Synthesis and Development of Lipoprotein-Based Nanocarriers for Light-Activated Theranostics

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Abstract: The design and synthesis of new light-activated contrast agents for theranostics (therapy/diagnosis) has the potential to facilitate multifunctional and improved personalized medicine. The use of light as a remote activation strategy provides spatial and temporal control of drug effect and nanotechnology can play a key role in this process. Lipoproteins (LDL and HDL), which transport water-insoluble cholesteryl esters and triacylglycerols in nature, have evolved to efficiently ferry exogenous hydrophobic compounds in vivo. They are naturally biocompatible, maneuverable due to their small size (< 30 nm), and can be loaded through various methods, and are therefore ideal vehicles to load and transport hydrophobic theranostic agents. This review examines the history and ongoing research activities regarding the design and synthesis of lipoprotein-based formulations, and their applications or potential applications as light-activated theranostic agents, with a main focus on photodynamic therapy. This field, while still in its infancy, will benefit from improved design and modulation of enhanced lipoprotein-based nanocarriers, with the ultimate goal of simultaneous imaging and photoactivation of therapeutic agents in a clinical setting.

Keywords: drug delivery · lipoproteins · imaging agents · photodynamic therapy · photosensitizers

1. Introduction

One of the central directions of research in medical theranostics is the design of agents that can simultaneously exert a therapeutic effect and enable molecular imaging. Although several types of small molecules (e.g., radiotracers and photosensitizers (PS)) have been successfully used for disease-related theranostics, nanocarriers have a variety of advantages over small molecules, such as enhanced pharmacokinetic profiles and drug-shielding ability.[1,2] In addition, nanocarriers are versatile and can be loaded with multiple agents in multiple ways (e.g., surface loading, core loading), which allows the packaging of several imaging and therapeutic agents and thus offers the potential for image-guided therapy. At the theranostics frontier, the loading of phototoxic agents into nanocarriers enables spatial and temporal control of therapeutic drug activation or release, with the goal of treatment of disease in a selective and specific manner, thus improving the therapeutic index.[3] Along with light-activated therapy, imaging modalities such as fluorescence imaging, radiography, magnetic resonance imaging (MRI) and computed tomography (CT) facilitate the understanding of drug distribution and can provide valuable information towards the treatment outcome. As natural and endogenous nanocarriers, lipoproteins have long been utilized in nanomedicine as delivery tools for therapeutics and imaging contrast agents.[4,5] They have several advantages for light-activated theranostics, such as excellent size control, high payload-carrying capacity, biocompatibility, biodegradability and nonimmunogenicity.

1.1. Photosensitizers

Over the past 40 years, photodynamic therapy (PDT) has emerged as a powerful cancer treatment modality with the ability to control the spatial activation of PS to produce cytotoxic singlet oxygen only at the site of the lesions being treated.[7] An ideal PS should have low levels of dark toxicity and low incident administrative toxicity, absorb light in the red or near-infrared range (600–900 nm) to allow deep tissue penetration, have a high extinction coefficient (> 20,000–30,000 M⁻¹ cm⁻¹) to minimize the dose of PS required for effective treatment, be

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easily prepared from starting materials, and exhibit high accumulation in lesion tissues.\cite{8,9} During the past decades, a number of PS have been approved for clinical trials in human patients for a variety of diseases, including skin-related carcinomas, T-cell lymphoma, Kaposi’s sarcoma, bladder and bone carcinomas, age-related macular degeneration, and cancers of the head, neck, prostate, lung, stomach, ovary, and cervix.\cite{10}

To date, there have been three generations of PS. First-generation PS (1970s to early 1980s), such as porfimer sodium (Photofrin), have limited red-shifted wavelength absorption and patients exhibit prolonged skin photosens-

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sitivity (up to 12 weeks). Second-generation PS (from late 1980s) are porphyrin derivatives or synthetics, including 5-ALA (Levulan), chlorins (temoporfin, Foscan), and benzoporphyrins (verteporfin, Visudyne), which have an improved absorption at longer wavelengths (>650 nm) and reduced patient photosensitivity. To exert the best use of such PS in treatment, increasing the selective accumulation of the PS in the diseased tissue can lead to improved PDT efficacy and reduced collateral damage to normal tissues. Thus, third-generation PS are designed to improve tumor cell-selective uptake by combining second-generation PS with appropriate carriers, of which lipoproteins are one of the most attractive candidates.

1.2. Lipoproteins for PS Delivery

Plasma lipoproteins are natural, spherical, and water-soluble macromolecules. Based on the density at which they float by ultracentrifugation, lipoproteins can be separated into various classes, including high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and chylomicron (Figure 1A). Lipoproteins have a high lipid content (phospholipids, cholesterol, and triglycerides) and one or more specific lipid-binding proteins, referred to as apolipoproteins (e.g., ApoB, ApoA-I), which are the most important components in lipoproteins. The apolipoproteins not only stabilize the core-shell lipid nanostructure of the lipoproteins, but also determine the recognition of lipoprotein receptors for the delivery of water-insoluble lipids to specific cells. This cellular-targeted delivery involves distinct mechanisms, such as typical receptor-mediated endocytosis for LDL and selective transportation of core components for HDL. In addition, these apolipoprotein-associated receptors are overexpressed in a variety of malignant cells and tumors. Thus, these two lipoproteins are appealing natural nanocarriers for delivery of hydrophobic theranostic agents (including PS) for cancer theranostics, as they presumably benefit from their favorably small size (7–30 nm), which enables the nanoparticle to escape kidney filtration, and also to penetrate through the restricted interfibrillar openings (<40 nm) that are commonly present in solid tumors. Furthermore, LDL and HDL are favorable candidates for PS delivery due to a rich understanding of their fundamental transportation roles in blood circulation.

2. LDL Nanoparticles as PS Carriers

2.1. PS-Lipoprotein Interactions

With a flat aromatic structure, porphyrin-based compounds are hydrophobic and tend to associate with lipophilic regions of serum proteins (lipoproteins and albumin) to increase their solubility in aqueous medium or the bloodstream. In the bloodstream, the selective binding ability of porphyrins to serum proteins is mainly determined by the degree of hydrophobicity of the porphyrin, in that highly hydrophobic porphyrins bind to lipoproteins, and porphyrins with moderate hydrophobicity are transported mainly by albumin. The binding constants of such porphyrins to lipoproteins and albumin are in the same order of magnitude. Although the exchange kinetics among the different plasma fractions seem to depend on the PS structure, the delivery systems (including liposomes and oil emulsions), which are often applied to solubilize the hydrophobic porphyrins and facilitate their in vivo delivery, may also be involved in this process.

Figure 1. (A) Diagram of lipoprotein sizes. LDL and HDL have diameters of less than 40 nm. (B) Approaches for cargo loading onto lipoproteins. Surface loading: intercalation of amphiphilic molecules into the phospholipid monolayer. Protein conjugation: covalent attachment of compounds to lysine residues of apolipoproteins or peptides. Core loading: reconstitution of hydrophobic molecules into the core of lipoproteins. Figure 1A was made from the corresponding references, and Figure 1B was adapted with permission from reference [46].
process. It was reported that over 80% of hematoporphyrin (Hp) loaded with dipalmitoylphosphophocholine (DPPC) liposomes became associated with lipoproteins, whereas only 30% associated with lipoproteins when Hp was administered in phosphate-buffered saline (PBS). Other factors, such as the serum protein composition, which vary between different animal species and individuals, further complicate the binding process. In addition, it was found that transportation of sulfonated tetraporphyrin (TPPS$_d$)$_n$ by albumin led to accumulation of PS mainly in the vascular stroma of tumor, resulting in photodamage to the extracellular matrix after PDT. Although albumin plays an important role as a porphyrin-binding protein in serum, there is mounting evidence that lipoproteins, especially LDL, are important carriers for the transportation of porphyrin in vivo. Several examples can illustrate this point: (1) the association of PS with plasma lipoproteins often results in increased accumulation of PS in tumors and enhanced tumor cell damage upon light exposure. (2) Photofrin II is delivered much more efficiently to cultured fibroblasts when complexed to LDL than when complexed to albumin or HDL, due to the rapid internalization through LDL receptors. (3) Several malignant tissues express an increased number of LDL receptors compared to their normal counterparts or regular tissues. (4) Reduction of the number of LDL receptors in targeted cells or blocking of the ApoB-binding activities by acetylation results in decreased recovery of PS in cells, when using LDL as the PS carrier. Despite the promise, serum protein composition of lipoproteins and albumin vary across different animal species and individuals. If a hydrophobic PS is applied as is, it will spontaneously complex with LDL as the PS carrier. Despite the promise, serum protein composition of lipoproteins and albumin vary across different animal species and individuals. If a hydrophobic PS is applied as is, it will spontaneously complex with LDL, as well as with other proteins in the circulation, thus complicating the delivery process. Therefore, using the preformed PS-LDL complex has the potential to be more efficient for targeted delivery.

2.2. PS-LDL Complexes in PDT Treatment

Having benefitted from research progress in lipoprotein metabolism and the identification of LDL receptor pathways, and the understanding of LDL and its roles in the transportation of photosensitizers, researchers were encouraged to use LDL as a nanocarrier for selective delivery of photosensitizers for PDT, taking advantage of its high loading capability, targeted delivery, and biocompatibility. A comparison of in vivo PDT efficacy between hematoporphyrin in PBS (Hp-aq) and Hp precomplexed with LDL (Hp-LDL) has been performed on mice bearing a transplanted MS-2 fibrosarcoma with Hp at a drug dose of 2 mg kg$^{-1}$. Electron microscopy studies of tumor tissues demonstrated that vascular damage was the main cause of tumor necrosis for mice treated with Hp-aq while a much faster rate of PDT response was observed in tumors with Hp-LDL, via a mechanism involving direct damage of neoplastic cells. These results suggest that the use of LDL for the delivery of PS fundamentally altered the target of PDT. An enhanced PDT efficacy when using LDL for benzoporphyrin derivative (BPD) delivery was also observed in M-1 tumor-bearing mice treated with BPD-LDL complex, BPD-plasma complex or aqueous BPD. For the control group of mice dosed with BPD-plasma, irradiation of tumors resulted in little or no effect in terms of tumor necrosis or eschar formation. The group treated with aqueous BPD showed slight tumor necrosis, but all tumors had recurred by nine days following treatment. However, for the group administered with the BPD-LDL complex, all of the treated mice exhibited considerable biological responses (including tumor cell necrosis and eschar formation) on the day following treatment and approximately 80% remained tumor-free in the following 18 days. However, some PS-LDL complexes may not be stable in vivo. AlPcS$_4$(C$_{12}$), a derivative of aluminum sulfolhalocyanine (AlPcS), was designed to target tumor cells for PDT. The MTT cell viability assay revealed a substantial increase in the vitro phototoxicity using AlPcS$_4$(C$_{12}$)-LDL complexes compared to the AlPcS$_4$(C$_{12}$) control group. However, both exhibited similar activities in vivo. This indicates that the PS-LDL complexes may suffer from stability issues in vivo if the formulations are not optimized. Based on these findings, there are several parameters that should be considered during the design of PS-LDL complexes for in vivo PDT. The first one is the loading capacity of LDL. The loading of PS on LDL should not extend beyond its maximum capacity to ensure the absence of any free unbound PS, which may diminish LDL receptor (LDLR)-targeting efficiency. De Smidt et al. reported LDL-TPPS-2A particles with PS:LDL molar ratios of up to 250:1 did not change LDL receptor recognition. Extremely high molar ratios (1000:1) resulted in loss of receptor recognition. In vivo studies demonstrated that complexes with molar ratios up to 100:1 behaved like native LDL. The second parameter to be considered is the binding stability. The PS-LDL complexes should have excellent stability to prevent the leakage of PS and transfer to other serum components or cell membranes. Allison et al. reported no direct dissociation of BPD from a BPD-LDL complex to cell membranes during incubation with cells for two hours at 37°C. However, using classical spectroscopy and stopped-flow experiments, Huntsosova et al. observed the transfer of hypericin (Hyp) from a saturated complex (Hyp:LDL = 200:1) to free LDL upon mixing the saturated complex in a cuvette with free LDL. The third parameter is the specificity of LDL receptor (LDLR)-targeting ability. The loading of PS to LDL should not interfere with the specific binding to LDL receptors. The Zn-phthalocyanine/LDL complex (10–12:1) was reported to internalize into LDL receptor-expressing cells through non-specific endocytosis and showed inefficient cell uptake due to the changes in the
ApoB structure induced by phthalocyanine association, according to spectroscopic studies.\textsuperscript{[46]} Thus, to achieve a potent therapeutic efficacy, an effective formulation is required that has an optimal payload, excellent serum stability and LDLR-targeting ability.

### 2.3. Stable PS-LDL Formulations

As we mentioned above, one of the main concerns of using PS-LDL complexes for PDT treatment is the transfer of PS from LDL to other membrane systems, thus reducing the PDT specificity and efficacy. There are at least three approaches that enable stable loading of therapeutic and imaging contrast agents (Figure 1B).\textsuperscript{[87]} Firstly, intercalation into the phospholipid monolayer (surface loading) is a common method to form the PS-LDL complexes. This requires the loading agents to have a certain degree of amphiphilicity, allowing the hydrophobic groups to be buried in the phospholipid monolayer. The loading efficiency may depend on the polarity of the loading agents, as well as the reaction conditions, such as incubation time and temperature. Secondly, covalent attachment to amino acid residues of apolipoproteins (protein conjugation) is another loading method. This process involves the modification of certain amino acid residues, such as lysine and arginine, using isothiocyanates, N-hydroxysuccinimidy (NHS) esters, or acid/l-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) groups. The final loading method involves reconstitution into the lipid core of lipoproteins (core loading). Typically, the lipoprotein core lipids are exchanged with the designated hydrophobic cargo through organic extraction followed by lyophilization. Other methods of formulation include the sonication or cholate dialysis methods which are also used to make core-loaded biomimetic lipoproteins.\textsuperscript{[48,49]} These methods require precise control of operating conditions, and the cargo recovery efficiency is largely affected by its inherent properties, such as structure and hydrophobicity. Overall, these diverse approaches for cargo loading provide opportunities for the design of stable PS-LDL formulations.

#### 2.3.1. Covalent Conjugation of PS to LDL

One of the approaches described above is to make stable PS-LDL formulations through covalent conjugation of PS to LDL. The methods for conjugation of porphyrins to peptides or proteins have been reviewed by Giuntini et al.\textsuperscript{[50]} For example, the photosensitizer chlorin e\(_6\) (Ce\(_6\)) was covalently bound to LDL via carbodiimide activation.\textsuperscript{[51]} The resultant Ce\(_6\)-LDL conjugate significantly increased the uptake of Ce\(_6\) into cells with high LDL receptor expression, with an optimum Ce\(_6\)/LDL binding ratio of 50:1, and receptor-mediated uptake was demonstrated by competitive inhibition using free LDL. The improved PDT efficacy was also demonstrated in cells using an MTT assay following PDT treatment. Cells treated with Ce\(_6\)-LDL conjugate had 80% reduced cell viability, while the free and Ce\(_6\)-LDL complex only induced a maximum of 10% reduction. These data suggest that covalent conjugation of PS to LDL could increase the efficiency and selectivity of PDT. However, this is not always the case. The covalent conjugation approach has limitations on PS loading capacity. When AlPcS\(_4\) was covalently conjugated to LDL via amide bonds and a 6-carboxylyaminosulfonyl linker molecule with over 50 molecules of PS per particle, the resulting AlPcS\(_4\)-LDL showed inefficient photodynamic cytotoxicity, even at tenfold higher light or drug doses,\textsuperscript{[43]} suggesting that covalent labeling of the protein moiety with a high payload of PS greatly reduced LDL receptor recognition, and thus decreased the delivery efficiency and PDT efficacy of the PS-LDL conjugate.

#### 2.3.2. Core-Loading of PS into LDL

Thirty years ago, Goldstein and Brown discovered a powerful LDL reconstitution method to enable the endogenous core lipids to be replaced with exogenous cholesterol (300–400 nm), which is far from the clinically practical window for in vivo PDT (600–900 nm). AlPcS\(_4\)-LDL complexes were used to make core-loaded biomimetic lipoproteins.\textsuperscript{[52,53]} A variety of hydrophobic drugs could be loaded into LDL nanoparticles through this approach, which expanded the usage of LDL for the delivery of hydrophobic theranostics. Pyrene covalently coupled to cholesterol oleate (CO) (Figure 2A) was the first PS incorporated into LDL by the reconstitution method, to generate r-(PCO)-LDL.\textsuperscript{[54]} It was found that cellular uptake and the related PDT effect of r-(PCO)-LDL was dependent on the LDL receptor expression level. In LDL receptor-positive cells (transformed human fibroblasts, human A-431, and mouse L cells), the uptake of r-(PCO)-LDL was significant. Subsequent photosensitization (irradiation at 300–400 nm) led to cell killing (Figure 2B and 2C). Mutant fibroblasts, which had a relatively low LDL receptor expression, exhibited less uptake of r-(PCO)-LDL and were not affected by PDT treatment. Moreover, when the pyrene-CO was reconstituted into methylated LDL, which no longer binds to LDL receptors, no toxicity was observed. Thus, functional core-loading of PS with LDL as a vehicle was achieved. However, this pioneering approach has never progressed to in vivo investigation, presumably due to the irradiation wavelength of pyrene (300–400 nm), which is far from the clinically practical window for in vivo PDT (600–900 nm).

### 2.4. Improvement of Reconstituted LDL (rLDL) for PS Delivery

The main advantages of LDL reconstitution for PS loading are the protection of the PS from serum degradation and prevention of PS leakage or membrane transfer, thus assisting targeted PDT. Recently, such a method has been utilized to incorporate porphyrin-based PS, the most common PDT agents. Pyropheophorbide cholesterol oleate (Pyro-CE) and bacteriochlorophyll cholesterol
oleate (BChl-CE) were developed (Figure 3) and successfully reconstituted into LDL, resulting in a PS payload of 50:1 and 20:1 (PS:LDL) respectively.\[55,56\] Further attempts to increase the PS payload were not successful, perhaps due to the propensity of these planar porphyrin macrocycles to aggregate, which limits their solubility. To deal with this issue, a lipid-anchoring strategy was developed to improve the PS core payload using bisoleate (BOA) conjugates of silicon phthalocyanine (Pc) and naphthalocyanines (Nc), termed SiPc-BOA and SiNc-BOA (Figure 3).\[57–59\] One of the advantages of this strategy lies in the silicon coordination, which allowed the binding of two axial oleate ligands at the top and bottom of the planar macrocycle to prevent Pc aggregation. Another advantage was the fact that the bisoleate conjugation provided strong lipid anchors to the LDL phospholipid monolayer. Through this approach, the formulated r-(SiPc-BOA)-LDL and r-(SiNc-BOA)-LDL could achieve a payload of 300:1 and 100:1, respectively (Table 1), while maintaining the LDL receptor–targeting specificity. Thus, the PS loading capacity of LDL was significantly improved. These PS-loaded LDL formulations could be

![Figure 2](image)

**Figure 2.** (A) Structure of pyrene-CO. The grey sphere represents the PS pyrene. (B) In vitro PDT efficacy of r-(PCO)-LDL on three types of cultured mammalian cells (SV40-transformed human fibroblasts, human A-431 cells, and mouse L cells). Cells were treated with LDL, r-(PCO)-LDL or r-(PCO)-MeLDL with a protein concentration of 10 pg/mL and were irradiated for 40 min following drug incubation. On day seven, the cells were fixed and stained with crystal violet. (C) Quantification of the toxic effect induced by PDT, using the [3H]-thymidine incorporation assay to evaluate the DNA synthesis rate. When CHO cells were incubated with r-(PCO)-LDL and then exposed to light, the incorporation of [3H]-thymidine was reduced compared to control groups. Figures 2B and 2C are reproduced with permission from reference [53].

<table>
<thead>
<tr>
<th>PS</th>
<th>Molecular weight (Da)</th>
<th>Payload</th>
<th>Size (nm)</th>
<th>Ex./Em. (nm)</th>
<th>Extinction coefficient (M⁻¹·cm⁻¹)</th>
<th>Ref.</th>
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<td>Pyro-CE</td>
<td>1070.7</td>
<td>-50</td>
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<td>667/720</td>
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<td>[55]</td>
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<td>-20</td>
<td>26.2 ± 4.8 (DLS)</td>
<td>752/762</td>
<td>97,800⁹[^2]</td>
<td>[54]</td>
</tr>
<tr>
<td>SiPc-BOA</td>
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<td>-300</td>
<td>23.2 ± 4.6 (TEM)</td>
<td>684/692</td>
<td>25,000⁹[^3]</td>
<td>[56]</td>
</tr>
<tr>
<td>SiNc-BOA</td>
<td>1528.2</td>
<td>-100</td>
<td>21.3 ± 3.4 (TEM)</td>
<td>811/826</td>
<td>365,700⁹[^4]</td>
<td>[58]</td>
</tr>
<tr>
<td>Bchl-BOA</td>
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<td>-50</td>
<td>30.9 ± 5.6 (DLS)</td>
<td>752/762</td>
<td>97,800⁹[^2]</td>
<td>[54]</td>
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used for targeted near-infrared optical imaging and as PDT agents for cancers. Using a two channel in-phase and in-quadrature (I&Q) spectrometer (Figure 4A), the selective accumulation of r-(SiNc-BOA)-LDL in a LDL receptor-overexpressing HepG2 tumor was observed by measuring the absorption, using the acetylated r-(SiNc-BOA)-LDL as a negative control. An in vitro clonogenic assay demonstrated that r-(SiPc-BOA)-LDL had potent PDT efficacy on LDLR-overexpressing human tumor cells. More recently, bacteriochlorophyll bisoleate (Bchl-BOA) was synthesized and reconstituted into LDL. The resulting r-(Bchl-BOA)-LDL displayed excellent tumor growth inhibition following treatment with a 750 nm diode laser, compared with the control groups, which were either dosed with drug alone or only irradiated with light (Figure 4B and 4C). Although these studies are still in the early stages, future research will focus on using these PS-loaded LDL nanoparticles in more meaningful orthotopic tumor models and taking advantage of interstitial lasers, which allow deeper and more specific light delivery.

2.5. New Directions in the Use of LDL for Theranostics

Although progress has been made towards the preparation of stable and high payload-containing PS-LDL formulations for PDT, there remain questions of how to further improve their formulation and how to explore new applications in theranostics.

2.5.1. Developing Multifunctional LDL Nanoparticles

The labeling of LDL with various imaging contrast agents facilitates the visualization and quantification of LDL uptake and biodistribution and is thus useful for theranostics. The use of LDL for fluorescent imaging has long been reported. In addition to the PS we mentioned above, a number of fluorescent dyes with different excitation and emission wavelengths have been used to label LDL for research into cellular pathways and biochemical mechanisms, atherosclerosis, and in vivo tumor imaging. Among these, the near-infrared probes are attractive because they allow deep penetration of light into
tissues with minimal optical interference from the blood and tissue components. Radioimaging is another imaging modality that was applied to LDL over 20 years ago. Several types of radiotracer were selected to bind to LDL by covalent conjugation to ApoB proteins for various applications, including indium-111 for atherosclerotic proximal aorta detection in hypercholesterolemic rabbits, iodine-125 for imaging of carotid lesions in patients with atherosclerosis, and 99m-technetium for tumor imaging in B16 melanoma–bearing mice. An LDLR-targeted MRI contrast agent has been prepared by incorporation of amphiphilic gadolinium–diethylenetriaminepentaacetic acid chelates into LDL. More recently, polyiodinated triglyceride molecules were successfully reconstituted into LDL for CT imaging. Although there has been limited research in combining the imaging and therapeutic modalities, simultaneous loading of phototoxic agents and imaging dyes into LDL is possible and in the future such combinations will provide more information about the behavior of LDL during treatment.

2.5.2. Redirecting LDL to Other Receptors of Choice

The use of PS-LDL in theranostics relies on the degree of expression of LDL receptors in targeted tissues or cells, which limits the range of targeted applications. To deal with this issue, several reports have described strategies for target exchange, such as lactosylated and acetylated lipoproteins with most reports targeting specific types of cells in liver tissue. We recently reported the concept of introducing tumor-homing molecules to lipoprotein-based nanoparticles and rerouting them from their normal lipoprotein receptors to other selected cancer-associated receptors. As a proof of concept, a folic acid–conjugated r-(SiPc-BOA)-LDL nanoparticle was prepared by attaching folic acid to the lysine residues of the ApoB protein, such that each particle contained 170 molecules of FA. The resulting nanoparticles no longer bound to the normal LDL receptors, but instead exhibited binding to folate receptor (FR)–expressing cancer cells. This demonstrated the possibility of rerouting LDL to alternative receptors by attachment of appropriate targeting molecules. Other targeting ligands, such as epidermal growth factor (EGF) and HER2/Neu could also be used for this purpose. More recently, this concept was successfully applied to HDL-based nanoparticles by the incorporation of exogenous targets, including FA, EGF, and arginine-glycine-aspartate (RGD) peptide, to reroute scavenger receptor class B type I (SR-BI) targeting.

Figure 4. (A) Measurement of the in vivo uptake of r-(SiNc-BOA)-LDL (left) and r-(SiNc-BOA)-AcLDL (right) in HepG2 tumor and in normal muscles using an in-phase and in-quadrature spectrum. (B) Tumor-bearing mice before and after PDT using r-(Bchl-BOA)-LDL. (C) Survival curves for PDT treatment. Tumor-bearing mice were administered 2 μmol/kg of r-(Bchl-BOA)-LDL, followed by a light dose of 125, 150, or 175 J/cm². Figure 4A is reproduced with permission from reference [56], and Figures 4B and 4C with permission from reference [54].
2.5.3. Cytosolic Delivery of LDL

It is well known that many drugs must escape endo/lysosomal compartments and reach the cytoplasm to be effective, and this is a major problem in using LDL for drug delivery. Photochemical internalization (PCI) is a technique that allows the light-induced release of endocytosed macromolecules into the cytoplasm. The mechanism involves breakdown of endosomal or lysosomal membranes using amphiphilic photosensitizers, such as disulfonated aluminum phthalocyanine (AlPcS$_2$) and meso-tetraphenylboron disulfonate (TPPS$_2$), which localize on endosomal and lysosomal membranes. The membranes of these organelles are then destroyed by singlet oxygen generated from photoactivation of the photosensitizers at a sublethal dose, resulting in the subsequent release of entrapped drugs into the cytosol.\cite{75} By confirming the endocytic location of the AlPcS$_2$-LDL complex, Bonneau et al. suggested that it might be possible to release the complex through PCI.\cite{76} However, there was no cargo loading into LDL except AlPcS$_2$. Our recent study demonstrated that the cytosolic release of LDL cargo using PCI was feasible and that the efficiency was dependent on the cargo loading method; surface-loaded and protein-conjugated cargo were more effective than core-loaded cargo in terms of cytosolic release through PCI.\cite{77} More recently, this method was adapted to assist in the cytosolic release of LDL-loaded siRNAs.\cite{78} This may provide a useful strategy to bypass the challenges of endolysosomal entrapment, making LDL-based nanoparticles a more efficacious platform for theranostics. Other methods should also be considered in the future to facilitate the LDL core-loaded cargo release.

2.5.4. Other Directions

Our group has reported that molecular beacons (MB) can be incorporated into LDL and selectively induce irreversible nanoparticle aggregation through target nucleic acid recognition.\cite{79} This suggested a new direction for targeted delivery of phototoxic MB through the manipulation of DNA-nanoparticle interactions. An additional challenge for the design and preparation of LDL-based theranostic agents is the requirement for isolation of fresh plasma from human donors. To circumvent this limitation, the use of peptides representing the LDLR-binding domain of ApoB has been reported.\cite{80,81} Developing such synthetic LDL using ApoB-mimetic peptides for PS delivery may provide a clinically transplantable approach for targeted PDT. In addition, the same principle of using lipoproteins as carriers of PS can be extended to other medically relevant applications, such as lipoprotein-conjugated catalytic antioxidants for attenuation of atherosclerosis and protection of the beneficial functionality of lipoproteins.\cite{82,83}

3. HDL-Based Lipoproteins as Vehicles for Light-Activated Theranostics

ApoA-I, which contains 243 amino acid residues and a rich $\alpha$-helical content, is the principal apolipoprotein of human HDL. HDL is heterogeneous in nature and can be separated into two types based on morphology, namely discoidal HDL and spherical HDL. Discoidal HDL comprises a small segment of phospholipid bilayer surrounded at the edge by two ApoA-I molecules. Spherical HDL is a 10 nm diameter sphere containing two to three molecules of ApoA-I, phospholipids, and a hydrophobic core loaded with cholesterol ester and triglyceride molecules.\cite{84,85} The HDL receptor SR-BI mediates the selective transport of core lipids from HDL to cells and such lipid transfers are fundamentally different from the typical receptor-mediated endocytosis observed for LDL, and a mechanism has been suggested that involves direct cytosolic delivery through a specific hydrophobic channel.\cite{86,87} Similar to LDL, HDL cholesterol levels also have been reported to decrease by approximately the same extent in patients with malignancies, and often this is accompanied by increased expression of SR-BI in tumor tissues and cells.\cite{88,89,90} In addition, HDL is involved in transportation of cholesterol in the body and removes cholesterol from peripheral cells, including macrophages and atherosclerotic plaques.\cite{91,92} These factors shed light on the design and use of HDL for treatment and diagnosis of cancers and atherosclerosis. Their small size, long blood circulation times,\cite{93,94} and direct cytosolic delivery mechanism make HDL an attractive nanocarrier for PS loading.

3.1. HDL as a Vehicle for PS Delivery

The use of native HDL for PS delivery has been reported in cancer treatment. One example investigated in vivo tumor photosensitization with BPD in M-1 tumor-bearing mice.\cite{95} The BPD-HDL complex resulted in increased in vitro cell killing and delayed in vivo tumor regrowth when light exposure was performed three hours after drug administration, compared to BPD in aqueous solution. However, it must be noted that when using HDL for PS delivery, the in vivo circulation time of HDL should be taken into account to decide the optimum treatment timeframe following administration, due to the observation of a two phase decay in the distribution of plasma porphyrins. The rapid phase of loss (2.4 hours) was suggested to be due to porphyrin bound to plasma LDL and albumin, while the slow phase of loss (24.5 hours) was due to porphyrin-HDL association.\cite{96,97} Instead of simple surface loading, the use of reconstituted or recombinant HDL (rHDL) provided an advanced method for the incorporation of a variety of drugs, including PS.\cite{98,99} Recently, we reported the successful loading of Bchl-BOA into HDL through the reconstitution method.\cite{100} The syn-
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Figure 5. (A) Synthetic HDL nanoparticles loaded with theranostic agents. Top: HDL loaded with Bchl-BOA. Bottom: HDL loaded with inorganic nanocrystals. (B) In vivo fluorescent images of tumor-bearing mice dosed with r-(Bchl-BOA)-HDL. (C, D) T2*-weighted images of an apoE KO mouse before and 24 hours after injection with FeO-HDL. (E) Significant uptake of Au-HDL was found in the excised aortas of mice using micro-CT images. (F) Quantum dot (QD)-HDL was taken up throughout the excised aorta of atherosclerotic mice as revealed by fluorescent imaging. Figures 5A (bottom), 5C, 5D, 5E and 5F are reproduced with permission from reference [91].

3.2. HDL-Mimetic Lipoproteins as Potential Carriers for Phototoxic Agents

To address some of the inherent challenges of HDL, isolation of recombinant ApoA-I from bacteria has been achieved \cite{97,98} and its use for the formulation of loaded therapeutics has been reported \cite{99}. However, biosafety issues (e.g., immunogenicity) of recombinant HDL are a concern. Another solution is the use of ApoA-I-mimetic peptides instead of the ApoA-I protein itself. Recently, we developed a HDL-mimicking peptide-phospholipid scaffold (HPPS) nanocarrier based on phospholipids, CO, and amphipathic α-helical peptides. HPPS is capable of core loading of hydrophobic fluorescent dyes, such as fluorescein isothiocyanate bisoleate (Fluo-BOA; ex.: 458 nm, em.: 518 nm) and 1,1'-dioctadecyl-3,3',3'-tetramethylindotricarbocyanine iodide bisoleate (DiR-BOA; ex.: 748 nm, em.: 782 nm) with a payload ranging from 15–100 molecules per particle \cite{100}. The SR-BI–targeting ability of (DiR-BOA)-HPPS was demonstrated using cell lines with differentiated expression of SR-BI, and inhibition using an excess of HDL \cite{101}. Moreover, one of the most attractive properties of the SR-BI pathway lies in its ability to directly transport the payload into the cytoplasm of targeted cells, thus bypassing endocytosis. This has been demonstrated by the use of HPPS to deliver therapeutic siRNAs and also a lipophilic drug, paclitaxel oleate (PTX-OL) \cite{102,103}. Since the structures of these modified fluorophores, drugs and porphyrins (including Bchl-BOA and SiPC-BOA) are similar, it is possible to load these PS into HPPS simultaneously with a considerable payload. Since mitochondria were considered as one of the optimal subcellular targets of PDT \cite{104}, the delivery of PS via a nonendocytic pathway may provide additional advantages.

4. Future Directions

Lipoprotein-based nanoparticles have been loaded with a diverse range of phototoxic and imaging agents for theranostics. The major challenge for the design and preparation of natural lipoprotein-based nanoparticles is their isolation from fresh plasma of human donors. To overcome this limitation, synthetic LDL, which contains peptides that mimic the LDLR-binding domain of the ApoB protein, has been reported \cite{80,81}. As a substitute for HDL, HPPS has been successfully created to mimic the behavior of HDL in many ways. HDL and HPPS benefit from the direct cytosolic delivery of core-loaded cargo, taking advantage of the SR-BI pathway. The creation of nanocrystal core lipoprotein nanoparticles using inorganic molecules has extended the application of lipoprotein nanoparticles in theranostics (Figure 5B, 5C, 5D and 5F) \cite{105,106}. These nanoparticles should not simply be limited to atherosclerosis since they can be easily rerouted to other targets of interest. The use of Au-HDL for therapeutic nucleic acid delivery has been achieved \cite{107}. As gold nanoparticles have been widely used as photothermal therapeutics due to the strongly enhanced absorption in the visible and near-infrared region that arises from their surface plasmon resonance (SPR) oscillations, \cite{108}
combination of photothermal and gene therapy will be another direction for light-activated theranostics. In addition, porphyrosome nanovesicles generated by porphyrin bilayers have been developed for use as multimodal biophotonic contrast agents.[10] The central components of these nanovesicles are porphyrin-phospholipids, which have great potential to be incorporated into lipoprotein-based nanoparticles to engage more potent mechanisms in light-activated theranostics.

5. Summary and Outlook

During the past 30 years, the use of lipoproteins has extended from their natural sources to lipoprotein-mimetic peptide-phospholipid scaffolds. Several types of light-activatable contrast agents, including PS and gold, have been successfully incorporated into the core of lipoprotein-based nanoparticles. Versatile imaging modalities have been built up that allow the imaging of drug distribution in vivo. The strategy for rerouting lipoproteins from endogenous receptors to other targets of interest will broaden the range of applications for disease-related theranostics. There are many avenues of research that warrant further investigation to make theranostic-loaded lipoproteins clinically relevant. Future work will address a number of critical issues, including large-scale production, construction of multifunctional modalities, and rigorous in vivo theranostic studies.

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