Highly Biocompatible Zwitterionic Phospholipids Coated Upconversion Nanoparticles for Efficient Bioimaging

Chi Yao, Peiyuan Wang, Lei Zhou, Rui Wang, Xiaomin Li, Dongyuan Zhao, and Fan Zhang*

Department of Chemistry, Laboratory of Advanced Materials, State Key Laboratory of Molecular Engineering of Polymers, Fudan University, Shanghai 200433, P. R. China

Supporting Information

ABSTRACT: The potential of upconversion nanoparticles (UCNPs) in various biomedical applications, including immunoassays, biomedical imaging, and molecular sensing, requires their surface derivatized to be hydrophilic and biocompatible. Here, a new family of compact zwitterionic ligand systems composed with functional phospholipids was designed and used for the surface modification of UCNPs. The zwitterionic UCNPs are hydrophilic, compact, and easily functionalized. It was proved that zwitterionic phospholipids could provide UCNPs with not only extended pH and salt stability but also little nonspecific interactions to positively and negatively charged proteins, low nonspecific adhesion in live-cell imaging process. Most notably, the efficient in vivo tumor imaging performance and long blood circulation half-life suggests the excellent biocompatibility for in vivo imaging of the zwitterionic UCNPs.

Lanthanide-doped upconversion nanoparticles (UCNPs) exhibit many advantages for biological applications over other luminescent reporters1−3 such as low toxicity, high chemical stability, high penetration depth in tissues, and high signal-to-noise ratio due to the absence of autofluorescence.4−11 Therefore, a lot of work has been carried out to exploit biological applications of lanthanide-doped UCNPs,12−14 including immunoassays, biomedical imaging, and molecular sensing via fluorescence resonance energy transfer (FRET).15−19

Like most of the synthesis methods for high-quality nanoparticles, synthetic approaches for UCNPs with the uniform size distributions usually involve the use of organic capping ligands and result in UCNPs that are hydrophobic as synthesized.20−25 Therefore, their hydrophobic ligands compromise their water solubility and their compatibility with the biological milieu. Exploring surface engineering that renders these UCNPs hydrophilic and biocompatible has been essential for demonstrating their potential uses in various biomedical applications. This goal, in turn, is almost completely dependent on the properties of the surface modification approach designed to provide aqueous stability to the UCNPs and facilitate their integration within biological environment.24,26−32 Strategies to make water-soluble and biocompatible UCNPs are guided by several criteria: (i) stability over a large pH range and at elevated salt concentrations, (ii) easy functionalization, (iii) minimally perturb inherent UCNPs properties or those of an attached biomolecule, and (iv) minimal undesired interaction with other molecules native to biological environments, that is, low nonspecific adsorption.

Although all are significant, it has been extremely challenging to design UCNP ligands that can provide a majority of these properties, let alone all, in a single molecule. For example, some of functional groups (such as −COOH or −NH₂) terminated amphiphilic ligands and cap exchange ligands have been utilized to realize the surface engineering of UCNPs through the encapsulation and cap exchanging process.3,12,13,15 However, aqueous solubility of carboxyl functionalized nanocrystals relies exclusively on deprotonation of the terminal carboxyl groups and their colloidal stability pH window is limited to the basic regime.33 In contrast, amine-coated nanocrystals are colloidally stable at moderately acidic pH; however, their stability drops when moving from neutral to basic conditions.34 Furthermore, the strong nonspecific electrostatic with their biological environment has been observed extensively for the carboxyl functionalized nanoparticles.35 Therefore, a major challenge for the bioapplications of UCNPs is still the lack of a ligand system retaining high aqueous solubility, biocompatibility with non-specific interactions, and long-term in vivo stability.

Researchers have revealed that zwitterionic ligands can improve the biocompatibilities of the nanoparticles with good water solubility and colloidal stability under a wide pH range and high salt concentration.33,35−39 Up to now, people have reported several ways to fabricate the zwitterion-coated quantum dots,33,35,36 iron oxide,38 and gold nanoparticles39 according to the characteristics of their respective surface nature and particle size. However, few works are focused on the zwitterionic UCNPs fabrication and application. If zwitterionic character can be realized for UCNPs by an extension of this strategy, their biocompatibility can be greatly enhanced for the...
bioapplications. For the lanthanide UCNPs, to realize proper zwitterionic coating, we also need to find facile and appropriate surface engineering method for their special surface property and particle size. Phospholipid micelles-encapsulated NPs can simultaneously provide good colloidal stability and low nonspecific adsorption in a variety of bioenvironments, and it can also afford biocompatibility by mimicking the composition and functionality of the cell external membrane.\(^5,40,41\) In light of the requirement to realize the zwitterionic coating for UCNPs, phospholipid surface engineering of UCNPs may offer an attractive method.

Herein, a new family of compact zwitterionic ligand systems composed with functional phospholipids were designed and used to coat on the surface of UCNPs to form UCNPs–phospholipids micelle complexes (UCMC). The zwitterionic UCMC are water-soluble, compact, and easily functionalized. We also demonstrate that zwitterionic phospholipids can provide UCNPs with extended pH and salt stability, and primary oleate ligands on the UCNP surface.\(^32\) In this way, the surfaces of UCNPs can be modified with various functional groups for specific biomedical applications. Moreover, commercial PEG2000 phospholipids with different heads (such as −COOH, −NH\(_2\), or −biotin) can offer the modified UCNPs with various functional groups for specific biomedical applications.

### RESULTS AND DISCUSSION

#### Design of the Compact Zwitterionic Ligands for UCNPs Surface Modification.

Efficient upconverting NaGdF\(_4\):20%Yb\(^{3+}/2%\)Er\(^{3+}\) UCNPs were synthesized using the successive layer-by-layer (SLBL) strategy reported previously by our group with oleic acid as the stabilizing agent\(^{25,42}\) and modified with a new family of compact zwitterionic ligands system composed with functional phospholipids. Herein the compact lipids system (Figure 1) containing 1,2-disteraroyl-sn-glycero-3-phosphoethanolamine-N-[carboxy (polyethylene glycol)-2000] (PEG2000-PE) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) were designed and used to coat on the surface of UCNPs to form the zwitterionic UCMC. PEG2000-PE acts as the stabilizer to ensure the monolayer cladding of the phospholipids onto the UCNPs, preventing the formation of lipid vesicles. The DOPC is used as the basic component to realize the zwitterionic surface. The hydrophilic region of DOPC contains two functional groups: a phosphate group acting as a negative charged group, and a quaternary amine group acting as a positive charged group. The acid dissociation constant (\(pK_a\)) of an alkyl phosphate acid (phosphatidylcholine) is \(\sim 1.0\), meaning that it can be mostly deprotonated (ionized) at pHs above the \(pK_a\) value. On the other hand, the \(pK_a\) of a quaternary amine group is \(\sim 11.0\), and it can be mostly protonated (ionized) at pHs below the \(pK_a\) value. When these two functional groups are combined within a single ligand, it is reasonable to expect that the resulting ligand can hold an ionized state over a wide range of pH, although the actual \(pK_a\) values in the microenvironments present on UCNPs surfaces may be slightly different from that of the individual group. Furthermore, the UCNPs would become water-dispersible, driven by the hydrophobic van der Waals interactions between the hydrophobic tail of the phospholipids and primary oleate ligands on the UCNP surface.\(^32\) In this way, the surfaces of UCNPs can be modified into hydrophilic and zwitterionic character. Moreover, commercial PEG2000 phospholipids with different heads (such as −COOH, −NH\(_2\), or −biotin) can offer the modified UCNPs with various functional groups for specific biomedical applications.

#### Fabrication and Characterization of Compact Zwitterionic Modified UCNPs.

The DOPC zwitterionic phospholipids here purposely designed to serve multiple simultaneous roles. To study the influence of zwitterionic charge phospholipid on the surface and colloidal properties, UCNPs coated with pure PEG2000-PE (PEGLipo-UCNPs), zwitterionic compact phospholipids containing 50% PEG2000-PE and...
50% zwitterionic DOPC (ZwitLipo-UCNPs), and negative charged compact phospholipids containing 50% PEG2000-PE and 50% negative charged 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (DSPE-PEG2000-COOH) (NegaLipo-UCNPs) are designed, respectively. Selected physical properties of the as-prepared phospholipid coated UCNPs are listed in Table 1. Some insights into lipid dissolution and interactions within aqueous environments were provided by probing the relative wettability of each UCMC, which was accomplished by drying uniform concentrations and volumes of each lipid-coated UCNP samples onto glass slide surfaces, later measuring the subsequent contact angles of water drops using sessile drop goniometry. Zwitterionic surfaces of ZwitLipo-UCNPs produced the smallest contact angle at 9.5°, while PEGLipo-UCNP surfaces show the largest value of 34.4°. The smaller the observed contact angle, the better the relative interaction of water is with a given surface coating. These results suggest that introduction of multiple ionizable zwitterionic groups onto the surface lipids can enhance the hydrophilicity of UCNPs and help improving aqueous solubility. The hydrodynamic diameters of the phospholipid modified UCNPs series were measured by using dynamic light scattering (DLS) analysis. As shown in Table 1, the hydrodynamic diameters of the ZwitLipo-UCNPs, NegaLipo-UCNPs, and PEGLipo-UCNPs were ranged from 39.2 to 47.1 nm. ZwitLipo-UCNPs show the smallest hydrodynamic diameter because the hydrophilic region of DOPC lipid is much shorter than that of PEG2000-PE or DSPE-PEG2000-COOH, and the complex of zwitterionic lipids offered the UCMC compact surfaces. The ζ potentials of the UCMC series in an acidic medium (pH ~4) and alkaline medium (pH ~8.3) are also shown in Table 1. Because of the existence of negative charged phosphate group in PEG2000-PE, there is little change for ζ potential at pH ~4 (~18.3 mV) and ~ 8 (~20.8 mV). It is revealed that the negative charged PEG2000PE-UCNP has little buffering capacity to acid and alkali. Respectively, at these acid and alkali conditions, the values of ~11.2 and ~25.0 mV were observed for the ZwitLipo-UCNPs, reflecting the balance between protonation of the phosphate group and ionization of the quaternary amines. In acidic medium, the phosphate group can be protonated, showing buffering capacity to acid; in alkali medium, the quaternary amine can receive hydroxide ions, showing buffering capacity to alkali. In contrast, NegaLipo-UCNPs were not stable in acid medium. When carboxyl was protonated, there were not any positive groups to stabilize the whole surface of UCNPs, resulting in the lack of buffering capacity to acid. The above results further confirmed that the DOPC and PEG2000-PE compact lipids indeed displayed the desired zwitterionic properties and, more importantly, could impart them to the UCNPs surface engineering to improve the biocompatibility.

A representative TEM image (Figure 2B) of the resultant ZwitLipo-UCNPs shows that they remain monodisperse in size without obvious change in shape and aggregation in comparison with the as-made hydrophobic UCNPs (Figure 2A). High-resolution TEM investigation (Figure 2B inset) confirms the UCNPs with an approximately 3 nm thick hydrophobic oleic acid/lipid layer around the surface. Upon continuous excitation at 980 nm, the luminescence of the ZwitLipo-UCNPs in water appears as predominantly green emission (Figure 2C). The corresponding upconversion luminescence spectrum of ZwitLipo-UCNPs in water is similar to that of the as-prepared NaGdF4:20%Yb3+/2%Er3+ samples in chloroform, with a slight decrease in the relative integrated

![Figure 2](https://example.com/figure2.png)
green/red emission ratio owing to the surface quenching effect of water molecules (Figure 2C). These results strongly indicate that the characteristic upconversion luminescent property of the nanoparticles is not affected obviously after being coated by the compact zwitterionic phospholipids. Dynamic light scattering (DLS) measurements indicate that the ZwitLipo-UCNPs are well-dispersed in water with a mean hydrodynamic diameter of ∼39 nm (Figure 2D). Compared with oleic acid capping UCNPs dispersed in chloroform (∼18 nm), this increase of approximately 21 nm in diameter is in agreement with a monolayer cladding of the phospholipids around UCNPs.

In the UCNP–phospholipid mixed system, the hydrophobic mismatch between the UCNPs and the phospholipid aggregated structure (liposome or micelle) is not expected because the surface chemical property of the nanoparticles is not affected obviously after being coated by the compact zwitterionic phospholipids. Dynamic light scattering (DLS) measurements indicate that the ZwitLipo-UCNPs are well-dispersed in water with a mean hydrodynamic diameter of ∼39 nm (Figure 2D). Compared with oleic acid capping UCNPs dispersed in chloroform (∼18 nm), this increase of approximately 21 nm in diameter is in agreement with a monolayer cladding of the phospholipids around UCNPs.

In the UCNP–phospholipid mixed system, the hydrophobic mismatch between the UCNPs and the phospholipid aggregated structure (liposome or micelle) is not expected because the surface chemical property of pure UCNPs is not amphiphilic but purely hydrophobic due to the presence of oleic acid molecules. However, because the hydrophobic UCNPs surface must be completely covered by the phospholipid monolayer in order to avoid the high energy penalty caused by exposure of the hydrophobic part to water, monolayer bending, and the conformational change of phospholipid hydrocarbon chains such as stretching, compression, and tilting are inevitable. As far as we know, any aggregated structures made of phospholipids and hydrophobic nanoparticles, except the NPs–liposome complexes (NLC) or NPs–micelle complexes (NMC), have not been reported yet. In the present work, we found that it is difficult to obtain the UCMC with the pure zwitterionic DOPC phospholipid. With 18 nm UCNPs as an example, DLS results reveal that UCNP–liposome complexes (UCLC) can be obtained in the presence of pure DOPC phospholipid (Figure 3B). To realize the uniform zwitterionic UCMC (ZwitLipo-UCNPs), the PEG2000-PE to DOPC has to be used as the stabilizer (Figure 1). With the increase of the PEG2000-PE ratio gradually, there is a critical transition point to obtain the uniform zwitterionic UCNP–phospholipids complex (Figure 3A). According to the optical photograph (Supporting Information, Figure S1) and DLS results (Figure 3B), we also found that this CR points were dependent on UCNPs particle size. For the 18 nm UCNPs, uniform ZwitLipo-UCNPs could be formed with the 50% DOPC and 50% PEG2000-PE. When the UCNPs size was decreased to 4 nm (Figure 3B, Supporting Information, Figures S2,S3), we had to increase the PEG2000-PE percentage to 70% to form the stable ZwitLipo-UCNPs, while only 20% PEG2000-PE was enough for the 40 nm UCNPs (Figure 3B, Supporting Information, Figures S2,S3) for this purpose. The aim of the present work is to obtain the uniform and dispersed zwitterionic UCMC by the phospholipid surface engineering while avoiding the formation of UCLC. Actually, previous work had demonstrated that the small NPs (<5 nm) cannot be covered with pure monolayer zwitterionic phospholipid such as 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) molecules. However, few work studied the size-dependent stability of NPs and zwitterionic phospholipid complexes before. Pak et al. proposed a theoretical model to explain the size-dependent stability of NPs and DOPC phospholipid complexes. In this model, the elastic interfacial energy changes related to deformations of the lipid monolayer around the NPs.
nonspecific affinity toward bovine serum albumin (C) and lysozyme serum proteins (D). Samples were measured at time point of less than 5 min, 1 h, 5 h, 12 h, 24 h, 48 h, and 72 h.

in the NMC and NLC systems were calculated and compared. According to this model, NPs below the critical size (<5 nm) are easily loaded into the lipid bilayer of NLC, but those above it prefer the micelle-like structure of NMC (>5 nm). Our results reveal that UCLC can be formed in the presence of small UCNPs and pure DOPC such as the uniform incorporation of 4 nm UCNPs (Supporting Information, Figures S2, S3) into the phospholipid bilayer of liposome (Figure 3C), which agrees well with the above theory model. However, we found that it is difficult to obtain the UCMC with the pure zwitterionic DOPC phospholipid even when the particle size was increased to 40 nm (Figure 3B, D, E), and the UCNPs can only be observed inside or outside the liposome vesicles (Figure 3D, E), which is totally different from the theory speculation proposed previously.49 This means that it is difficult to obtain the NMC for the pure DOPC even for the large size NPs due to the difficulty in accommodating the high curvature of the nanoparticles with the bulk molecular structure of DOPC with double hydrocarbon chains. In other words, the hydrocarbon chain must be accompanied by conformational changes such as stretching and compression during the high curvature micelle formation from the zwitterionic DOPC phospholipid. To obtain the UCMC, the PEG2000-PE lipid has to be used as the stabilizer to ensure the monolayer cladding of the lipids onto the UCNPs, preventing the formation of lipid vesicles. The uniform UCMC also can be obtained with the pure PEG2000-PE even for the 4 nm UCNPs. We estimated the larger headgroups of the PEG2000-PE lipid lead to the decrease of the bending energy cost in comparison with the DOPC. Furthermore, according to the theoretical packing parameter consideration,50 the intrinsic curvature of the multicomponent lipid with different headgroup size is proper for covering a high curvature surface corresponding to the NPs size. Therefore, the multicomponent nature of the PEG2000-PE and DOPC system can contribute to lowering the stretching energy and ensure the monolayer cladding of the lipids onto the UCNPs, preventing the formation of lipid vesicles.

**Stability of Zwitterionic Modified UCNPs.** To further study the influence of zwitterionic phospholipids on the surface properties of UCNPs, the long-term colloidal stability of the Lipo-UCNPs series across a wide pH range was also evaluated. The representative images in Figure 4A, B show the luminescence images of ZwitLipo-UCNPs and NegaLipo-UCNPs, respectively, dispersed in buffer solutions increasing from pH 3 up to 12 at a time point of less than 20 min and 4 weeks following sample preparation approach. ZwitLipo-UCNPs were colloidally stable in all of these pH conditions without apparent fluorescence quenching during this period. While colloidally stable in basic media, NegaLipo-UCNPs became unstable in acidic media below pH ~5. The results reveal that the pH stability of ZwitLipo-UCNPs is better than that observed for NegaLipo-UCNPs. We attribute this to their zwitterionic nature of DOPC phospholipid, which displays pK_a's in both acid and basic regime and thus should be ionized across a broad pH range. Furthermore, the acid dissociation constant (pK_a) of an alkyl phosphate acid (phosphatidylcholine) (~1.0) is evidently lower than that of the alkyl carboxylic acid (~5.5),43 which can explain why the ZwitLipo-UCNPs are more stable than NegaLipo-UCNPs for the low pH value solution (Figure 3A, B). The colloidal stability of the zwitterionic UCNPs was also examined under high salt concentration and in bovine serum (Supporting Information, Figure S4). ZwitLipo-UCNPs were respectively dispersed in 3 M NaCl solutions (pH ~6) and bovine serum (pH ~7) and monitored over time. Both of the samples showed good colloidal stability and dispersibility for at least 4 weeks (Supporting Information, Figure S4). This suggests that compact zwitterionic UCNPs may be quite tolerant of similar ion-rich biological environments for long time periods.

To evaluate the stability of UCNPs coated with different phospholipid in protein solutions, DLS was used to track the size change process of the UCMC during their incubation in protein solutions. Lysozyme and bovine serum albumin (BSA), representative positively and negatively charged proteins at neutral pH, were chosen for protein binding tests. The stability of the UCMC was tested in 10 mg/mL lysozyme and BSA solution incubated at room temperature, respectively. Figure 4C shows the hydrodynamic diameters of the ZwitLipo-UCNPs and NegaLipo-UCNPs in 10 mg/mL BSA solution. Both kinds of the UCMC showed excellent stability without an obvious increase in size during a 72 h incubation period. These results indicate that zwitterionic surface indeed protects the ZwitLipo-UCNPs against aggregation under the existence of BSA protein. There are repulsive interactions between the

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**Figure 4.** Luminescence images for a set of 10 mM ZwitLipo-UCNPs (A) and NegaLipo-UCNPs (B) in different buffers at pH 2–12. The green emission of UCNPs was excited with a NIR laser at 980 nm. Images were taken <20 min and ~4 weeks after sample preparation. DLS point plots of nonspecific affinity toward bovine serum albumin (C) and lysozyme serum proteins (D). Samples were measured at time point of less than 5 min, 1 h, 5 h, 12 h, 24 h, 48 h, and 72 h.
negative surface of NegaLipo-UCNPs and the negative BSA, which can also make the NegaLipo-UCNPs stable for a long time in the BSA solution. The DLS results of the ZwitLipo-UCNPs and NegaLipo-UCNPs in lysozyme solution are shown in Figure 4D. ZwitLipo-UCNPs show excellent stability during the period of 72 h incubation. However, due to the negative surface and its attraction with positively charged lysozyme, NegaLipo-UCNPs formed a white precipitate in several hours when exposed to the lysozyme solution. The excellent stability of ZwitLipo-UCNPs in both negative and positive protein solutions shows that the zwitterionic surface is more effective in protecting UCNPs from binding with nonspecific protein and more suitable for bioapplications in complex physiological conditions.

Chemical Conjugation to Compact Zwitterionic Modified UCNPs. The ZwitLipo-UCNPs can be easily designed for further bioconjugating and specific biomedical applications. For example, a certain percentage (e.g., 5 wt % in the present work) of commercial PEG2000-PE lipids with biotin heads can take the places of PEG2000-PE lipids in the compact zwitterionic lipid system (Figure 1), without changing the colloidal properties of the whole ZwitLipo-UCNP, offering the UCMC (Biotin-ZwitLipo-UCNP) functional biotin groups. Biotinylated single strands of DNA with designed aptamer sequence can be attached to streptavidin-functionalized Biotin-ZwitLipo-UCNP through the well-established specific interaction between streptavidin and biotin to prepare single-strand DNA modified ZwitLipo-UCNP (sDNA-ZwitLipo-UCNP). These nanoparticles were then incubated with complementary strand DNA with TAMRA dyes labeled at 3′-termini (TAMRA-cDNA). The hybridization format of sDNA-ZwitLipo-UCNP and TAMRA-cDNA induced close proximity between UCNPs to TAMRA under illumination at 980 nm, which can be demonstrated with an appearance of a broad and characteristic TAMRA emission band (~577 nm) (Figure 5A) accompanied by the simultaneously decreasing of the green upconversion emissions bands between 514 and 560 nm of UCNPs. Furthermore, red emission between 635 and 680 nm of UCNPs was also quenched to a small extent, which is related with the upconversion mechanism: among other feeding channels, the population of the red emission level ($^{4}F_{9/2}$) is partially derived from the green emission level ($^{4}S_{3/2}$, $^{4}F_{7/2}$) via a nonradiative relaxation process.51

Nonspecific Cell Binding and Efficient Cell Targeting. After demonstrated the general and versatile method to prepare
functionalizable ZwitLipo-UCNPs, we then explored the usage of the antibody conjugated ZwitLipo-UCNPs for targeted imaging of cancer cells (Figure 5B). The specificity of antibody conjugated ZwitLipo-UCNP binding to the surface of living cancer cells was confirmed using HeLa cell lines. DSPE-PEG2000-biotin (5%), PEG2000-PE (45%), and DOPC (50%) composite lipids were used for coating of NaGdF4:20%Yb3+/2%Er3+ UCNPs to form the Biotin-ZwitLipo-UCNPs. The biotinylated zwitterionic UCMC can bind to streptavidin and rabbit Anti-EGFR antibody (biotinylated) to conjugate the targeting antibody on the surface of ZwitLipo-UCNPs to get antibody-ZwitLipo-UCNPs. Because anti-EGFR antibody can bind to overexpressed EGFR receptors on HeLa cancer cell lines, the HeLa cells treated with the resulting antibody-ZwitLipo-UCNPs were then imaged with wide field scan excitation under 980 nm NIR laser. As shown in Figure 5B and Supporting Information, Figure S5, luminescence spots are mainly observed on the cell membrane and cytoplasm, thus indicating excellent binding of antibody-ZwitLipo-UCNPs to the cancer cells. One of the main challenges in nanoparticle cell staining is the mitigation of the nonspecific interactions. In the present work, zwitterionic UCNPs remarkably minimized nonspecific binding to cell membranes, as demonstrated by control cells exposed to ZwitLipo-UCNP and Biotin-ZwitLipo-UCNP (Figure 5B), which exhibited indistinguishable luminescence signals from the background.

To investigate the cytotoxicity of ZwitLipo-UCNPs, a CCK-8 assay with the HeLa cells was used to determine the effect of ZwitLipo-UCNPs on cell proliferation after 24 h. No significant differences in the proliferation of the cells were observed in the absence or presence of 0.05−0.5 mg/mL ZwitLipo-UCNPs (Supporting Information, Figure S6). The cellular viabilities were estimated to be greater than 89% after 24 h. These data show that ZwitLipo-UCNPs (<0.5 mg/mL) can be considered to have low cytotoxicity.

Biocompatible Zwitterionic Surface Coating of UCNPs for Efficient in Vivo Bioimaging. To evaluate the effectiveness of the biocompatible zwitterionic surface coating of UCNPs for in vivo bioimaging, we then monitored the upconversion bioimaging signals of ZwitLipo-UCNPs and NegaLipo-UCNPs by enhanced permeability and retention (EPR) effect of the tumor vasculature. Here the NaGdF4:20%Yb3+/0.2%Tm3+ UCNPs were prepared for the bioimaging because they have efficient emission at ∼800 nm under 980 nm laser excitation (Figure 6A), which has been demonstrated with deeper tissue penetration depth previously. Before the in vivo experiments, we compared the in vitro tissue bioimaging performance between the ZwitLipo-UCNPs and NegaLipo-UCNPs by depositing the two samples beneath the animal tissues (Figure 6B). When irradiated with 980 nm laser, almost the same signal-to-background ratio (SBR) could be observed for the two samples with different surface coating, indicating that the different surface coating has no effect on the bioimaging performance. For the in vivo experiment, mice bearing a S-180 tumor on the right foreleg for targeted imaging were administered a 100 μL solution of ZwitLipo-UCNPs or NegaLipo-UCNPs intravenously. Then the mice were imaged 8 h postinjection of the liposome-UCNPs. As shown in Figure 6C, the SBR was significantly higher for the ZwitLipo-UCNPs than for the NegaLipo-UCNPs (Figure 6D).

Figure 6. (A) Upconversion luminescence spectra of blue-emission UCNPs in chloroform (black trace) and ZwitLipo-UCNPs in water (blue trace). (B) Upconversion luminescence imaging of ZwitLipo-UCNPs (a, left) and NegaLipo-UCNPs (a, right) covered with a ∼1.5 mm piece of pork slice. All images were acquired under the same conditions (power density ∼200 mW/cm²). (C) In vivo upconversion luminescence imaging of subcutaneous S-180 tumor (right foreleg) borne by mice after intravenous injection of ZwitLipo-UCNPs (left) or NegaLipo-UCNPs (right) in 8 h. Both images were acquired under the same conditions (power density ∼200 mW/cm²). (D) The intense signal-to-background ratio (SBR) observed in the in vitro tissue bioimaging in (B) and the in vivo imaging in (C). (E) Blood circulation results of the ZwitLipo-UCNPs and NegaLipo-UCNPs based on ICP-MS analysis of Gd element concentration of the blood samples. A first-order exponential fits the data points with a half-life of circulation for the ZwitLipo-UCNPs and NegaLipo-UCNPs of 6.1 and 5.1 h, respectively. (F) Biodistribution of particles in organs of tumor-bearing mice sacrificed at 24 h postinjection of Lipo-UCNPs. Error bars were based on triplet samples.
NegaLipo-UCNPs at a concentration of 2 mg/mL (approximately ≈200 µg per animal) through tail vein injection. After 8 hours of blood circulation, the mice were injected using the modified upconversion luminescence in vivo imaging system (Figure 6C). Under the 980 nm laser excitation, the SBR in the S-180 tumor of the mice injected with ZwitLipo-UCNPs was compared with that of the NegaLipo-UCNPs injected ones (Figure 6D). The SBR of the former is significantly higher than that of the latter due to the lower nonspecific affinity of ZwitLipo-UCNPs toward the components of blood, indicating that the zwitterionic coating can enhance the in vivo bioimaging efficiency. Furthermore, this result is consistent with results of the long-term in vivo blood circulation experiment. Blood circulation half-life of the ZwitLipo-UCNPs was determined to be 6.1 h based on a fit to first-order exponential decay of the Gd element concentration of the blood samples (Figure 6E), suggesting the higher biocompatibility and slower uptake by the reticuloendothelial system (RES) compared to the half-life of NegaLipo-UCNPs (5.1 h).

To further investigate the zwitterionic surface modification effect on the biological compatibility, we performed quantitative biodistribution studies to detect the amount of Gd element in tissue samples following ZwitLipo-UCNPs and NegaLipo-UCNPs injection using ICP-MS analysis. ZwitLipo-UCNPs exhibited an enhanced accumulation at tumor sites by 8 h and presented a much higher concentration than NegaLipo-UCNPs by 24 h (Figure 6F). Moreover, the data from ICP-AES analysis of the Gd concentration within the tissues indicated that accumulation of ZwitLipo-UCNPs in the S-180 tumor was much higher than that of the NegaLipo-UCNPs (Figure 6E), further demonstrating they have less nonspecific interactions with blood components and less loss in the process of circulation of the blood. It is also worth noting that nonspecific UCNP uptake and retention took place primarily in the liver and the spleen, with little accumulation in the kidney or the lung. This pattern of organ uptake and distribution was similar to that of nanoparticle probes in the previous reports.54

CONCLUSIONS

By designing and fabricating the zwitterionic phospholipid-based compact ligands, we have developed water-soluble, biocompatible, and easily functionalized UCNPs for biosensing, cellular imaging, and in vivo imaging applications. Because of the zwitterionic nature, inherent benefits that these compact surface modification provide for biological applications include: (i) more colloidal stability under a wide pH range and high salt concentration, (ii) the functional groups on ZwitLipo-UCNPs (i) more colloidal stability under a wide pH range and high salt surface modiﬁcation, (iii) excellent colloidal stability and reduced non-speciﬁc protein adsorption in protein solutions, (iv) utility with low toxicity in cellular environments, and (v) excellent in vivo bioimaging performance and longer blood circulation half-life by comparing with those of negative charged UCNPs. Moreover, because the hydrocarbon chain must be accompanied by conformational changes such as stretching and compression during the high curvature micelle formation from zwitterionic DOPC phospholipid, the PEG2000-PE lipid has to be used as the stabilizer to ensure the monolayer cladding of the lipids onto the UCNPs, preventing the formation of lipid vesicles. The intrinsic curvature of the multicomponent lipid with different headgroup size is proper for covering a high curvature surface corresponding to the NPs size. Furthermore, we also believe that the multicomponent lipid system can be extended to other hydrophobic NPs for the zwitterionic modification. Therefore, the designed zwitterionic compact surface modification approach developed here has a strong potential to expand UCNPs and other NP capabilities in many biological applications.

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