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Lipid-Based Nanoparticles as Pharmaceutical Drug Carriers: From Concepts to Clinic

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Abstract

In recent years, various nanotechnology platforms in the area of medical biology, including both diagnostics and therapy, have gained remarkable attention. Moreover, research and development of engineered multifunctional nanoparticles as pharmaceutical drug carriers have spurred exponential growth in applications to medicine in the last decade. Design principles of these nanoparticles, including nano-emulsions, dendrimers, nano-gold, liposomes, drug-carrier conjugates, antibody-drug complexes, and magnetic nanoparticles, are primarily based on unique assemblies of synthetic, natural, or biological components, including but not limited to synthetic polymers, metal ions, oils, and lipids as their building blocks. However, the potential success of these particles in the clinic relies on consideration of important parameters such as nanoparticle fabrication strategies, their physical properties, drug loading efficiencies, drug release potential, and, most importantly, minimum toxicity of the carrier itself. Among these, lipid-based nanoparticles bear the advantage of being the least toxic for in vivo applications, and significant progress has been made in the area of DNA/RNA and drug delivery using lipid-based nanoassemblies. In this review, we will primarily focus on the recent advances and updates on lipid-based nanoparticles for their projected applications in drug delivery. We begin with a review of current activities in the field of liposomes (the so-called honorary nanoparticles), and challenging issues of targeting and triggering will be discussed in detail. We will further describe nanoparticles derived from a novel class of amphipathic lipids called bolaamphiphiles with unique lipid assembly features that have been recently examined as drug/DNA delivery vehicles. Finally, an overview of an emerging novel class of particles (based on lipid components other than phospholipids), solid lipid nanoparticles and nanostructured lipid carriers will be presented. We conclude with a few examples of clinically successful formulations of currently available lipid-based nanoparticles.

Keywords

lipid-based nanoparticles; drug delivery; solid lipid nanoparticles; liposomes; nanostructured lipid carriers; bolaamphiphiles; cancer therapy

I. INTRODUCTION

The formulation of bioactive molecules into relatively inert and non-toxic carriers coupled with site-specific targeting ligands for in vivo delivery constitutes a promising approach to

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improving their therapeutic index while reducing their side effects.[1,2] In past years, extraordinary efforts have been made toward improving efficacy and bioavailability of drugs and pharmaceuticals by developing nanotechnology platforms.[3–10] These efforts are further aided by the creation of outstanding excellence centers and other initiatives by the National Institutes of Health (NIH), including a national network of eight nanomedicine development centers, which serve as the intellectual and technological centerpiece of the NIH Nanomedicine Roadmap Initiative. In addition, the National Cancer Institute (NCI) Alliance for Nanotechnology in Cancer has established eight Centers of Cancer Nanotechnology Excellence (CCNEs), which are multi-institutional hubs that focus on integrating nanotechnology into basic and applied cancer research and providing new solutions for the diagnosis and treatment of cancer. Other initiatives of the NCI alliance include the establishment of 12 Cancer Nanotechnology Platform Partnerships that are designed to develop technologies for new products in molecular imaging and early detection, in vivo imaging, reporters of efficacy, multifunctional therapeutics, prevention and control, and research enablers; multidisciplinary research training and development teams; and the Nanotechnology Characterization Laboratory (NCL), which performs and standardizes the pre-clinical characterization of nanomaterials intended for cancer therapeutics and diagnostics developed by researchers from academia, government, and industry and funding opportunities worldwide.[11]

A diagram presented in Figure 1 shows the design principle of an ideal, multifunctional nanoparticle for targeted delivery of therapeutics that can also be envisioned as a nanobiologist's "Holy Grail." This nano-assembly includes imaging molecules, a payload of drugs, ligands for site-specific targeting, and a destabilizing lipid that allows for on-demand drug release at the desired site, as well as sensors that probe the efficacy of the drug in real time. Some widely examined nano-carriers aimed at delivering DNA, pharmaceuticals, and/or imaging agents include dendrimers,[12,13] nano-gold shells,[14,15] nano-emulsions,[16] drug-polymer conjugates,[17,18] drug-antibody conjugates,[19] and quantum dots.[20–22] Each of these is based on unique properties of the structural components used in fabricating the delivery vehicle and relies on self-assembly of the structural motifs, while accommodating the pharmaceutical agent and the targeting ligand. In spite of these efforts, only a limited number of the drug-loaded nanoparticles are successful for their clinical applications,[23,24] suggesting that these nanotechnology platforms deserve a closer look to overcome several technical roadblocks to become ready for clinical applications.[25] An important parameter of the delivery vehicle pertains to low or no toxicity of the carrier itself either in vivo or in the environment as a by-product. Therefore, nanoparticles fabricated using an assembly of natural biomolecules such as lipids, proteins, and carbohydrates are expected to be an appropriate choice for clinical applications.[24]

Among various lipid-based formulations, classical examples are "liposomes," which primarily consist of phospholipids (major components of biological membranes) and have been extensively studied.[26,27] Lipoplexes (lipid-based assemblies of non-covalently associated DNA by charge-charge interactions) are used in gene-targeting studies.[28,29] Recently, studies by Torchilin et al. reported novel micelle-like nanoparticles loaded with plasmid DNA and based on a covalent conjugate of DNA and polyethylenimine, which is coated with a lipid monolayer and polyethylene glycol (PEG) molecules. Their study suggests that micelle-like nanoparticles have architecture and properties suitable for in vivo application.[30] Another well-studied example is tumor-targeted, liposome-based systemic gene delivery.[31–35] Interestingly, bioactive lipids such as ceramide that are involved in cell signaling pathways have also been examined via nanoliposome-delivery systems.[36,37] The above-mentioned nano-systems have been described elsewhere in detail.

In this review, we will limit ourselves to the history, current status, and future of lipid-based nanoparticles as carriers of drug delivery. The first section will deal with the drug-delivery status of liposomes, with a special focus on the principles of lipid packing, strategies for optimal formulations, phospholipid structural modifications, in vitro and in vivo triggering, and tumor-targeting modalities. In the second section, we will provide details about a novel class of amphipathic lipids, "bolaamphiphiles," which bear distinct non-bilayer-forming properties and may become suitable drug-delivery platforms. The last section will briefly summarize current research activities in lipid-based nanoassemblies based on molecules other than phospholipids. These include solid lipid nanoparticles (SLN) and nano-structure lipid carriers (NLC); both systems are relatively new and present opportunities for further investigations for their application in drug delivery.

II. LIPOSOMES AS DRUG CARRIERS

The preparation of liposomes with entrapped solutes was first demonstrated in a published paper[38] by Prof. A.D. Bangham of the United Kingdom. Since their inception, liposomes have been explored as carriers for delivering drugs and pharmaceuticals.[4,39–41] They present a well-studied class of drug carriers generally characterized by the presence of a lipid bilayer that is primarily composed of amphipathic phospholipids (chemical structures shown in Fig. 2A) enclosing an interior aqueous space. Currently, a number of liposome formulations are in clinical use to combat cancer and infectious diseases, while others await clinical trial outcomes (for updated information, please visit the website www.clinicaltrials.org). Table 1 summarizes a partial list of liposomes approved for clinical applications, and this list is growing at a steady pace; Table 2 summarizes a partial list of liposomes that are currently undergoing clinical trials. It is notable to mention that Doxil®/caylex (a liposome-based formulation of the anticancer drug doxorubicin, Ortho-Biotech) was the first formulation approved for application in the clinic and therefore may be considered an honorary nanoparticle for patient care.[23,24] Historically, the important milestones that led to the research and development of clinically suitable liposome formulations can be summed up in two major technological achievements: i) inclusion of pegylated lipids in the liposomes to bypass reticulo-endothelial system, resulting in significant accumulation in the tumors[42,43]; and ii) the strategic development of a remote drug-loading process based on the ammonium sulfate gradient method to achieve significantly high quantities of doxorubicin in the interior of the liposomes.[44,45] These issues have been widely discussed elsewhere[46] and therefore will only be briefly covered here.

II.A. Design Principle of Liposomes and Assembly of Lipid Molecules

Phospholipids (phosphatidylcholines, usually called "lecithins") are the main constituents of liposomes (Fig. 3). Due to their amphipathic properties, they readily form concentric bilayers (also initially called "bangosomes" by A.D. Bangham). There are several protocols and techniques available to convert these multilamellar lipid dispersions into single bilayer structures (called unilamellar liposomes or vesicles). The most commonly used laboratory methods include sonication, extrusion, reverse-phase evaporation, and solvent injection. The formulations that meet the regulatory standards by the Food and Drug Administration (FDA) require consideration of important parameters including their size, stability in circulation, batch-to-batch variations, efficiency of drug loading, etc.[47] In addition to these features, the ability of liposomes to destabilize their membranes for localized drug delivery (triggerable liposomes) is another highly crucial aspect for improving the efficacy of liposome-entrapped drugs and pharmaceuticals. "Triggerable liposomes" will be discussed later in this section.

Liposomes have also long served as excellent tools for model membranes and therefore lipid molecules other than phosphatidylcholine (PC) that may direct changes in the bilayer structure have been well studied.[48] The polymeric structures of some of the phospholipids used in liposome formulations for biomedical applications are shown in Figure 2B. Among these, PC,

which has zero spontaneous curvature, favors assembly in a lipid bilayer, whereas lyso-PC (usually called "lyso-lecithin"), which has positive spontaneous curvature, forms micelles when dispersed in an aqueous solution. Phosphatidylethanolamine (PE), which has negative spontaneous curvature, assumes a non-bilayer (inverted micellar or the hexagonal, H-II phase) structure at a given temperature and degree of hydration. If the desired outcome of a liposome formulation is fusion of its membrane with that of a cell, incorporation of PE into that formulation would favor that outcome because negative curvature lowers barriers to fusion.[49] When lyso-PC is incorporated into a bilayer, its positive spontaneous curvature will lead to the formation of lipidic pores. Therefore, lyso-PC has been incorporated into thermosensitive liposomes, which have been designed to leak their contents when the tumor site is heated a few degrees above the physiological temperature. Thermosensitive liposomes are currently undergoing clinical trials,[50,51] as discussed in detail below.

An alternate strategy to developing "designer liposomes" bearing desired properties includes discrete chemical modifications in the phospholipid structure, primarily PC. The PC molecule can be divided into three major parts, the head group, the glycerol backbone, and fatty acyl chains (Fig. 3). Each of these regions has been modified either by the introduction of additional groups or modification of existing chemical bonds. The head-group modifications include introduction of ligands,[52–55] functional groups (such as malemide) for chemical conjugation of ligands (such as antibodies),[56] and/or polymerizable moieties[57] to produce stable liposomes. The carbonyl ester bonds of the glycerol backbone of PC (at both the sn-1 and sn-2 position) have been the choice for modifications with either ether[58,59] or carbamyl esters,[60–62] resulting in modulation of stability and in vivo circulation of these liposomes. The fatty acyl chain length and degree of unsaturation are important factors that govern bilayer packing properties of liposomes and contribute to the observed phase-transition (Tm) effect.

Thermosensitive liposomes were initially developed in early 1980s based on the Tm release properties of phospholipids,[51] and since then have been further developed for their applications in the clinic (discussed in detail later in the review). Fatty acyl chains of the phospholipids have been further explored to introduce chemical modifications, and the resulting modified phospholipids have primarily yielded photo-activable liposomes.[63] Design principles and current work on photo-triggerable liposomes will also be discussed later in this review.

II.B. Liposome-Triggering Modalities

Although the liposome drug-delivery field has made strides in overcoming crucial pitfalls (such as clearance rates, in vivo stability, etc.), the overall deliverable drug by these nanocarriers is expected to significantly increase by designing modalities to release drugs within a defined space and time in a localized area (such as the site of a tumor). It can be envisioned that strategic development of drug-loaded nanocarriers tuned to trigger drug release would significantly improve the efficacy of drugs and pharmaceuticals, potentially obliterating drug-resistance problems. Various triggering modalities for site-specific release of drugs from liposomes have been developed.[64–69] The principles underlying these approaches revolve around one common feature, creating defects in the liposome membrane (Fig. 4A), and can be broadly classified into two main approaches: external and internal triggers (Fig. 4B).

Examples of external triggers include the utilization of two forces of nature, heat and light. Among these, liposomes sensitive to mild hyperthermia (currently under clinical trials) are the best-studied examples for triggered drug delivery, whereas light-sensitive liposomes (explored since early 1980s) have lately regained attention. Another relatively new modality at a very preliminary stage of development involves the concept of alternate magnetic field (Fig. 5A); however, the potential success of the approach is subject to future investigations. In addition to utilization of external triggering of liposomes, alternate triggering strategies are primarily based on exploiting the abnormalities in the biology of diseased cells, tissue, and/or organs.

One well-studied example in this class includes utilization of enzymes that are up-regulated in certain tumors to cleave liposomal lipids and create defects in the membranes. Several excellent reports have recently dealt with enzyme-triggered release of liposomal contents.[70–73] Among these, secretory phospholipase A2 appears to be a promising target.[68,71,74] Specific examples in this class include generation of a pore-forming lipid (e.g., lyso PC, Fig. 2A) mentioned above. Significant efforts have also been made to develop liposomes that will undergo membrane reorganization in a low-pH environment (predominantly from a bilayer to a hexagonal HII lipid phase, Fig. 2). The lipid formulations for "pH-sensitive" liposomes typically include PE and cholesterol hemi-succinate for membrane destabilization in the endosome, resulting in localized drug release.[3] A number of reviews on pH-sensitive liposomes and other nanoparticles have been published previously.[75–78] In this review, we will only elaborate on the recent updates on thermo- and light-sensitive liposomes.

Thermosensitive liposomes are based on lipid-destabilizing mechanism(s), and thermal melting temperatures (Tm); this concept was first described by Yatvin et al.[51] Since then, progress in the development of these liposomes has been substantial. Figure 5B shows a timeline of step-wise progress in the field of thermosensitive liposomes. It is now well-documented that local hyperthermia can be used to selectively enhance both the delivery and the rate of drug release from thermosensitive liposomes to targeted tissues.[50,65] Recently, the Center for Interventional Oncology was established to develop and translate image-guided technologies for localized cancer treatments. For example, using thin needles, sound waves can be used to ablate tumors and enhance drug delivery from thermo-sensitive liposomes. Energy sources include high-intensity focused ultrasound, freezing, microwaves, laser, and radiofrequency. The advances in focused high-frequency ultrasound technologies has been used for noninvasively enhancing drug delivery and the clinical applications of liposomes.[79] Thermosensitive liposomes are currently in clinical trials to treat hepatocellular carcinoma liver neoplasms and breast cancer (www.clinicaltrials.gov, identifier #NCT00441376 and #NCT00346229, respectively). The latest developments in this field include vascular targeting of thermosensitive liposomes.

Electromagnetic radiation-triggered release of liposomal drugs presents a promising approach that involves strategically designed phospholipid molecules to respond to a light trigger. These liposomes[63] are based on the principle of photo-polymerization of lipids,[80] photo-sensitization by membrane-anchored hydrophobic probes,[72,73,81–83] or photo-isomerization of photo-reactive lipids.[84] The design principles and mechanisms of light-triggered chemical changes in photo-reactive segments of these lipids have been recently reviewed in detail. Figure 5C and 5D show some of the designer lipids used for the development of light-activated liposomes. However, none of the formulations developed so far has been successful for in vivo applications, presumably due to the lack of adequate photon energy produced by the radiation source(s) or the inability of radiation to penetrate into biological tissues. We believe that the development of novel and innovative strategies to combine the unique chemistry of photoactivable lipids with "helper" components (such as metal ions) is needed to acquire high energy radiations. Therefore, we have recently pursued an alternative approach to create phase boundary defects in lipid model membranes using mixtures of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and a photo-polymerizable phospholipid, 1,2 bis(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine ($DC_{8,9}PC$) (Fig. 5D). This lipid bears highly reactive diacetylenic groups that can be polymerized by UV irradiation to form chains of covalently linked lipid molecules in the bilayer.[80,85] We hypothesize that $DC_{8,9}PC$ is likely to form aggregates in the bilayer of phospholipids containing saturated acyl chains. We have recently reported that light-triggered calcein release occurs from liposomes containing a mixture of saturated phospholipids and $DC_{8,9}PC$. The packing properties of $DC_{8,9}PC$ in triggerable formulations were in agreement with the modulation of the melting phase transitions (Tm) in these liposomes as determined by differential scanning calorimetry. Our initial in vivo

experiments indicated that liposomes containing $DC_{8,9}PC$ are not toxic to mice, and these formulations have similar biodistribution to that of DPPC liposomes alone (unpublished observations). We surmise that the ability of diacetylenic groups to undergo chemical modifications in the presence of metal ions may result in the development of the next generation of radiation-sensitive liposomes for drug-delivery applications in the clinic.[86]

II.C. Liposome Targeting

As discussed in the previous sections, triggerable liposomes may serve as improved drug-delivery systems. It can be envisioned that grafting specific ligands on the liposome surface will further improve the delivery of drugs to targeted cells and/or tissues. To this end, antibody-coated liposomes (immunoliposomes) have been explored for decades. However, success of immunoliposomes in the clinic still remains to be seen. In addition to using antibodies as ligands,[4,40,52,87] a number of other small molecules such as vitamins,[17,18,88] peptides,[89–92] aptamers,[93] and Affibodies[94–96] have also been examined to improve targeting of liposomes. However, the notion that "targeted liposomes" bear an advantage over non-targeted liposomes has been challenged and is subject to intense debate. In this section, we will provide an overview of various strategies used for liposome targeting (including specific examples of ligands, conjugation strategies, and biological systems used), and discuss the pros and cons of targeted versus non-targeted liposomes and their ability to be targeted to specific molecular signatures expressed on cell surfaces.

1. Targeted Liposomes: General Considerations—The advantage of using nanocarriers, including liposomes (size range 100–200 nm), to deliver anticancer agents to tumor tissue has been extensively discussed.[2] This approach for anticancer therapy involves "passive targeting" of the drug-loaded nanocarriers, because these particles are known to accumulate in the tumor area due to the leaky vasculature-enhanced permeability and retention (EPR) effect (non-targeted liposomes). This effect represents the anatomic differences between normal and cancerous tissue because capillaries in the tumor area possess increased permeability. This passive targeting effect is highly dependent on a number of characteristics, including the degree of tumor vascularization and angiogenesis and the porosity and pore size of tumor vessels, which vary with the type and status of the tumors.[97] These factors contribute to the pharmacokinetics and biodistribution of the liposomes. Additional issues related to "passive targeting," such as the kinetics of drug release, efflux of released drug into the tumor cells, and tumor retention, are important factors that will dictate the outcome of effective treatment (Fig. 6A). For example, liposomes in the range of 100 to 150 nm have been shown to preferentially accumulate in tumors due to the EPR effect.[98,99]

The design and development of ligand-bearing liposomes for "active targeting" involves specific interactions of liposomes via the receptors, followed by uptake of liposomal drugs by receptor-mediated endocytosis (targeted liposomes) (Fig. 6A). The development of targeted liposomes has been ongoing since the 1970s/1980s,[40,87] while taking into consideration that liposome targeting does not compromise pharmacokinetics and the efficacy of drugs.

Ligands such as antibodies, peptides, and vitamins (e.g., folic acid), which can bind to up-regulated/overexpressed receptors on tumor tissue, have been investigated as biomarkers for targeted drug delivery. It is important to mention here that the concept of improving drug delivery by means of "targeted liposomes" has met with skepticism and has been challenged[100,101]; ligand-bearing liposomes showed improved efficacy of encapsulated drugs in some systems, whereas such an improvement was not observed in other experimental systems. We believe that a detailed analysis of the kinetics and extent of: i) liposome accumulation, ii) liposome internalization, iii) intracellular liposome degradation, and iv) intracellular fate of the drug are the factors that will play an important role in establishing the

validity of targeted liposomes.[102] The surface density of receptors, affinity with ligands (most importantly, the effect of multimerization of ligand binding), receptor recycling, and state of tumor development also contribute in determining the fate of targeted liposomes. Current studies using targeted liposomes are summarized in Table 3. For example, earlier work by Gabizon et al. (ref. [124]) showed that attaching folate to liposomes did enhance uptake of liposomes in folate-expressing tumors in mouse biodistribution studies, but HER2-targeted immunoliposomes did not show any difference in biodistribution and tumor accumulation compared with non-targeted liposomes.[103] However, targeted liposomes in the latter case had sixfold increased intracellular localization, suggesting that they are likely to bear an advantage for drug targeting. Recent studies by Laginha et al. support this notion, because their experiments, which were based on the examination of the HER2-targeted liposomes, showed improvement in doxorubicin-mediated cell killing and tumor regression in mice.[104] Another example in support of targeted liposomes includes a number of recent studies by Torchilin's group demonstrating improved targeting of doxorubicin using the anti-nucleosome antibody.[105] Therefore, it can be postulated that the targeting molecules contribute (via the ligand-receptor interactions) subsequent to the nanocarrier accumulation in the tumor tissues.[106,107]

2. Targeting Ligands—Various candidate ligands have been examined to target liposomes to tumors that are aimed at exploiting overexpressed receptors (Table 3); these include antibodies, affibodies, and small ligands such as folate, aptamers, peptides, and lectins. Properties of a viable ligand that will bear the potential to succeed in targeting liposomes for site-specific drug delivery can be broadly classified based on the following factors: i) methodology of ligand production in large scale, ii) ease of purification and stability, and iii) ligand-liposome conjugation strategies without compromising the properties of either the ligand and/or liposomes. Among the various ligands examined thus far, antibodies have been extensively studied and will be discussed in detail. We will also provide an overview of affibodies and folate targeting in this section.

a. Immunoliposomes: Immunotherapy has been explored since detailed structural analysis of antibodies, hybridoma technology (monoclonal antibodies, mAb), and phage display technology (single-chain antibodies (scFv) became available (for details on therapeutic antibodies, the reader is referred to *Therapeutic Antibodies: Methods and Protocols* by A.S. Dimitrov, Humana Press, NY). Currently available therapeutic antibodies include mAbs (such as Herceptin for breast cancer and Epratuzumab for B-cell lymphoma), and therefore have been the preferred choice of molecules for generating immunoliposomes. One of the advantages of using mAbs is their stability and higher binding avidity that comes from the presence of two binding sites on the molecule. The Fc-receptor binding of mAb can lead to complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity, which may enhance tumor-cell killing. However, the Fc receptor-mediated responses may lead to high liver and spleen uptake of the immunoliposomes and might increase the immunogenicity of the molecule. The modifications in the whole antibody molecule include F(ab′)2, Fab′, and scFv fragments (Fig. 6B) that lack the Fc domain and the complement-activating region, which might reduce their immunogenicity.[108] F(ab′)2 fragments retain two binding regions that are joined by disulfide bonds and can be quite stable during storage. Under reducing conditions, the disulfide bonds are cleaved to yield two Fab′ fragments, which is very useful for coupling the fragments to lipid based nano-particles. Fab′ fragments and scFv fragments have only one binding domain, which reduces their binding avidity; however, by attaching several fragments at the surface of immunoliposomes or by engineering bivalent or multivalent fragments, the multivalency and hence the avidity can be restored.[109–111] The use of scFv fragments is attractive because of their ease of identification (from phage display) and production and because they decrease immunogenicity. However, these small fragments might be less stable during storage than Fab′ fragments or whole mAbs.[109,110]

Tremendous efforts are being made to apply antibody-directed nanotechnology platforms for diagnostics, imaging, and therapy. Among these, antibody-coated liposomes (immunoliposomes) are one of the long-studied nanoparticles and, as discussed previously, the practical applications of these particles are still subject to intense debates. Historically, initial attempts to link the whole-antibody molecules to the liposome surface involved chemical modifications of amine using bifunctional reagents. This method suffered drawbacks due to compromised active domains of the antibodies and lack of specificity.[112] Since the availability of scFv (with engineered cysteines at the C-termini), antibody conjugation methods are now based on the maleimide-cysteine reaction, resulting in the formation of thio-ether bonds between proteins and liposomes, and are presently the preferred method of choice.[56]

Liposomes can be converted to targeted liposomes using two strategies. The first method (Fig. 7A) uses a versatile "post-insertion technique" in which ligands are coupled to end-functionalized groups in PEG lipid micelles. The ligand-PEG lipid conjugates are then transferred in a simple incubation step from the micelles into the outer monolayer of pre-formed, drug-loaded liposomes. This method allows for a combinatorial approach to the design of targeted liposomes that minimizes manufacturing complexities, allowing ligands to be inserted into pre-formed liposomes containing a variety of drugs; this process has been reviewed in detail previously.[6,113] To date, HER2 scFv[114,115] conjugated liposomes for drug delivery and anti-TfR scFv-lipoplexes for gene delivery have been successfully developed.[31,32] In addition, an alternate protocol is based on preparing liposomes with grafted malemide-containing PEG lipid in the lipid bilayer. Antibodies bearing a cysteine (such as scFv) at the C-terminus are first reduced and then conjugated to the outer surface of liposomes using the same chemistry as above (Fig. 7B). The first method typically relies on using Doxil®, a formulation already being used clinically.[104,114] However, insertion techniques are rather uncontrolled, and separation of micellar scFv-PEG lipid conjugate from the liposomes may become a technical challenge. On the other hand, direct conjugation of scFv to the exposed malemide groups on the liposome surface is relatively controlled and allows for only antibody conjugation on the outer surface.

To date, the issue of optimal ligand density per liposome remains to be resolved, mainly because of technical challenges in directly quantitating ligand density on liposomes. This is an underdeveloped research area and requires focused efforts. It is logical to predict that immuno-liposomes with high antibody densities may be desirable for antibody fragments, because this will lead to better binding avidity of the immuno-liposomes for the target antigen. In addition, high antibody densities on the surface of liposomes will provide multimeric binding sites to the cell surface receptors, potentially leading to the initiation of signal transduction mechanisms. Although high binding affinities are desirable, low-affinity ligands may be preferred in certain scenarios because they may allow liposomes to penetrate further into the tumor interior, decreasing the "binding site barrier."[116]

b. Affisomes: Recently, a novel class of small molecules called "affibodies," which can be considered antibody mimics, have been examined for liposome targeting. Affibody molecules are relatively small proteins (6–8 kDa) that offer the advantage of being extremely stable, highly soluble, and readily expressed in bacterial systems or produced by peptide synthesis. The binding affinities of affibody molecules are considerably higher compared with the corresponding antibodies. (Detailed information about the production and characterization of affibody molecules can be found at www.affibody.com).

Recently, we conjugated an 8.3-kDa HER2-specific affibody molecule (Z(HER2:342)-Cys) to the surface of thermosensitive liposomes (called "affisomes") aimed at improving the targeting efficacy of these liposomes for breast cancer treatment.[96] Another study by Beuttler et al.[95] used a bivalent, high-affinity epidermal growth factor receptor (EGFR)-specific affibody

molecule (14-kDa) for targeting PEGylated liposomes to EGFR-expressing tumor cell lines. Enhanced cytotoxicity toward EGFR-expressing cells was detected with mitoxantrone-loaded affibody targeted liposomes compared with untargeted liposomes in these studies.[95] In another study, HER2-specific affibody molecules were used to fabricate nanoparticle-affibody conjugates composed of poly(D,L-lactic acid) and pegylated lipids. The resulting nanoparticles showed specific binding and uptake by $HER2^+$ cells.[117] Since the receptor-binding domains of affibodies may differ from that of antibodies, affisome uptake mechanisms may result in altered outcomes. Therefore, further studies in vitro and in animals are needed to establish the projected advantage of affibodies as targeting ligands for liposomes.

c. Folate as a Targeting Ligand: Folate receptor (FR) expression is known to be up-regulated in many human cancers, such as ovarian, lung, breast, kidney, brain, and colon cancers, and receptor density appears to increase as the disease progresses.[117,118] FR has two glycosyl phosphatidylinositol-anchored isoforms: alpha and beta. FR-alpha expression is frequently amplified in epithelial cancers, whereas FR-beta expression is found in myeloid leukemia and activated macrophages associated with chronic inflammatory diseases. The vitamin folic acid binds to FR with high affinity and results in efficient internalization into cells. Therefore, FR is an attractive target for tumor-specific drug delivery.[119] Interestingly, another feature of FR is its location on the apical membrane of epithelial cells, where it is not accessible from blood in normal cells. Studies by Low et al. show that folic-acid-drug conjugates are attractive molecules for folate targeting in vivo.[118,120,121]

In the field of lipid-based nanoparticles, folate lipid-bearing, drug-loaded liposomes have been investigated.[122–127] Initial studies using the doxorubicin-loaded liposomes (folate $lipid^+$) showed promising data; these liposomes displayed a 45-fold higher uptake than their non-targeted controls, and cytotoxicity of targeted liposomes was found to be 85 times greater than similarly loaded controls.[122] These observations stimulated tremendous interest in the liposome field as well as other nano-carriers.[88] Although initial animal studies showed an early increase in folate-targeted liposomes, overall uptake and tumor regressions were not significantly improved in vivo, and were tumor-type dependent.[127] However, the same group has recently reported that folate-targeted liposomal doxorubicin formulations bear significant advantage, especially for intracavitary therapy, further suggesting tumor biology will be a major determinant in the treatment outcome.[126] Therefore, the future of folate-targeted liposomes in the clinical setting remains uncertain at present.

III. BOLAAMPHIPHILES AND NANO-STRUCTURES FOR DRUG DELIVERY

III.A. Monolayer Membrane from Bolaamphiphiles

Bolaamphiphiles (also called bolalipids) are a unique class of lipids that bear two hydrophilic head groups situated at both ends of a hydrophobic domain (Figs. 8A and B). In contrast to single-hydrophilic head amphiphiles such as phospholipids (Fig. 2A), which form bilayer membranes, bolaamphiphiles may form monolayer membranes[128] (Fig. 8A). Studies demonstrate that membranes made from bolaamphiphiles are less permeable and more durable than membranes composed of monopolar lipids.[129,130] This unique combination of properties has ignited interest in the potential use of bolaamphiphiles as membrane-stabilizing agents in applications such as drug delivery and membrane-protein-based bio-sensors.[131–133]

Unsymmetrical bolaamphiphiles bearing two different hydrophilic head groups are attracting considerable attention in the field of nano- and biotechnologies because they can self-assemble in water to form unsymmetrical monolayer lipid membranes with parallel molecular packing,[134–139] which results in nanostructures that possess different inner and outer surfaces covered with each head group. These nanostructures are applicable to construct delivery and medical

diagnosis systems based on selective and effective encapsulation of functional nanomaterials and biomolecules into their inner space.[137,140]

In nature, bolaamphiphiles are found in archaebacteria, organisms that survive in volcanic environments under extreme conditions.[141,142] The monolayer architecture of the archeabacteria enables them to survive in high-temperature and low-pH conditions,[143] meaning that their bolaamphiphiles are excellent candidates as building blocks of nanoparticles for drug delivery.[144,145] However, the chemical synthesis of bolaamphiphiles such as those found in archaebacteria is faced with technical challenges[146–147]; extraction techniques from the organism itself result in limited recovery of these lipids and therefore they are not yet commercially viable. Therefore efforts are focused on synthetic novel bolaamphiphiles and their ability to aggregate into nano-sized particles.[148]

III.B. Synthetic Bolaamphiphiles and Their Aggregation into Nano-structures

Synthetic analogs of the membrane-spanning lipids of archaebacteria have been prepared by Fuhrhop et al.[149] In addition to Fuhrhop's extensive work, efforts have been made by other groups to design and synthesize bolaamphiphiles with different structures (Fig. 8B) and to characterize their aggregation behavior in aqueous solutions.[150–155] Depending on their molecular structure, bolaamphiphiles can form micelles, vesicles, multilayered sheet rings, and a variety of micro- and nano-structures with cylindrical geometry such as rods, tubules, ribbons, and helices (Fig. 8C).[128] Polidori et al.[154] reported the synthesis of symmetric bolaamphiphiles with systematic changes in their molecular structure, such as length and rigidity of the aliphatic chain, polar head volumes, and the presence of hydrogen-bonding groups. Studies with these synthetic bolaamphiphiles showed that the primary structures into which they aggregate are micelles. However, bolaamphiphiles with two hydrocarbon chains, as well as some single-chain bolaamphiphiles, formed spherical vesicles in aqueous solution.[154] Studies with asymmetrical bolaamphiphiles showed that 1-galactosamide bolaamphiphile self-assembles to form a multilayer structure comprising unsymmetrical monolayer lipid membranes linked via a sugar–carboxylic acid H-bonding interface.[156] The molecular parameters that seem to determine the natural shape and stability of the particles depend on the number and the length of the aliphatic chains, saturation versus unsaturation of the C-C bonds within the aliphatic chain, sizes of the head groups relative to the hydrophobic domain (see below), and the presence of hydrogen bond forming groups.

Typically, short-chain bolaamphiphiles form micelles, whereas long-chain bolaamphiphiles form vesicles.[128] However, an incorporation of a diamide midsection into the hydrophobic chain of bolaamphiphiles[157] caused short-chain bolaamphiphiles to form vesicles and long-chain bolaamphiphiles to form fibers. By comparison, when bolaamphiphiles with diester midsections[157] were examined for their ability to form nanostructures, both short- and long-chain bolaamphiphiles formed vesicles, which were more stable than vesicles made from bolaamphiphiles with diamide midsection and therefore are more adequate for drug delivery.

III.C. Formation of Vesicles from Bolaamphiphiles for Drug Delivery

Liposomes made from phospholipid amphiphiles that aggregate into bilayer membranes have been extensively investigated as drug-delivery systems.[158–162] However, in many cases, liposomes injected into the bloodstream are rapidly cleared from the system and only a fraction reach the target site, even when PEG-coated liposomes are used.[163] Promising alternatives include vesicles composed of monolayer membranes made from bolaamphiphiles, which are potentially more stable than the classical bilayer liposomes and less likely to fuse with each other or with cell membranes due to reduced lipid exchange.[150,164] This feature of reduced lipid exchange enables vesicles to cross biological membranes while maintaining their structure (see below). However, not all bolaamphiphiles aggregate into vesicles and some may form

other nanostructures. Amphiphiles that form vesicles are mainly those having a ratio of the cross-sectional area of the apolar to polar regions of 0.74 to 1.0.[165–167] When immersed in an aqueous solution at a concentration higher than the critical aggregation concentration (CAC) and at a temperature above the solid-ordered to liquid-disordered phase transition, the lipids aggregate spontaneously and form vesicles.[167,168] Bolaamphiphiles generally have higher water solubility and a high CAC in the range of 10^{-4} to 10^{-6} M[154,169] due to the presence of two head groups, resulting in vesicle membranes that are less stable upon dilution. In comparison, phospholipids have lower CAC values within the range of about 10^{-8} M,[159] and from that point of view, they may form more stable vesicles than bolaamphiphiles. This point has to be taken into account when designing bolaamphiphiles to produce stable vesicles for drug-delivery applications—their CAC should be sufficiently low in order to maintain the vesicle structure independent of vesicle concentration.[170] The choice of the fatty acyl chain length and the head group properties are important determinants to ensure stability of vesicles prepared from bolaamphiphiles. Indeed, it has been demonstrated that vesicles from bolaamphiphiles with short aliphatic chains were less stable than vesicles made from similar bolaamphiphiles with longer aliphatic chains.[171]

Other structural features of the bolaamphiphiles that are known to influence vesicle formation are the number of the aliphatic chains within the amphiphiles and the presence of symmetrical versus non-symmetrical head groups. Some single-chain bolaamphiphiles with symmetrical head groups were shown to form spherical vesicles, but only when they were formulated with single head amphiphiles and constituted no more than 20% of the formulation.[172] Other single-chain symmetric bolaamphiphiles form tube-like vesicles[136] or fibrous[173] or helical[175] structures. Investigators [154] have succeeded in preparing vesicles with monolayer membranes from a symmetrical single-chain bipolar ammonium salt. Monolayer membrane vesicles were shown to be formed from symmetrical bolaamphiphiles with relatively short (C16 and C20) membrane-spanning alkyl chains, but these vesicles were stable only when cholesterol was used in the formulation, presumably by adopting an asymmetric distribution of this component at opposing membrane interfaces, thus allowing the formation of highly curved surfaces.[175] Alternatively, asymmetric bolaamphiphiles can be used to improve the curvature of the bolaamphiphiles within the membrane instead of using additives such as cholesterol. In this context, a highly stable monolayer membrane was obtained from asymmetric bolaamphiphiles extracted from the thermophilic archaeobacterium *Sulfolobus solfataricus*.[132,176] Kai et al. [132] demonstrated the formation of vesicles with high thermal stability with synthetic asymmetric bolaamphiphiles with long hydrophobic chains. Recently, Grinberg et al.[157] demonstrated the formation of stable cationic vesicles from synthetic short-chain asymmetric bolaamphiphiles. The improved stability of vesicles made from short-chain asymmetric bolaamphiphiles can be attributed to the ability of the different-sized head groups to accommodate differences in the radii of curvature of the inner and outer surfaces of the monolayer membrane. Symmetric bolaamphiphiles do not generally form stable spherical vesicles because the same head group cross-sectional areas and volumes cannot accommodate the difference in the radii of the inside and outside surfaces of the monolayer membrane.[128] Therefore, it can be predicted that a molecular design that results in adequate packing of bolaamphiphiles to form stable monolayer membrane vesicles will be well-suited for targeted drug delivery. From the studies described above, it can be assumed that monolayer membrane vesicles may have the characteristics needed for targeted delivery of drugs, as well as for releasing the encapsulated drug in a controlled manner. This assumption is based on the following considerations.

Bolaamphiphiles are expected to form more stable vesicles than liposomes made of bilayer membranes due to the high activation barrier towards pulling the inner charged head group through a hydrophobic matrix of a monolayer membrane.[128,150] By comparison, bilayer vesicles and liposomes can grow by fusion because of an easy amphiphile exchange with the

exterior. Although PEGylation of liposomes is used to reduce fusion, it does not completely eliminate it,[177,178] and aggregation and fusion may still be a problem with PEGylated liposomes. In fact, under certain conditions, PEGylation may even increase fusion.[179] Therefore, from this point of view, vesicles made from bolaamphiphiles that do not easily fuse may be superior to PE-Gylated liposomes.

A reduced lipid exchange is also deemed especially important for the transport of the liposomes intact through biological barriers. Because vesicles made from bolaamphiphiles are characterized by reduced lipid exchange, they may be superior to bilayer membrane liposomes when passage via a biological barrier is required. PEGylation of bilayer membrane liposomes is not sufficient to solve the problem, because steric hindrance by the PEG-shielding layer may interfere with cellular uptake.[180–182]

Monolayer membranes made of bolaamphiphiles with long aliphatic chains and polar groups within the chains offer the possibility of improved flexibility and elasticity (because they will be more resistant to shear forces) over that of bilayer membrane liposomes. Lipid bilayer membranes do not exhibit surface shear rigidity above the order-disorder transition temperature.[183,184] Because the lipids in biological membranes exist above the order-disorder transition, such membranes may not tolerate shear forces well, which hampers their elasticity. Monolayer membranes, on the other hand, have higher elasticity[185] because they tolerate greater shear forces. This would allow for shape change while penetrating though biological barriers, enabling the vesicles to remain intact during the penetration process.

The ability to obtain a more effective disruption mechanism in monolayer membranes compared with bilayer membranes is another advantage of bolaamphiphiles. This is based on the observation that bolaamphiphiles are known to form self-aggregating structures that readily convert from vesicles to fibers or sheets upon small changes in their structure,[128,151] especially with small vesicles due to curvature considerations.[186] Thus, vesicles made from bolaamphiphiles will have superior stability as described above, and yet, upon removal of the head groups, the content of the vesicles will be readily released.

Based on a calculation of the inner vesicle volume, a higher encapsulation volume can be achieved in nano-sized vesicles made from bolaamphiphiles. This is because the monolayer membrane is thinner than the bilayer membrane, resulting in a larger inner volume in monolayer vesicles compared with bilayer vesicles of the same size.

To date, only a few attempts have been made to obtain monolayer vesicles from bolaamphiphiles for targeted drug delivery, because the structural requirements for the formation of stable vesicles from such bolaamphiphiles were not well-understood. When analyzing lipid layer stability for drug-delivery applications, two main factors should be addressed: permeability of the vesicle membrane and prevention of delamination.[186] Permeability in lipid membranes is related to the packing of the hydrocarbon chains. In the case of bolaamphiphiles, it was found that the permeability of the bolalipid layer is reduced compared with that of a monopolar lipid bilayer.[187] Bilayer membranes can easily undergo delamination, since they are weakly bonded at the bilayer midplan, resulting in low mechanical stability.[188] Delamination can also be prevented by using bolaamphiphiles because they have the ability to adopt a transmembrane configuration that completely spans the hydrophobic region of the lipid layer by placing the polar head groups at opposite membrane-water interfaces,[189,190] thus making such bolaamphiphiles appropriate for several biotechnological applications because they can stabilize the membrane. Recently, a series of novel symmetric bolaamphiphiles that form stable vesicles with potential applications in targeted drug delivery have been described.[157] Based on systematic changes in the bolaamphiphile structure and the relationships to the performance of the vesicles, new bolaamphiphiles that may form improved

vesicles for targeted drug delivery can now be synthesized.[171,191] Vesicles made from similar amphiphiles have already been shown to be effective in gene transfer[192,193] and in the delivery of peptides to the brain[192], the latter probably due to the stability and the flexibility of the monolayer membrane vesicles that allowed them to penetrate intact via the blood-brain barrier.

In summary, bolaamphiphiles are potential candidates for the formation of nanoparticles for targeted drug delivery and therefore deserve a closer look for the development of novel lipid-based nanoparticles. The suitability of bolaamphiphiles as building blocks of vesicles for drug delivery was well demonstrated with archeosomes, which are made from bolalipids extracted from archaebacteria,[194–197] and with synthetic bolaamphiphiles based on the bolalipids of archaebacteria.[145,154] These studies show that the structures of bolaamphiphiles determine the nature of the nanoparticles they form and their advantage in targeted drug delivery. In order to design optimal nanoparticles for drug delivery, understanding the rules of molecular self-organization is important. To this end, the structural requirements needed for bolaamphiphiles to form vesicles for targeted drug delivery are under investigation, and new efforts are focusing on rational design of bolaamphiphiles for targeted drug delivery.

IV. SOLID LIPID NANOPARTICLES (SLN) AND NANOSTRUCTURED LIPID CARRIERS (NLC)

Although liposomes have been the hallmark of lipid-based nanoparticles for site-specific delivery of drugs and pharmaceuticals, there is a need to develop alternate approaches for nanoparticles based on lipid components other than phospholipids. It is hoped that these drug carriers may allow for higher control over drug release and delivery of therapeutics, which may not efficiently load in to liposomes. Compared with other drug-delivery systems, SLN and NLC have been developed very recently and are potentially attractive, marketable choices due to their natural components and are easily scaled-up synthesis processes. Both SLN and NLC are well positioned for large-scale manufacturing, as solvent use can be avoided using the high-pressure homogenization method with extant machinery.[198] In addition, their hydrophobic core provides a suitable environment for entrapment of hydrophobic drugs. This is important, as approximately 40% of newly developed drugs are hydrophobic in nature.[198,199]

Numerous reports have described various SLN formulations since the early 1990s, and NLC formulations since the late 1990s, that may find applications in drug-delivery systems. The SLN structure is composed of a solid lipid core, which may contain triglycerides, glyceride mixtures, or waxes that are solid at both room temperature and human body temperature.[200, 201] The diagram in Figure 9 depicts the possible assembly of triglycerides to generate SLN and NLC. SLN are interesting lipid-based drug-delivery carriers for a number of reasons, including: i) particle size is on the nano- to sub-micron scale (50–1000 nm) after drug encapsulation; ii) they are composed of biocompatible and biodegradable components (i.e., physiological lipids or lipid molecules) and do not require the use of organic solvents for their assembly; and iii) the particle synthesis process (e.g., high-pressure homogenization) can be performed at a lower cost and are easily scaled up. Therefore, these nanoparticles bear the positive aspects of other nano-lipid carrier systems, and they also overcome several of their disadvantages. For example, SLN are similar in nature to nanoemulsions, but feature a solid lipid core as opposed to a liquid lipid version. As a result, drug mobility decreases in the solid lipid state compared with the oily phase, thereby enhancing the controlled release of loaded drugs.[198] SLN stability can be further improved by the addition of a surfactant coating.[198] An additional advantage involves the production of SLN in a powder form, which may be loaded into pellets, capsules, or tablets for further enhancement of drug delivery. It is important to mention that applications of SLN formulations may be limited due to the undesired particle growth by agglomeration or coagulation resulting in rapid “burst release” of the drug. SLN have perfect crystal lipid matrices that accommodate the loaded drug in its molecular form

between fatty acid chains. The formation and enhancement of the crystal structure during both production and storage of SLN often results in expulsion of the loaded drug solution, a major disadvantage of the nanoparticle.[198,201]

NLC, often referred to as the second generation of SLN, were first developed by Müller et al. in the late 1990s. In contrast to the lipid crystal matrix of SLN, the lipid matrix of NLC has an imperfect crystal or amorphous structure, which allows for drug loading in both the molecular form and in clustered aggregates at lattice imperfections (Fig. 9). As a result, NLC show enhanced drug loading and less pronounced drug expulsion by avoidance of a crystal structure.[198]

NLC, similar to SLN, are colloidal particles that typically range in size from 100 to 500 nm, depending on production parameters.[199,200,203] A blend of solid- and liquid-phase lipids, NLC are generally solid at temperatures above 40°C. They have been successfully multifunctionalized to capture a payload of drugs, to target specific cells, and to release entrapped drugs in a controlled manner.[198,199] NLC have been mostly researched for oral or dermal drug delivery applications, with little focus on parental administration; however, recent literature has demonstrated their potential as attractive candidates for the delivery of anticancer agents, as well as therapeutic proteins and peptides.[198,199,202]

IV.A. Formation of SLN and NLC

1. SLN—Encapsulation of drug solutions into SLN can be performed using numerous methods, including high-pressure homogenization, microemulsion formation, emulsification-solvent evaporation (precipitation), solvent injection (or solvent displacement),[203,204] phase inversion,[205] the multiple emulsion technique,[206,207] ultrasonication,[209] and the membrane contractor technique.[209–211] A typical SLN formulation includes 0.1% to 30% solid lipid content, including one or more of the base ingredients (trimyristin, tristearin, trilaurin, stearic acid, glyceryl caprate as Capmul®MCM C10, theobroma oil, triglyceride coconut oil, 1-octadecanol, glycerol behenate as Compritol® 888 ATO, glycerol palmitostearate as Precirol® ATO 5, and cetyl palmitate wax); 0.5% to 30% surfactant stabilizer (examples previously mentioned); and 5% of the incorporated drug. For longer circulation time in vivo, curdlan and PEG molecules have been used.

Encapsulation of drug molecules can occur at various locations within SLN depending on their chemical properties. Lipophilic drugs will disperse well due to their miscibility in the lipid matrix, whereas hydrophilic drugs are thermodynamically immiscible and will separate to the outside of the lipid matrix. Typically, the SLN assembly process involves dispersion of drugs into a melted-lipid phase (precursor emulsion) either by using the appropriate solvent(s) or application of mechanical forces. For successful drug loading into SLN, the drug needs to adequately partition into the lipid droplets. In the synthesis step of SLN, fast cooling creates an unstable and disordered α-crystalline structure, which allows the desired drug to be stored in to the nanoparticle's amorphous areas. During the storage period, this α-crystalline state can be converted to a thermodynamically stable state, which is β-crystalline in nature. The exact partitioning of the drug in SLN depends on the recrystallization rate of the lipid matrix and the resulting crystalline structure. Because drug molecules incorporate between the fatty acid chains, lipid layers, and in areas of crystal imperfections, a highly ordered and organized crystalline structure is not desirable for higher drug-loading capacities. It is important to note that the structural transformation from α-crystalline to β-crystalline can result in a "burst release" upon administration into the body, a significant drawback for SLN in the clinical setting. Recently, reverse micelle formation by the amphiphilic lipid lecithin within the lipid matrices has been shown to increase SLN drug-loading capacity.[204]

2. NLC—Similar to SLN, a mixture of solid- and liquid-phase lipids are used to create NLC. Usually, about 5% of drug (by weight) is incorporated in the lipid mixture upon initial NLC production, and approximately 3% to 4% drug loading is achieved (with typical encapsulation efficiencies of approximately 70%).[199,212] Examples of solid-phase lipids typically utilized include monostearin, stearic acid, glyceryl dilaurate, hydrine, glyceryl, monostearate, cetyl alcohol, and imwitor 900; typical liquid phase lipids used are oleic acid, capmul glyceryl mono-dicaprylate, and caprylic/capric triglycerides.

Appropriate lipid selection is crucial for creating a stable drug-loaded NLC. The chemical stability of the drug is dependent on the type of solid lipid incorporated into the NLC. Similarly, the incorporation of drug into lattice defects of the NLC may alter particle stability (most likely enhancing stability). In addition, possible lipid interactions with the drug during and after NLC production should be considered. For example, auto-oxidation of lipid may cause drug degradation.[213]

Similarly, the percentage of liquid-phase lipid incorporated can influence the size and surface morphology of particles. Hu et al. showed that as the concentration of the liquid-phase lipid oleic acid increased up to about 30%, particle size decreased, and particle morphology became more spherical, smooth, and regular. In addition, their studies suggest that oleic acid concentration controls the initial rate of drug release.[214]

NLC may be subdivided into three categories based on the structure of their lipid matrix: the imperfect type, multiple types, and amorphous or structureless type (Fig. 9). The imperfect type of NLC has the least amount of liquid-phase lipid (oil) and is composed of saturated and unsaturated lipids with varying fatty acid chain lengths, which lead to defects in the lipid matrix and compartments for drug storage.[213] However, less pronounced than in SLN, the imperfect NLC is prone to an expulsion of drugs during the crystallization process of production. Toward the end of production, the temperature is lowered, lipids transition from their melted state to a solid phase, and particles crystallize. This causes drug solubility to decrease and the subsequent release of entrapped drug from the lipid matrix.[215]

The multiple type of NLC avoids this drug expulsion by incorporating a higher concentration of liquid-phase lipids in the lipid matrix. During the cooling process, oil reaches its solubility limit and precipitates into nanocompartments. Compared with other SLN and NLC formulations, these nanocompartments can accommodate a higher drug concentration.[216] In addition, a higher oil concentration is associated with faster drug release.[217] The amorphous type of NLC forms a solid lipid that lacks any crystalline structure. This is achieved through the use of lipids such as hydroxyoctacosanylhydroxystearate and isopropyl-myristate.[215] As expected, the lack of a crystalline structure avoids undesired drug expulsion during the cooling process.

3. Stabilization—The in vitro and in vivo stabilization of SLN and NLC is commonly achieved through PEGylation or polymer coating (e.g. PEG2000, PVA, poloxamers),[218–221] which has been already used for doxorubicin-formulated liposome stabilization (i.e., Doxil®). The addition of PEG molecules prevents immunoprotein adsorption and minimizes phagocytic uptake by macrophages, thus increasing blood plasma circulation time.[222,223] PEG has been successfully incorporated into lipid matrices by its conjugation to monostearate (PEG-SA). However, the incorporation of PEG-SA into NLC reduced drug encapsulation efficiency and increased the rate of drug release.[217]

Typically, PEG lipids are mixed with other solids and melted together. Past studies have found a five-fold enhancement of doxorubicin plasma concentration by a SLN carrier, and a seven-fold enhancement for PEG-stabilized stearic acid SLN have been reported.[224] PEGylation has

also been shown to increase oral delivery of peptide drugs, including calcitonin.[225] However, literature studies show that SLN themselves increased drug circulation time significantly, providing higher drug concentration in the bloodstream when lecithin-based (e.g., Epikuron 200) surfactants were used for stabilization. The "stealth effect" was not found to be significant in these SLN, and more intensive studies are required to understand this effect with SLN through the use of PEGylation.

IV.B. Therapeutic Drug Delivery

There are several advantages of therapeutic drug delivery by SLN and NLC: i) they can control and extend drug release, ii) they can encapsulate various drugs, and iii) they can extend blood circulation time and utilize the EPR effect for enhancing treatment. Hydrophobic drugs with short circulation half-lives are ideal candidates for delivery via SLN and NLC. Many pharmaceutically active peptides and proteins are being developed. However, they are often characterized by a short half-life in the body and a limited ability to cross cell membranes. NLC may be an ideal carrier for their delivery, because it can protect the protein or peptide from degradation and may transport the therapeutic into the cell interior. However, the encapsulation of peptides and proteins into these lipid carriers is not always realizable. High temperatures associated with HPG and solvents associated with other production methods may denature and degrade proteins.[199] Hydrophilic drugs are also candidates for delivery by lipid nanoparticles by using lipid drug conjugates.[226]

Therapeutic compounds can oftentimes be chemically reactive. SLN and NLC have the ability to protect labile anticancer drugs known to be susceptible to hydrolysis and, as a result, the active drugs remain in the bloodstream for a longer period of time. One example is the SN-38 compound, a relatively hydrophilic pro-drug of irinotecan that also carries a labile lactone structure such as camptothecin. SLN loaded with SN-38 protected the hydrolysis of the drug and increased the treatment effect. In addition, an extended half-life of the active lactone drug form in the whole blood was observed in in vivo nude mice studies.

A wide variety of drugs, such as prednisolone, doxorubicin, and retinol, have been successfully incorporated into SLN.[227–231] Similarly, the anticancer therapeutics paclitaxel and doxorubicin have been successfully loaded into NLC.[199] Compounds with the deaza skeleton of the antitumor drug temozolomide have also shown promising anticancer results when loaded in NLC.[232] In addition, progesterone, valdecoxib, clobetasol propionate, closporine, retinol, Celecoxib, and etomidate have all been successfully loaded into NLC (some for transdermal drug delivery).[212,214,217] The anticancer therapeutics paclitaxel and doxorubicin loaded into NLC have been found to overcome cell-multi drug resistance.[199] In their study on the effects of a SLN formulation on the human colorectal cancer cell line HT-29, Serpe et al.[233] demonstrated that SLN containing cholesteryl butyrate and doxorubicin showed significantly higher cytotoxic effect than the equivalent amount of free drug. In cancer chemotherapy, cancer cells continuously exposed to sub-optimal levels of cytotoxic agents may induce the expression of membrane-associated drug transporters (e.g., P-glycoprotein), thus rendering the cells more drug resistant. The enhanced chemotherapeutic effect by the lipid nanocarriers could be the result of efficient endocytosis into the cells, thereby bypassing the P-glycoprotein drug efflux mechanism. This effect has also been reported in doxorubicin-loaded SLN on murine and human breast cancer cell lines.

Drug administration can be facilitated by the submicron size and controlled drug release of SLN. Various administration routes (parenteral, pulmonary, mucosal, and topical) have been studied. Due to their nano-scale size, SLN can be delivered parenterally and can increase circulation times of therapeutic agents. Parenteral administration is suitable for drug targeting by SLN, and peptide and protein drugs are commonly supplied by this administration method due to the avoidance of enzymatic degradation in the GI tract, which is possibly in oral dosage.

Initial work on SLN on the oral delivery of lipid nanopellets was reported, and a cyclosporine SLN formulation has been introduced to the market for oral administration. Interest in ocular administration of SLN has peaked due to their biocompatibility and mucoadhesive properties. Tobramycin delivery in rabbit eyes suggested that the drug bioavailability was significantly enhanced by SLN. Additionally, SLN formulations in a nebulized form have been used to carry anti-tubercular drugs for treatment of pulmonary tuberculosis, and showed an improved dug treatment effect. Finally, SLN have also been extensively applied to dermal applications in prolonging the shelf lives of sensitive compounds such as retinol and vitamin E.[234]

Entrapped therapeutics are released from NLC through natural diffusion and lipid degradation. Most NLC exhibit a biphasic drug release pattern consisting of an initial release burst followed by prolonged release afterward. The initial burst release rate can be controlled by the concentration of liquid lipid.[199] An energetic impulse given to an NLC is expected to convert the disordered lipid matrix into a more crystal structure. In turn, this may drive out entrapped therapeutics. This phenomenon is evident in the transdermal delivery of cyclosporine in lipid particles. Burst release of the drug is initiated upon application to the skin, where it is exposed to body temperature and nearby evaporation of water, which convert the lipid matrix to a higher crystalline order, expelling the drug.[198]

IV.C. Targeting Ligands: SLN

Targeting strategies for tumor sites by SLN can also be combined to minimize side effects and enhance specificity to sites of interests. Liposomal-targeting approaches have been adapted directly to SLN formulations (i.e., ligand binding to the surface of nanoparticles). In one approach, researchers have prepared docetaxel-loaded SLN with a galactosylated conjugated DOPE lipid to specifically target the asialo-glycoprotein receptor on hepatocellular carcinoma cells.[235] Additional work has been performed on a folate-targeted SLN system, which was developed for the delivery of the drug paclitaxel.[121] The targeted formulation resulted in greater drug uptake and cytotoxicity in folate receptor cell lines than non-targeted SLN, and significantly improved in vivo tumor growth inhibition and tumor-bearing animal survival. For liver targeting in particular, SLN containing galactosylated or mannosylated lipids have been employed. Although SLN with and without galactosylation showed higher liver targeting compared with free drug solutions, galactosylated SLN displayed further enhancement when comparing in vitro activity and in vivo biodistribution of all formulations.[236]

Finally, these nanoparticles have also been utilized for drug delivery to the brain. Developments of nanocarriers to deliver drugs to the central nervous system have been limited due to their inability to cross the restrictive blood-brain barrier.[237] On the other hand, the highly vascularized nature of brain tissue makes it an attractive choice for intravenous delivery of therapeutic drugs. SLN and NLC take advantage of the high capillary density of the brain, and at the same time possess the ability to overcome blood-brain barrier limitations.[238] These formulations have been utilized to enhance delivery of antiretrovirals to the brain, such as the HIV protease inhibitor Atazanavir[239] and drugs to treat cardio-cerebrovascular diseases, including daidzein.[240] Treatment of Parkinson's disease has also involved the use of SLN to deliver dopamine agonists to the brain. In comparing bromocriptine (BK)-loaded SLN to free BK in both in vitro and in vivo studies, Esposito et al. reported a faster preliminary release and subsequent slower gradual release of the drug associated with the SLN formulation.[241] In 2007, Gupta et al. reported findings on the treatment of cerebral malaria with transferrin conjugated-SLN encapsulated with quinine hydrochloride.[242] Drug release was found to be greater in the unconjugated SLN, whereas transferrin-SLN showed greater accumulation in brain tissue. Researchers have also demonstrated that thiamine-coated SLN successfully bind to the blood-brain barrier thiamine transporter, and result in a gradual buildup of SLN that is responsible for increased brain uptake.[243] SLN and NLC are seen as promising drug-delivery systems, but

in the case of the brain, they are even more important in terms of successfully overcoming the restrictions imposed by the blood-brain barrier.[244]

IV.D. Future Applications

The versatility of SLN and NLC as drug-delivery systems makes them an attractive choice as carriers of diverse anticancer cytotoxic agents and peptide drugs. The use of these lipid nanoparticles in drug vectorization is now being tested in both in vitro and in vivo studies for commercial applications. Surface modifications may make the SLN delivery system even more attractive, for example, through increased bioavailability with PEG coating.[221] However, some disadvantages such as polymorphism and crystalline rearrangements in SLN and some formulations of NLC must be overcome to achieve controllable and stable drug delivery. Moreover, the release of the active molecules incorporated into these solid nanoparticles should be further investigated. For commercial applications, the quality control of the natural components in the SLN production has to be considered more seriously. NLC appear to be attractive vehicles for intravenous drug delivery; however, their application in this field is in the early exploratory stages of research. Future studies may enhance the controlled release of therapeutics from these carriers and improve the production process for more homogeneous samples pure of toxic compounds. Furthermore, their applications in cancer drug delivery remain to be seen.

V. FUTURE PERSPECTIVES OF LIPID-BASED NANOPARTICLES IN DRUG DELIVERY

Various nanotechnology platforms are currently being developed with the aim of improving drug delivery, especially to combat cancer. Among these, lipid-based nanoparticles present one of the promising drug-delivery candidates and have been the longest-studied nanocarriers. The various systems discussed here are summarized in Table 4. Despite tremendous efforts, only a few formulations are approved for clinical use thus far. In addition, the clinical applications of targeted nanoparticles remain to be seen. Therefore, it is imperative to re-visit considerations of current approaches and strategies employed in the design and development of these anticancer nanocarriers. In our opinion, efforts could be focused primarily on two areas: i) technical aspects such as fabrication strategies, the development of techniques for reproducible nanocarriers, large-scale production, and the conjugation of targeting molecules such as scFvs and peptides to the nanoparticle surface; and ii) novel concepts and approaches to accomplish on-demand release of drugs from the nanoparticles (based on the unique properties of the assembly components of lipid-based nanocarriers). We surmise that the future for on-demand drug-phospholipid assemblies (liposomes) is promising as strides are being made for on-demand drug release from targeted nanoparticles. Bolalipids bear several unique properties compared with glycerol-based phospholipids (used to fabricate liposomes), and their applications as nanocarriers are currently in the infant phase. Thus, the future of bolalipid drug-delivery assemblies remains to be seen. Similarly, SLN and NLC are very attractive drug-delivery candidates, primarily due to their relatively stable constituents and probable ease of drug encapsulations. However, their future in the clinical setting is also subject to extensive research.

Additionally, it is our viewpoint that two important aspects for clinically viable nanocarriers will facilitate their usefulness in the clinic: the development of triggering modalities that are amenable to human applications and the development of alternate strategies for in vivo stabilization of drug-delivery vehicles. Although the concept of PEGylation to increase half-life of nanoparticles revolutionized the nanoparticle-mediated drug-delivery field, significant improvements are warranted in this area.

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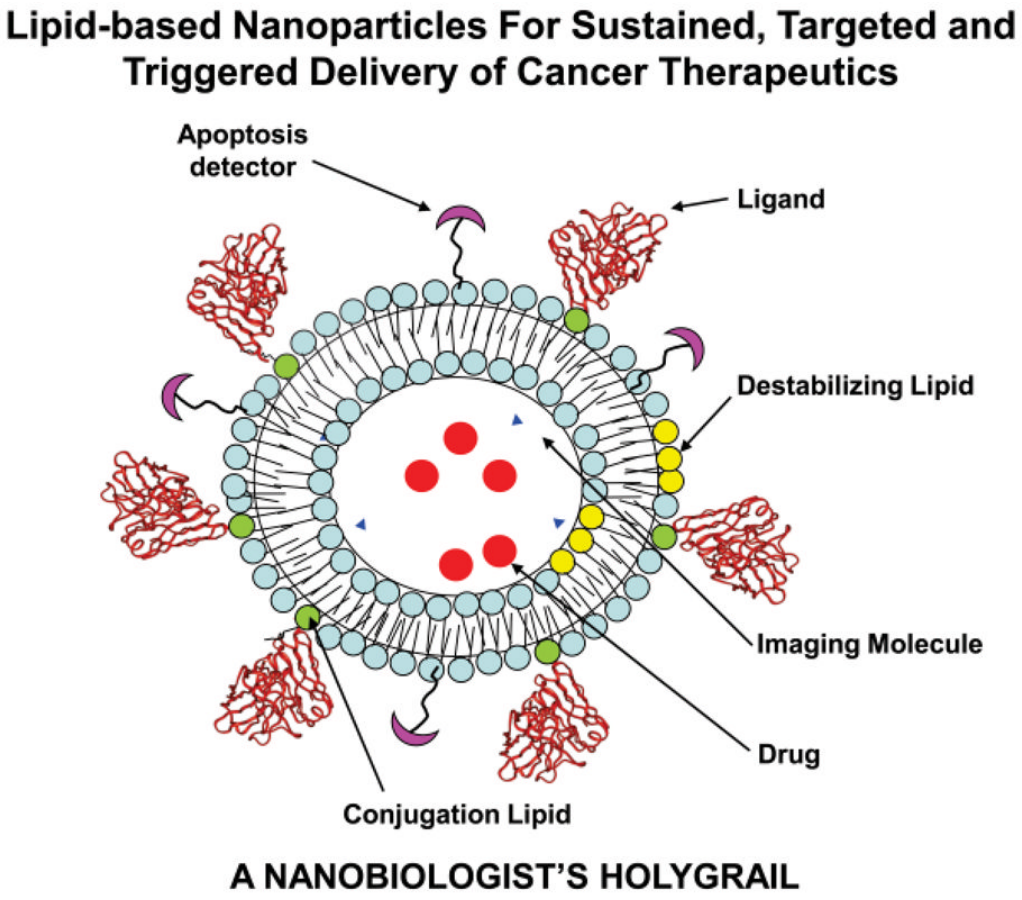

FIGURE 1.

Design principle of an ideal multifunctional lipid-based nanoparticle for targeted and triggered drug delivery. Liposomes consist of a matrix phospholipid (cyan), a destabilizing (pore forming) phospholipid (yellow), conjugation lipid (green), ligand attached via the conjugation lipid (brown), and a cell death marker such as an apoptotic detector (pink). The nanoparticle is loaded with a chemotherapeutic agent (red) and an imaging agent (blue) in the aqueous milieu.

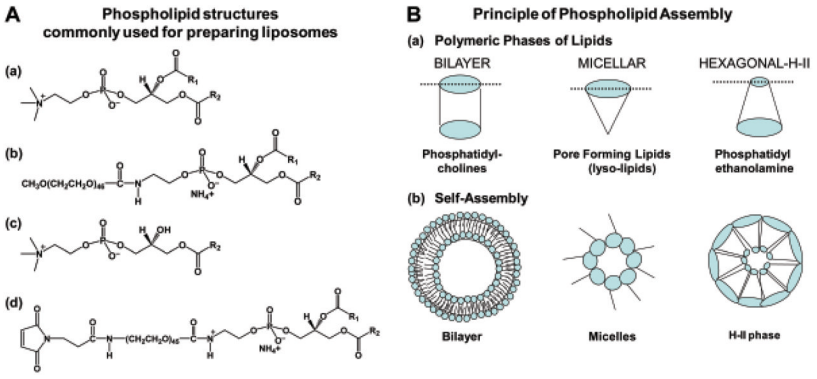

FIGURE 2.

A, Chemical structures of commonly used phospholipids to prepare liposomes: Matrix lipid such as DPPC or DSPC (a); PEGylated lipid (usually DSPE-PEG2000) for longer circulation in vivo (b); lipid bilayer destabilizing lipid, such as lyso-lecithin or pore forming photoactivable lipid (c); and a functionalized lipid such as maleimide-DSPE-PEG2000 for conjugating ligands such as antibodies and/or peptides for site-specific targeting (d). R1 and R2 represent fatty acyl chains at sn-1 and sn-2 positions, respectively, and usually vary from C16 to C18. The selection of R1 and R2 will depend on the type of liposomes needed. B, Principle of assembly of various lipid molecules. The lipids assume various physical structures depending on their chemical structures. Top panel shows polymeric phases (PC, bilayer, lyso-PC, micellar, PE, hexagonal HI); bottom panel shows the corresponding self-assembly of these lipids as indicated.

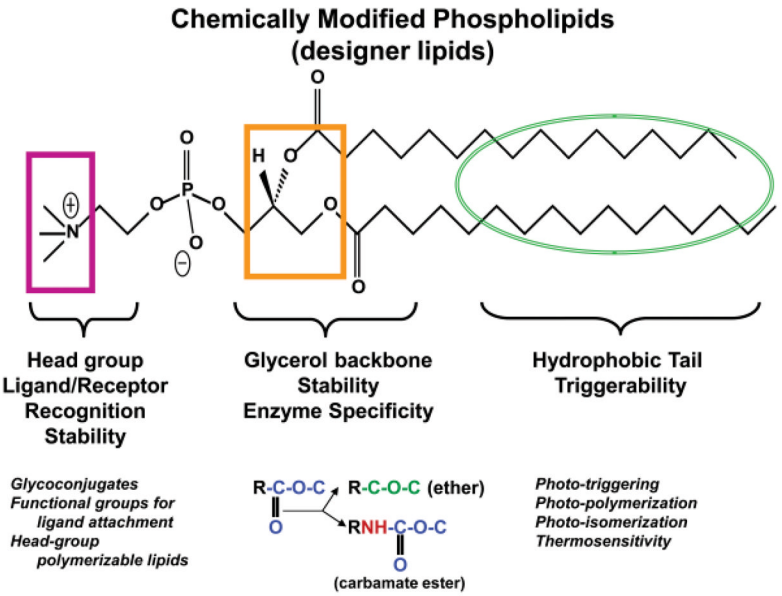

FIGURE 3.

Sites of chemical modifications in the phospholipid molecules for tunable liposomes. The chemical structure of a typical PC is shown and the sites of various modifications in three major portions of this molecule are as follows: pink, head group modification; orange, glycerol backbone modifications; and green, fatty acyl modifications. The specific examples of modifications are given at the bottom.

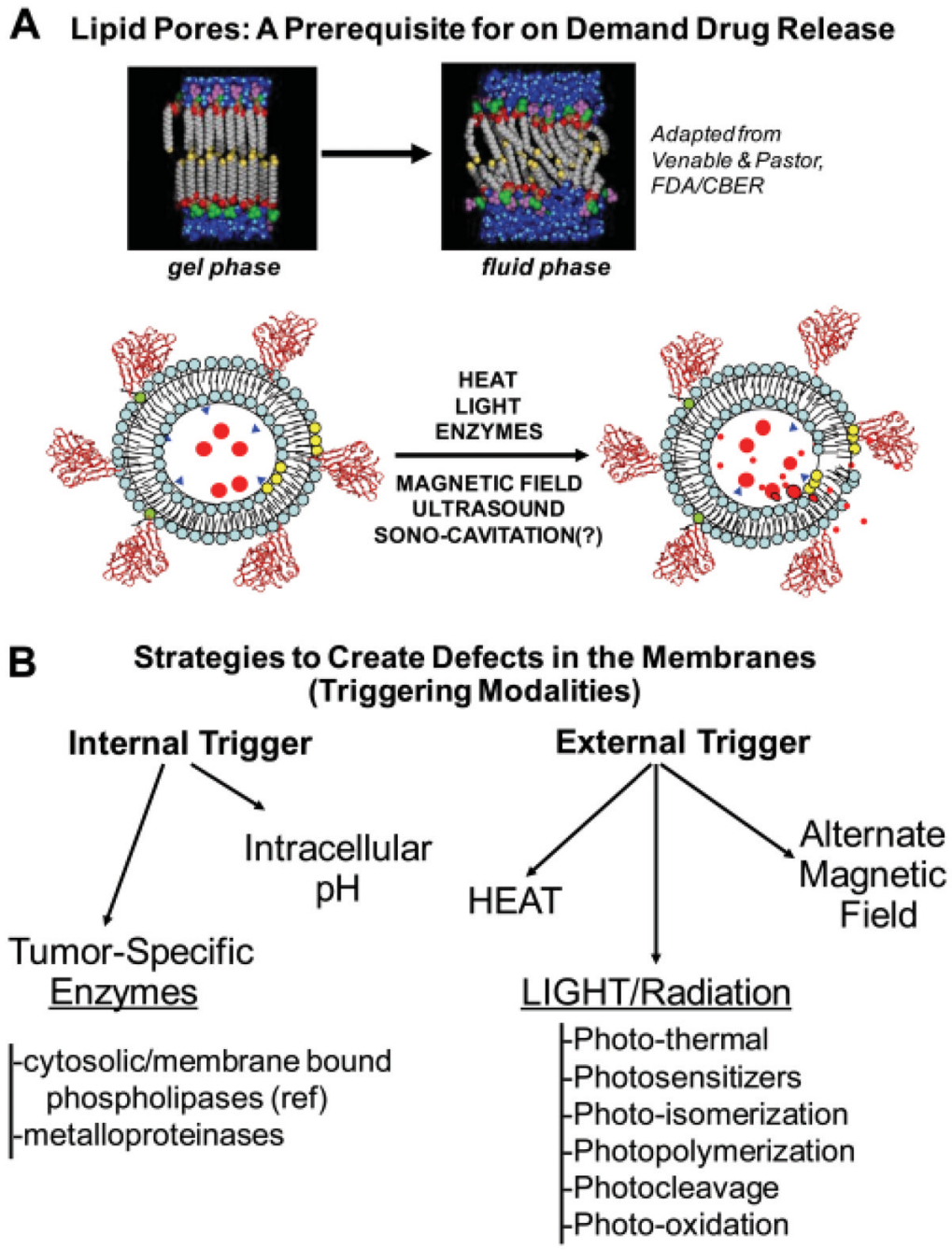

FIGURE 4.

A, Principle of creating defects in the lipid bilayer. Top panel shows fatty acyl chain organization at the Tm temperature. The diagram was adapted from the website of Venable & Pastor (FDA/CBER). Lower panel shows release of drugs through localized defects in the membrane. B, Various strategies used for localized drug delivery.

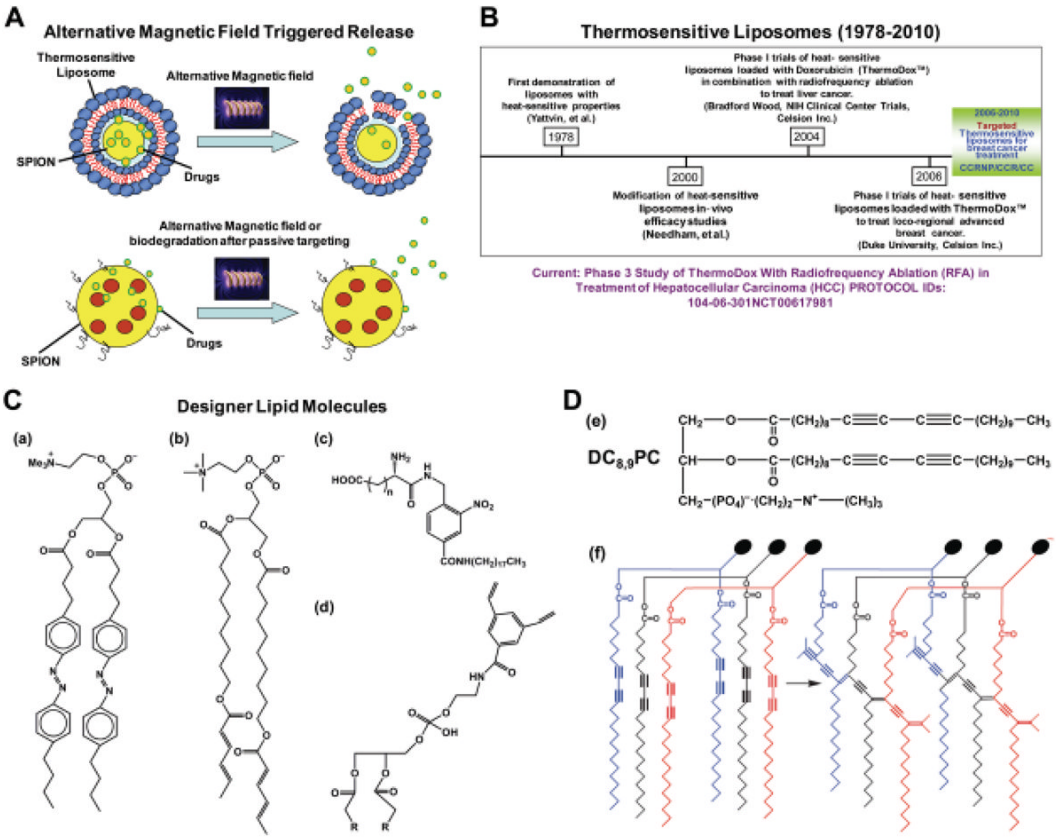

FIGURE 5.

Examples of external triggering modalities. A, Drug-loaded superparamagnetic iron oxide nanoparticles (SPIONs) encapsulated in liposome membrane (top panel). Exposure of these liposomes to alternative magnetic field results in local disruption of liposomes releasing SPIONS (step 1), and the drug is then released from the SPIONS (step 2). B, Timeline for development of thermosensitive liposomes. C and D, Chemical structures of designer lipids used for development of photo-activable liposomes: (a) Bis AzoPC, (b) Sorbyl PC, (c) o-nitrobenzyl conjugated lipid, (d) head-group polymerizable lipid, (e) $DC_{8,9}PC$ (1,2 bis (tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine), (f) principle of photopolymerization of $DC_{8,9}PC$.

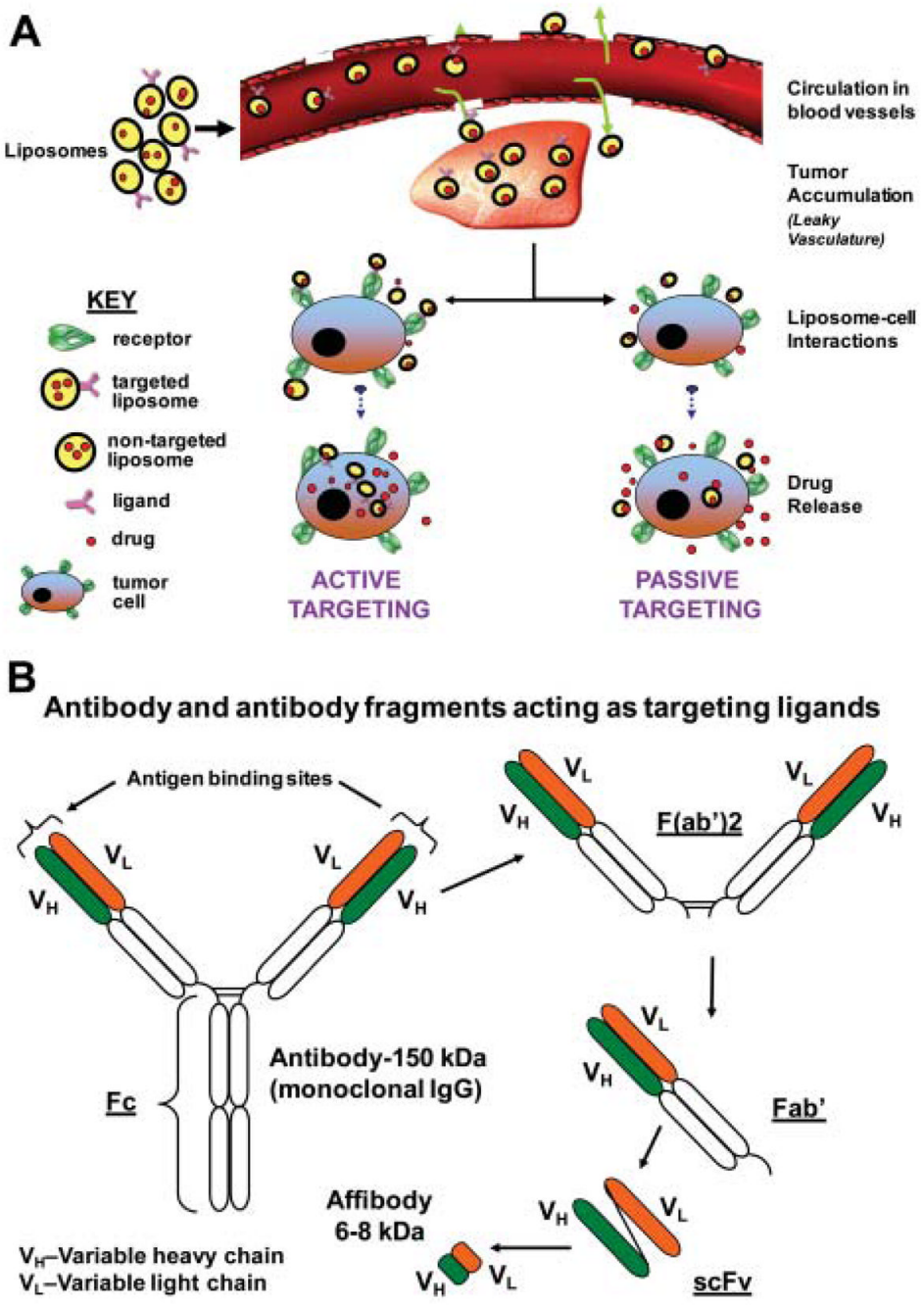

FIGURE 6.

A, Targeting pathways of lipid-based nanoparticles to tumors. Schematic presentation of nanoparticles that passively or actively target tumors. These particles accumulate in the tumor area due to the leaky vasculature surrounding the tumors. However, targeted nanoparticles bind to the target tumor cells via ligand-receptor interactions and are internalized, whereas non-targeted nanoparticles are less efficient in interacting with tumor cells. B, Antibodies and antibody fragments used for targeting of lipid-based nanoparticles.

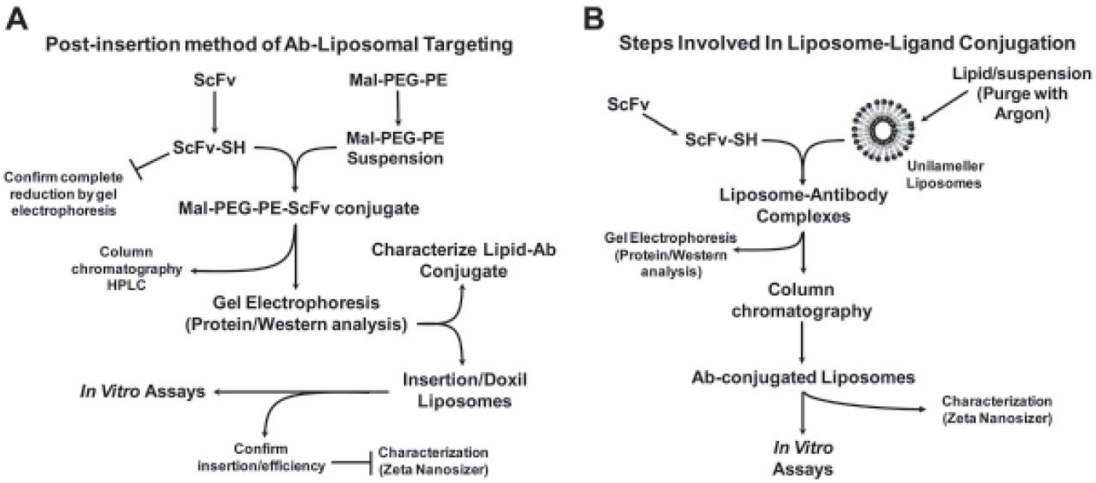

FIGURE 7.

Scheme presenting steps in the conjugation of antibodies/targeting molecules to the liposomes. A, Post-insertion protocol. B, Antibody conjugation on the surface of liposomes.

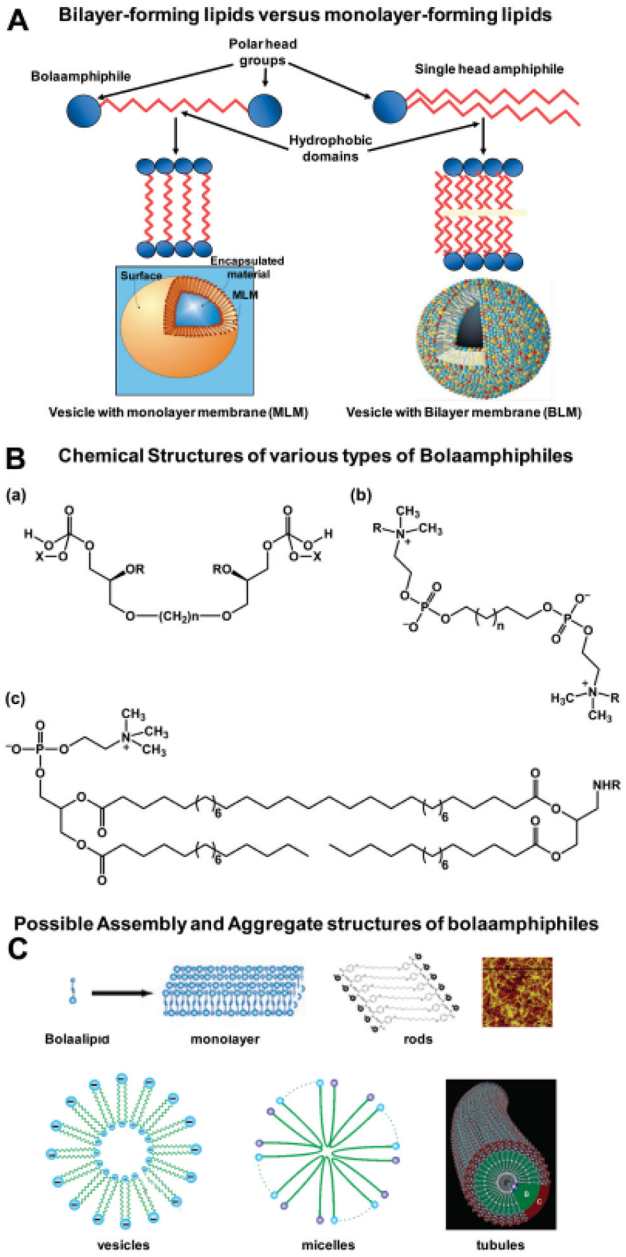

FIGURE 8.

Bolaamphiphiles. A, Self-assembly of bolalipids into monolayers and comparison with bilayer forming lipids such as phosphatidylcholine. B, Chemical structures of various types of bolalipids: (a) bolaamphiphilic phosphocholine, (b) symmetrical bipolar phospholipid, and (c) unsymmetrical bipolar phospholipids. C, Possible assembly and aggregate structures of bolaamphiphiles.

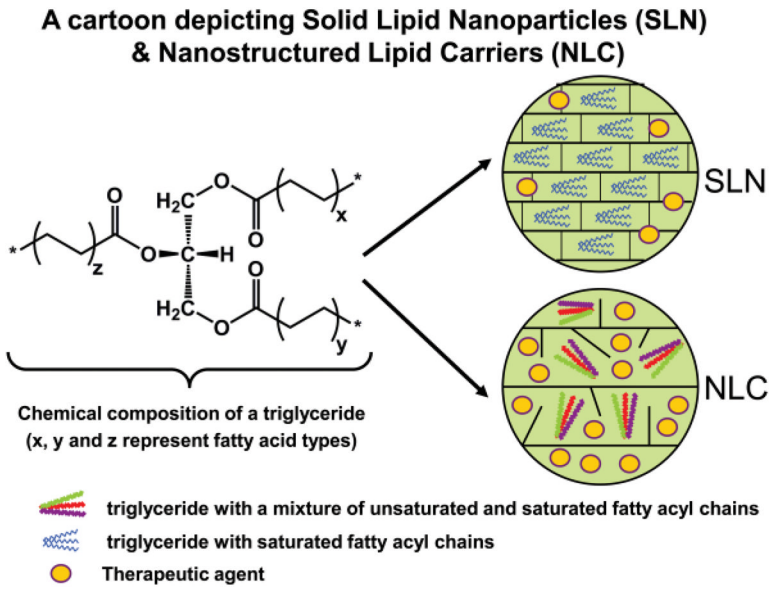

FIGURE 9.
Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC).

TABLE 1

Federal Drug Administration (FDA)-Approved Liposomes

Drug name and company	Indications/Target	Reference
Marqib-Vincristine sulfate liposomes injection: Vincristine-encapsulated liposomes in a lipid bilayer of sphingomyelin (Hana Biosciences)	Treatment of adult patients with acute lymphoblastic leukemia	245
DaunoXome: Daunorubicin citrate-liposome injection (NeXstar Pharmaceuticals)	Advanced HIV-associated Kaposi's sarcoma for first-line use	246
AmBisome®: Lipid-based formulations, including liposomal amphotericin B (Fujisawa Healthcare)	Treatment of fungal infection and visceral leishmaniasis	247,248
Doxil®, caylex: PEGylated liposomal loaded with doxorubicin (PLD) (Ben Venue Laboratories for Johnson & Johnson)	Treatment of metastatic breast cancer, advanced ovarian cancer, multiple myeloma, and AIDS-related Kaposi's sarcoma	249–252
Amphocil: Lipid form of amphotericin B stabilized with cholesteryl sulfate (Samaritan Pharmaceuticals)	Amphocil binds to lipoproteins and ergosterol in cell membranes of infecting fungi; also indicated for the treatment of invasive aspergillosis and leishmaniasis	253,254
ABELCET®: Amphotericin B lipid complexed with two phospholipids (DMPC:DMPG, 7:3 molar ratio) in a 1:1 drug-to-lipid molar ratio (The Liposome Company)	ABELCET® consists of amphotericin B, a polyene, antifungal antibiotic for invasive fungal treatment	255
Depocyt™: Liposomal cytarabine or liposomal Ara-C are antimetabolite cytarabine, encapsulated into multivesicular lipid-based particles (Enzon Pharmaceuticals)	DepoCyt is a sustained release formulation of the chemotherapeutic agent cytarabine, used for the treatment of patients with lymphomatous meningitis	256

TABLE 2

Drug-Loaded Liposome Formulations Currently in Clinical Trials

Drug name and company	Treatment	Status	Reference
Liposomal-annamycin semi-synthetic doxorubicin analog: Annamycin intercalates into DNA and inhibits topoisomerase II (Aronex Pharmaceuticals)	Acute myeloid and lymphoid leukemia (drug-resistant tumors)	Phase I/II	257,258
Lipoplatin: A new cisplatin-encapsulated liposome composed of DPPG, soy phosphatidyl choline (SPC-3), cholesterol, and mPEG2000-DSPE, designed to reduce cisplatin toxicities without reducing efficacy (HBio)	Carcinoma of the head and neck, pancreatic cancer, and continuing in advanced breast cancer and gastrointestinal cancers	Phase II clinical trial and one phase III non-inferiority clinical study	259,260
NX 211 (liposomal lurtotecan): Liposomal formulation of the lurtotecan a topoisomerase I inhibitor (Gilead and Glaxo)	Advanced or recurrent ovarian epithelial cancer	Phase II	261,262
Liposomal vincristine (ONCO-TCS™): Vincristine encapsulated in liposomes composed of sphingomyelin and cholesterol (INEX Pharmaceuticals)	Treatment of relapsed aggressive non-Hodgkin's lymphoma and other cancers	Pivotal phase II/III	245,263–265
Doxorubicin HCL liposome for injection: Non-PEGylated liposomal doxorubicin Myocet made by Enzon Pharmaceticals for Cephelon in Europe and for Sopherion Therapeutics in the United States and Canada	Treatment for patients with stage II or III invasive breast cancer and with tests showing an overexpressing of human epidermal growth factor receptor 2; Myocet® is approved in Europe and Canada for treatment of metastatic breast cancer	Pivotal phase III global registrational trial for treatment of HER2-positive metastatic breast cancer	266,267
VentusTM: Prostaglandin E1 liposomes (The Liposome Company)	Pretreatment of acute respiratory distress syndrome	Phase III clinical trial	268
ThermoDoxTM: Lysolipid thermally sensitive liposomes are heat-activated liposomal encapsulation of doxorubicin (Celsion Corporation)	ThermoDoxTM can be used as treatment for hepatocellular carcinoma (primary liver cancer) and recurrent chest wall breast cancer	Phase III	269
Protein-stabilized liposome encapsulation of active drug Docetaxel (ATI-1123): Liposome product made in a single step and encapsulates Docetaxel a clinically well-established anti-mitotic chemotherapy medication (Azaya)	Docetaxel (trade name Taxotere) is used mainly for the treatment of breast, ovarian, and non-small cell lung cancer	Pre-clinical studies to FDA phase I clinical trial	270

TABLE 3

Ligand-Targeted Lipid-Based Particles for Anticancer Drug-Delivery Antibodies

Targeted Agent/Antigen	Encapsulated Marker/Drug	Model	Reference
Anti CD-19 (Ab)	Doxorubicin	Human multiple lymphoma, ARH77 cells	271, 272, 273
Anti CD-19 (scFv)	Doxorubicin	Raji human lymphoma	274
Anti HER2	Paclitaxel	HER2+ human breast cancer cells	275
Anti HER2 Fab′ or scFv		Human breast BT-474 adenocarcinoma cancer cells	103,276
Recombinant human, anti-HER2-Fab′ or scFv C65	Doxorubicin	HER2+ human breast cancer cells	277
Anti-nucleosome 2C5 mAb	Doxorubicin	Murine LLC, 4T1, C26; human BT-20, MCF-7, PC3	278,279
scFv A5 with encapsulated Endothelin	Doxorubicin	Endothelial cells HU-VEC, HDMEC	280
Anti-GD2 and anti-GD2-Fab′	Doxorubicin	Human neuroblastoma	281
Anti-idiotype mAb, S5A8 targeted to 38C13	Doxorubicin	Murine D-cell lymphoma	282
Anti-51-kDa Fab′	Doxorubicin	Mouse model of visceral leishmaniasis	283
Anti-MT1 (Fab′) (Metalloproteinase)-MMP-	Doxorubicin	Human HT1080 fibro-sarcoma	284
Anti-CD74 LL1	Doxorubicin	Raji human B lymphoma	285
C225 mAb or Fab′ against EGF receptor	Doxorubicin	Human MDA-MB-468	286
Anti-VCAM-1 targeted to Vascular cell adhesion molecule-1	--	Human endothelial cells	287
Anti epidermal growth factor receptor (EGFR)	Doxorubicin, epirubicin, or vinorelbine	EGFR-overexpressing MDA-MB-468 tumor cells	287
Peptides			
Antagonist G targeted to vasopressin	doxorubicin (DXR)	Human small cell lung cancer H69	288,289
Vasoactive intestinal peptide targeted to VIP receptors	(fluorescent cholesterol)	Rat breast cancer	290
P0-protein targeted to intracellular adhesion molecule-1	(Radioactive lipids)	Human M21 and A-375 melanoma	291
Arg-Gly-Asp peptide	Doxorubicin	Murine B16 melanoma	292
Small Molecules			
Folate vitamin targeted to folate recptor	Doxorubicin, anti-sense oligodeoxy-ronucleotides against growth factor receptor	M-109-R mouse carcinoma tumor, Chinese hamster ovary and KG-1 human acute myelogenouse leukemia cells	121,122,124
Folate vitamin targeted to folate receptor β	Doxorubicin	Murine acute myelogenouse leukemia cells	293
Affibody			
HER2-specific affibody-conjugated thermosensitive liposomes (affisomes)	Calcein/rhodamine	Breast cancer (formulation development)	96
Epidermal growth factor receptor-affibody liposomes	Mitoxanthrone	Breast cancer (cellular toxicity)	95
HER2-specific nanoparticles	Paclitaxel	Breast cancer (cellular toxicity)	117

TABLE 4

Overview of Currently Available Lipid-Based Nanoparticles

Lipid carrier	Base composition	Assembly	Clinical applications	References
Liposomes (elastic, transfersomes, magnetic), 25–200 nm, 1965 to date	Phospholipids, sphingolipids, cholesterol, PEGylated lipids	Phospholipid bilayer surrounding an aqueous core (well-characterized)	• Doxil • Ambiosome	52,294–297
SLN, 50–1000 nm, 1990s to date	Pure triglycerides, glyceride mixtures, waxes (primarily saturated fatty acid chains)	Solid lipid core, hydrophobic in nature, with a surfactant coating	• Mouse studies • Remains to be tested	201,221,229,298
NLC, 100–500 nm, 2000s to date	Pure triglycerides, glyceride mixtures, with saturated and unsaturated fatty acid chains, waxes	Second-generation SLN; blend of solid and liquid lipid phases	• Mouse studies	212,299
Monolayer membrane structures: archaeosomes, vesicles from synthetic bolalipids and micelles	Natural bolaamphiphiles from archaebacteria, synthetic bolaamphiphiles with and without cholesterol, cholesteryl hemisuccinate, PEGylated lipids, and chitosan-lipid conjugates	Monolayer membrane surrounding an aqueous core, micelles, and a variety of cylindrical and fiber structures	• May be potentially used for targeted drug delivery and imaging • Under development • Mouse studies	157,171,300