The asterisk (*) refers to optimal NP formulations. (D) A transmission electron microscopy (TEM) image shows the core–shell structure of the NPs. NPs were negatively stained with uranyl acetate to enhance electron contrast between the polymers and the lipids.

sPC can be totally biodegraded and metabolized since it is an integral part of biological membranes. PEG and PLGA are FDA-approved polymers and biocompatible [24,25]. Hence, these three materials are regarded as well tolerated for potential pharmaceutical applications.

3.2. Characterization of PLGA–lecithin–PEG NP properties

DLS was used to characterize NP hydrodynamic size, polydispersity and zeta potential in each preparation. The average diameter of synthesized NPs ranged between 60 and 70 nm. The zeta potential ranged between −40 mV and −60 mV, depending on the size and composition of the NPs. Regardless of Dtxl loading, the particle sizes and zeta potentials remained in the same range. A schematic shows the core–shell structure of the NPs (Fig. 1B), while TEM was used to examine the morphology of the NPs (Fig. 1D). The TEM images revealed that the NPs are dispersed as individual NPs with a well-defined spherical shape and homogeneously distributed around 60–70 nm in diameter, and that the incorporation of Dtxl did not seem to cause morphological changes.

3.3. PLGA–lecithin–PEG NP lipid formulation parameters

Taking PLGA/acetonitrile concentration (1 mg/mL of 0.82 dl/g PLGA) and lipid/lipid–PEG molar ratio (9:1) to be constant, we first investigated the effect of total lipid (lecithin + DSPE–PEG–COOH)/polymer mass ratio on NP size. At 0% total lipid/polymer mass ratio, the formulation was pure PLGA polymeric NPs, and these NPs aggregated immediately in PBS to form ~2 μm particles (data not shown). An increase in total lipid/polymer mass ratio to 10%, 15%, 20%, or 100% did not change the ~2 μm aggregation (Fig. 1C). This suggests that even good coverage of the PLGA polymeric core by a phosphatidylcholine monolayer is insufficient for charge screening in PBS.

Next, we investigated the effect of lipid/lipid–PEG (lecithin/DSPE–PEG–COOH) molar ratio on NP size. We expanded it to include lipid/lipid–PEG molar ratios of 8:2, 7.5:2.5 or 7:3, against total lipid/polymer mass ratio of 10%, 15% or 20% (Fig. 1C). In contrast to ~2 μm aggregation observed with 9:1 molar ratios, an increase in the lipid–PEG representation in the lipid monolayer showed a significantly better PBS stability. As denoted by asterisks (*) in Fig. 1C, we chose formulations with diameters less than 100 nm as suitable for further characterization.

The end result of investigating the lipid/polymer mass ratio and lipid–PEG/lipid molar ratio formulation parameters was the identification of a minimum requirement of a 15% lipid/polymer mass ratio and 7.2:2.5 lipid/lipid–PEG molar ratio to form sub-100 nm NPs (Fig. 1C). Hence, an optimal NP formulation consists of sufficient total lipid coverage so that the PLGA hydrophobic core is not exposed, and also sufficient PEG surface density to provide charge screening in PBS. With the addition of other types of drugs, higher

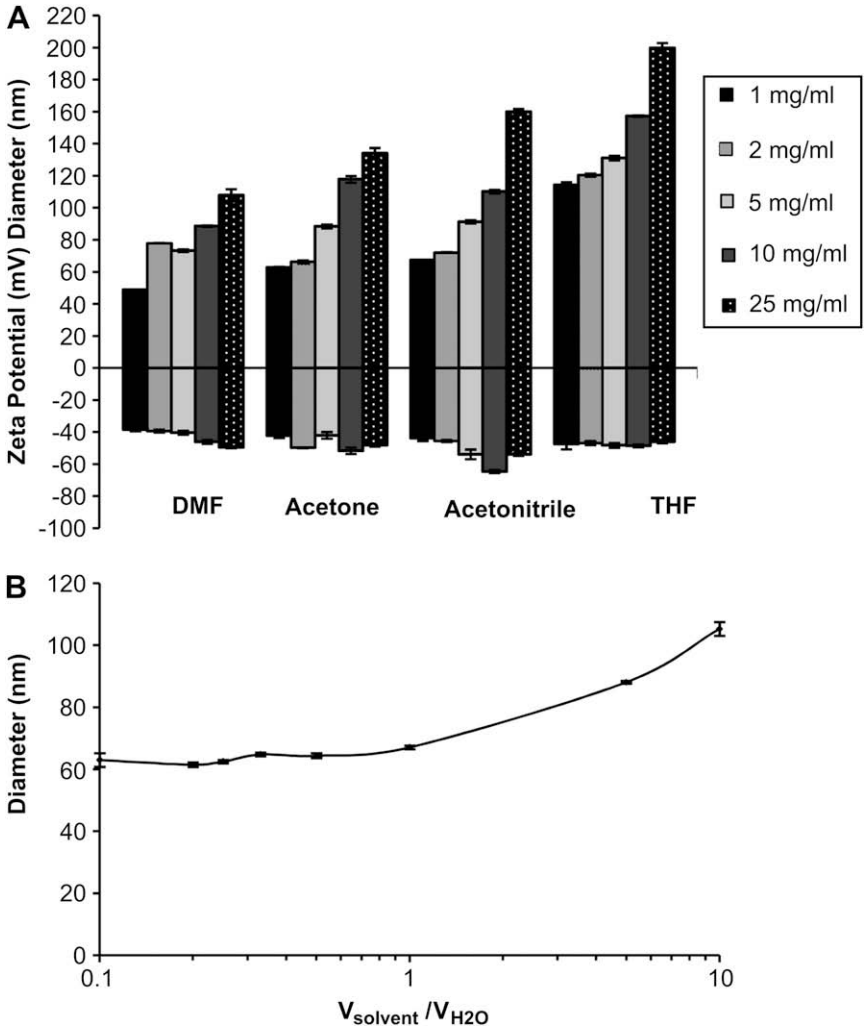

Fig. 2. The effect of formulation parameters on the size and zeta potential of PLGA–lecithin–PEG NPs. (A) Effect of polymer concentration on NP size and zeta potential in various organic solvents with different water miscibilities. (B) Effect of solvent–water ratio on NP size, taking acetonitrile as the organic solvent.

drug loadings, or functionalized PEG end-groups, it may be necessary to recalibrate the formulation as done in Fig. 1C to derive a new optimal formulation.

3.4. PLGA–lecithin–PEG NP polymer formulation parameters

Another parameter we can use to control NP size is polymer inherent viscosity. Using an optimal formulation of 20% total lipid/polymer mass ratio with 7:3 lipid/lipid–PEG molar ratio as denoted by an asterisk (*) in Fig. 1C, we could vary the PLGA polymer inherent viscosity (which corresponds approximately to polymer molecular weight). The NPs were stable in PBS and had relatively similar polydispersities, and showed a gentle decrease in size with increased polymer inherent viscosity (data not shown). PLGA with 0.82 dl/g polymer inherent viscosity for the polymeric core was used in the formulation for all subsequent experiments.

Organic solvents of increasing dielectric constants exhibit increasing polarity and water miscibility. We chose four organic solvents: tetrahydrofuran (THF), acetone, acetonitrile, and dimethylforamide (DMF) which have been commonly used to solubilize and formulate NPs, to study the effect of the type of organic solvent used to solubilize the PLGA polymer. The trend towards larger NPs was observed as the solvent used became less water miscible and the polymer concentration was raised (Fig. 2A). Interestingly, when very non-polar organic solvents such as THF was used, we obtained extremely low polydispersities on average around ~0.100, whereas when very polar organic solvents such as DMF was used, polydispersities were higher at ~0.300; acetonitrile and acetone gave moderate polydispersities around ~0.200. The effect of vortexing for 3 min after nanoprecipitation should not only disperse random lipid micelles but also disperse the solvent droplets. Presumably, the PLGA/THF droplets showed inefficient solvent dispersion and in a more predictable fashion than PLGA/DMF droplets, giving us the difference in polydispersities and sizes observed. Subsequently, we used acetonitrile as a model organic solvent, as it is a good solvent for our model chemotherapy drug, Dtxl.

With a change in polymer concentration during preparation, we could control the size and zeta potentials of NPs. As polymer concentration was increased from 1 to 25 mg/mL in acetonitrile (with a corresponding scale-up of lipid added to the aqueous phase), we observed the trend of increasing NP size from 65 to 160 nm with a corresponding increase in zeta potential from −40 mV to −70 mV (Fig. 2A).

Finally, we tested the effect of varying solvent–water ratios on the size and zeta potentials of NPs. When our typical solvent–water ratio of 0.33 was modified from 0.1 to 1, and we observed NP sizes to be almost constant at ~65 nm, with a slight trend of increase in size. However, when the solvent–water ratio was increased to 5 and

10, NP sizes increased to 88.1 ± 0.4 nm and 105.3 ± 2.2 nm, respectively, (Fig. 2B).

3.5. *In vitro* controlled drug release kinetics of PLGA–lecithin–PEG NPs

We next wanted to understand how the three functionally different layers of our PLGA–lecithin–PEG NPs controlled the release of a model chemotherapeutic drug Dtxl, namely, the total lipids present at the interface, the length of the PLGA polymer in the core, and the PEG surface density (Fig. 1B). This experiment was performed in H_2O instead of PBS buffer solution as we took into consideration that some were not optimal formulations with sufficient stability in PBS. This experiment characterizes the fundamental properties of the NPs as opposed to simulating a physiological situation. An inset in Fig. 3A–C shows the diameters and zeta potentials of the NPs in H_2O.

When we changed total lipid/polymer mass ratio on three log scales from 0.1% to 100% (wt/wt), we observed significantly different *in vitro* drug release rates. Drug release rates for 0% lipid compared to 100% lipid differed as much as 20 h for 50% release (Fig. 3A).

In a separate experiment, we changed the PLGA polymer length in the core. Previously, PLGA polymeric NPs with higher MW were shown to release drugs more slowly than NPs with shorter polymer chains. Our study shows a small contribution of polymer inherent viscosity in controlled drug release, but the dominant contribution in these core-shell NPs come from the lipid monolayer density (Fig. 3B).

Likewise, in an experiment to examine the contribution of PEG surface density to drug release, with the number of total lipid and lipid–PEG molecules remaining constant, the PEG surface density was changed from 0% to 40%. Drug release was also observed to be relatively similar (Fig. 3C).

In Fig. 3A–C, initial drug loading was quantified by docetaxel drug loading of three samples at $t = 0$ h. In Fig. 3A, when lipid concentration was varied, drug loading at $t = 0$ h ranged from 29 to 38 μg/mL; In Fig. 3B, when PLGA polymer length was varied, drug loading at $t = 0$ h was about 31–32 μg/mL; In Fig. 3C, when PEG surface density was varied, drug loading was also about 31–32 μg/mL. Taking an initial polymer concentration of 0.5 mg/mL, 10 wt% docetaxel added (docetaxel concentration of 50 μg/mL) can give ~62% encapsulation efficiency for optimal formulations.

Based on the observations in Fig. 3A–C, we propose a model where the lipid monolayer is acting as a molecular fence and contributes to keep the drug molecules in the hydrophobic core, as well as keep H_2O out of the core which would hydrolyze the PLGA polymer and increase erosion and drug release. The lipid monolayer contributes to being a limiting factor in controlled drug release, considering that in the range of polymeric inherent viscosities and PEG surface densities tested we were unable to observe changes in drug release if the lipid monolayer coverage is kept constant (Fig. 3B and C). Hence, controlled drug release from the NPs can be manipulated by lipid coverage, with minor contributions by PLGA inherent viscosity and PEG surface density.

3.6. *In vitro* stability of PLGA–lecithin–PEG NPs

We performed *in vitro* assays of long-term stability and protein binding on a formulation of 20% total lipid/PLGA mass ratio, 7:3 lipid/lipid–PEG molar ratio, and 0.82 dl/g PLGA polymer inherent viscosity core.

PBS stability assays for long-term stability were carried out where the NPs were dialyzed in PBS over 120 h at 37 °C and withdrawn at 24 h intervals. DLS sizing measurements demonstrate that the NPs remain stable over 5 days with no significant change in size and polydispersities (Fig. 4). This result suggests that the DSPE–PEG_{2000}–COOH density on the NPs does not drop below the range of full electrostatic and steric stabilizations over the course of 120 h. It could be that the DSPE–PEG covalent bond in PEGylated lipid molecules is very stable and the PEG group is not hydrolyzed off, or that the lipid monolayer does not peel off over 120 h. Thus, the NPs prepared in this study should remain stable *in vitro* for relatively long periods.

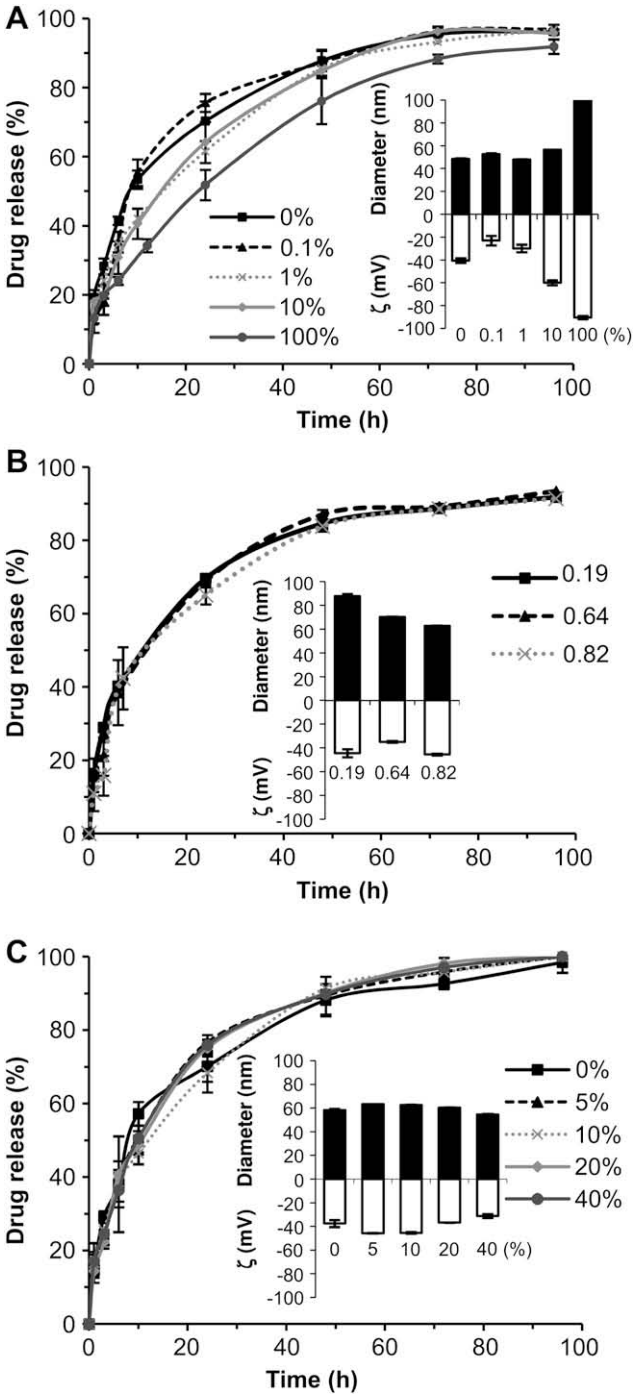

Fig. 3. Controlled and sustained drug release profiles of PLGA–lecithin–PEG NPs. (A) Effect of total lipid/polymer weight ratio on drug release profile of the NPs. Percentage units indicate total lipid/PLGA mass ratio. (B) Effect of PLGA inherent viscosity on drug release profile of the NPs. Units are in dl/g. (C) Effect of lipid–PEG/lipid molar ratio on drug release profile of the NPs. Percentage units indicate lipid–PEG/lipid molar ratio. The inset of each panel shows the diameter and zeta potential (denoted as ζ) of the corresponding NP formulations.

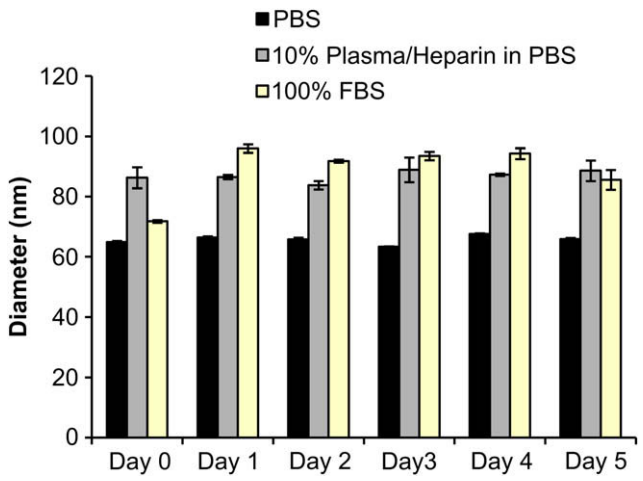

Fig. 4. *In vitro* stability of PLGA–lecithin–PEG NPs over 120 h. NPs were incubated with PBS, 10% (v/v) plasma/heparin in PBS and 100% FBS, respectively, at 37 °C over five days. An aliquot of NP suspensions was collected to measure NP size using ZetaPALS dynamic light scattering.

Plasma stability assays for protein binding were performed in which NPs were incubated in 10% (v/v) human plasma solution (diluted in PBS) or 100% fetal bovine serum (FBS) for 120 h at 37 °C. After an initial ~20 nm increase in size, the NPs maintain size stability throughout the 120 h study (Fig. 4), suggesting that plasma protein binding was not a significant factor. This result suggests that the current formulation has sufficient stability derived from steric repulsion due to the hydrodynamic diameter of the PEG chain and electrostatic repulsion from the negatively charged carboxylic-end group, as well as adequate lecithin coverage of the polymeric core.

The mechanism of drug release from a polymeric core is typically bulk or surface erosion, or in some cases a combination of both. These *in vitro* stability assays also suggest a mechanism of drug release from the PLGA–lecithin–PEG NPs. Surface erosion of particles will result in a gradual decrease in NP mean diameter over 120 h, while bulk erosion of particles results in constant NP mean diameters over 120 h. Our data show almost constant mean diameters with no gradual mean diameter reduction over 120 h (Fig. 4). We postulate that the mechanism of drug release from PLGA–lecithin–PEG NPs occurs by bulk erosion in which Dtxl diffuses out through channels that form in within the NP core before bulk degradation, as opposed to gradual surface erosion from the PEG shell.

3.7. Post-formulation purification and storage of PLGA–lecithin–PEG NPs

NPs made by nanoprecipitation contain 10–15% (v/v) organic solvent which did not evaporate during the self-assembly stage. Post-formulation purification of the NPs is necessary to remove trace amounts of organic solvent contamination which causes NP instability via degradation. We analyzed the diameter, polydispersity and zeta potential of our NPs after purification by dialysis in H_2O and ultrafiltration using a commercially available centrifuge filtration device. We found there to be no observed difference using either methods, even with multiple washing steps using the ultrafiltration method (Fig. 5A). These two purification methods can be used together to achieve gentle organic solvent removal and to change NP concentrations or buffer solutions.

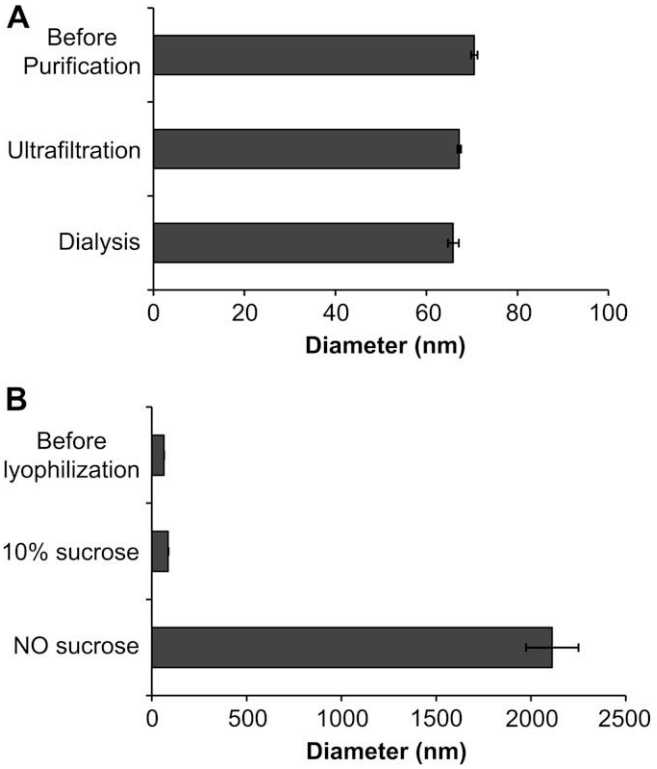

Fig. 5. Post-formulation stability of PLGA–lecithin–PEG NPs. (A) Post-formulation stability of the NPs upon purification by ultrafiltration or dialysis methods. (B) Post-formulation stability of the NPs upon liquid nitrogen freeze-drying and lyophilization with 10% sucrose cryroprotectant.

Potential pharmaceutical use of these NPs requires scalable processes for the storage of larger batches of NPs. We encapsulated Dtxl in the NPs (5 wt%), freeze-dried the NP solution in liquid nitrogen and lyophilized the NPs at −80 °C. Addition of cryoprotectants such as 10% (wt/vol) sucrose allows for recovery of 87.0 ± 0.6 nm NPs, similar to the original 64.5 ± 0.5 nm diameters measured by DLS. In the absence of 10% sucrose, NPs aggregated significantly to ~2.1 μm. As a first attempt to demonstrate the potential scalability of this process, we increased the formulation volume to synthesize 300 mg of NPs. The NPs maintained the biological and physicochemical properties as previously described (data not shown).

3.8. In vitro cytotoxicity of PLGA–lecithin–PEG NPs

To assess the cellular cytotoxicity of the NPs, HeLa and HepG2 cell lines were used. Cytotoxic activity was evaluated at twofold dilutions in triplicate ranging from 25 mg/mL to 0.1 mg/mL. Following 24 h exposure to the NPs, cell viability was assessed by the MTT assay. The results were plotted onto a fitted curve and expressed as treatment over control (T/C) values for cell survival. The NPs did not cause significant cytotoxicity against either cell line. Experimental TC_{50} values of the NPs grown on HeLa cells were found to be 5.55 mg/mL for HeLa cells (Fig. 6A) and 4.58 mg/mL for HepG2 cells (Fig. 6B), which translates to ~200–300 mg/kg body weight in an adult human, suggesting low *in vitro* cytotoxicity as it represents a much higher intravenous material dose than required for *in vivo* drug delivery. Representative photographs of HeLa and HepG2 cells are shown with no NPs added (left column), incubated with NPs at TC_{50} values (middle column), and incubated with NPs at TC_{100} values (right column) (Fig. 6C).

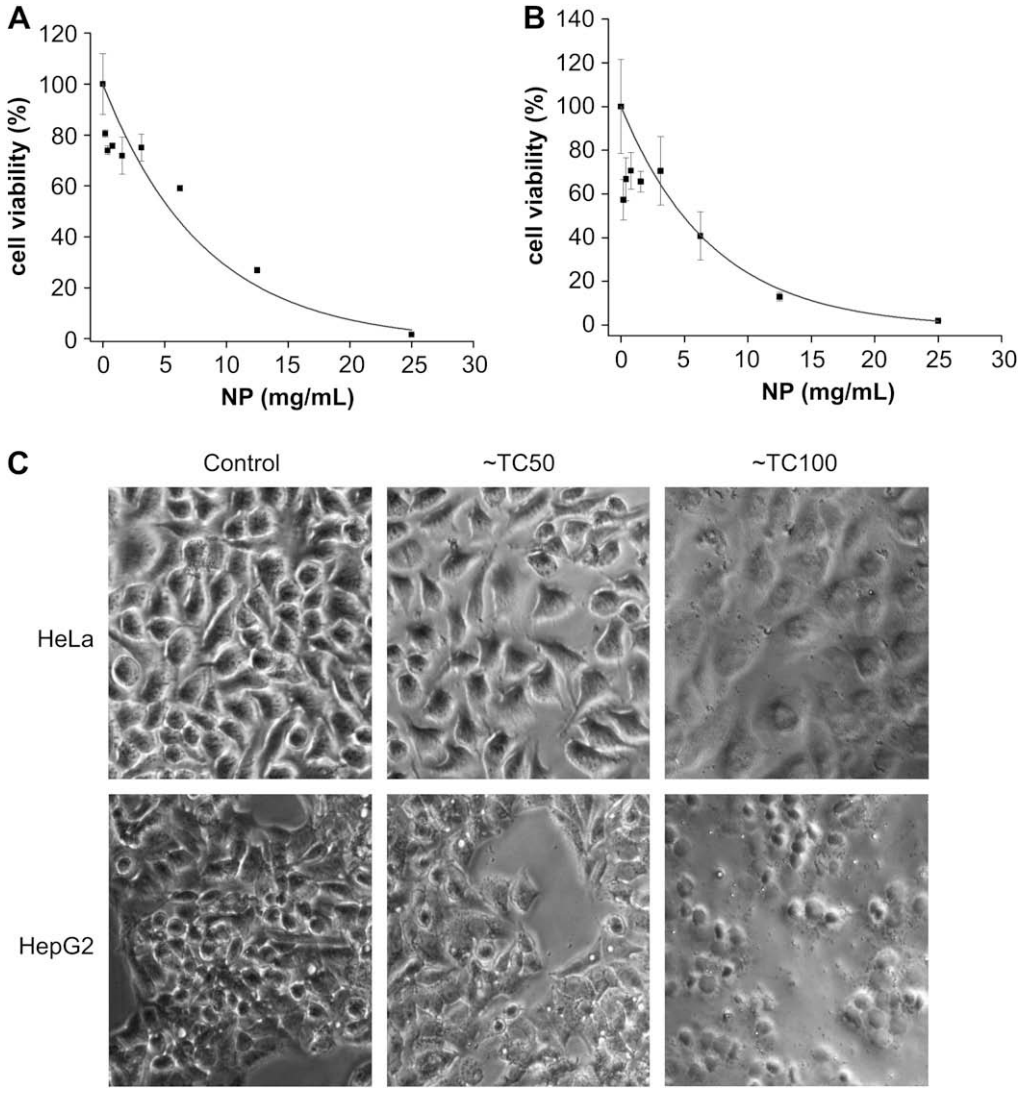

Fig. 6. Cytotoxicity of PLGA–lecithin–PEG NPs on HeLa and HepG2 cell lines using the MTT assay. Fitted curves show cell viability of (A) HeLa and (B) HepG2 cell lines with twofold dilutions of NPs from 25 mg/mL to 0.1 mg/mL. (C) Photographs of control HeLa and HepG2 cells without NP incubation (left column); HeLa and HepG2 cells at TC50 concentrations of NPs (middle column); HeLa and HepG2 cells with the highest concentration (25 mg/mL) of NPs (right column).

4. Conclusion

We developed PLGA–lecithin–PEG core–shell NPs which contain a hydrophobic PLGA core, a soybean lecithin monolayer and a hydrophilic PEG shell. The NP formulations were characterized and evaluated for controlled drug release kinetics and physical stability in PBS and plasma. The NPs were well tolerated by human cell line models, HeLa and HepG2. The NPs were prepared by a potentially scalable nanoprecipitation process with predictable and controllable outcomes and thus may be suitable as a potential drug delivery system. Further *in vivo* studies are planned to demonstrate the efficacy of these NPs as a drug delivery system for a variety of applications.

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