Liposomes: Biomedical Applications

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Liposomes, with their flexible physicochemical and biophysical properties, continue to be studied as an important potential a critical drug delivery system. Liposomes have overcome the challenges of conventional free drug therapy by encapsulating therapeutic agents, thereby improving *in vivo* biodistribution and reducing systemic toxicity. New imaging modalities and interpretation techniques, as well as new techniques for targetable system formulation technique, and tumor environmental information, have affected the search for a means of overcoming the difficulties of conventional liposome formulation. In this review, we briefly discuss how liposomal formulation has been applied across the biomedical field, particularly as a therapy, and the role it may play in the future, when paired with new developments in diagnosis and theranostics. The biological challenges that still remain and the translational obstacles are discussed.

Key Words: *Liposomes; Drug Delivery Systems; Precision Medicine; Neoplasms*

INTRODUCTION

The number of potential drug delivery systems that have been studied is vast, and includes carriers such as metallic, organic, inorganic, nanohybrid, and polymeric nanoparticles like micelles, dendrimers, and liposomes. Among these, liposomes, which have lipid bilayer spherical nanovesicles that resemble the structure of cell membrane, are a very promising carrier.[1-3] Since the 1964 discovery of liposomes by Prof. Bangham, numerous studies have sought to understand their possible clinical applications. Liposomes have a number of advantages, which explains why they are present in a significant percentage of clinical-stage nanotherapeutics and pharmaceutical preparations. They are biodegradable, biocompatible, non-toxic, and composed of non-immunogenic compounds. They also improved solubility of lipophilic and amphiphilic drugs, passively target immune system cells, permit sustained release for systemic and local administration, and offer improved tissue penetration.[3,4] To date, more than eighteen liposomal drugs have been approved by FDA for the treatment of cancer, infectious disease, pain management, and age-related macular degeneration.[2]

Liposomes are comprised of a lipid bilayer structure and lipid monolayer structures called micelles (Fig. 1). A liposomal bilayer is typically composed of cholesterol and phospholipids.[5] Due to their unique biphasic nature, these can be used to deliver both hydrophilic as well as lipophilic drugs. Liposomes can vary in size from very small to large (20 nm-2.5 μm). Vesicle size is a main parameter for determining the *in vivo* circulation half-life, as both the size and number of bilayers affects the amount of drug encapsulation possible in the liposomes.[6,7] Liposomes can be classified into two categories on the basis of their size and number of bilayers: multilamellar vesicles (>500 nm) referred as
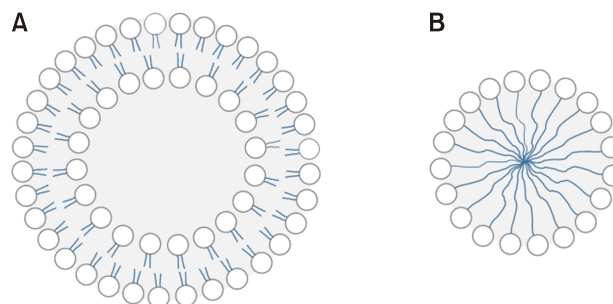

FIG. 1. Liposome (A) and micelle (B) structure.

MLV and unilamellar vesicles. Unilamellar vesicles can be further classified as large (LUV, >100 nm) and small unilamellar vesicles (SUV, <100 nm).[8] These structures are depicted in Fig. 2.

There are several methods of liposome preparation and drug loading, including passive loading techniques and an active loading technique. The most common passive loading methods are thin-film hydration, microemulsification, sonication, membrane extrusion, microfluidizer, freeze-thawing, ether injection, ethanol injection, reverse phase evaporation, dehydration-rehydration, and calcium-induced fusion.[9-11] Of these, thin-film hydration is the most widely used. In this passive loading method, a water-soluble drug is encapsulated in the aqueous phase of a liposome, while a lipid-soluble drug is loaded into the lipid layer. With active loading, drugs are loaded by creating a diffusion gradient for the ions or drugs across the external to internal phases. This method, called "remote loading", is used in liposomal formulations of doxorubicin, and offers significant improvements in the drugs' pharmacokinetics and safety profiles in humans.[12-16]

Although liposomes have been intensely investigated for 50 years, they continue to be the object of vigorous research. Liposomes have a reputation as an optimal delivery system for drugs or biologically active substances, and are regarded one of the most successful drug delivery systems identified to date. Despite their success, liposomes still face problems related to their stability, storage, drug leakage, rapid clearance, and specific targeting ability. In this review, we provide a brief overview of liposomes as they are applied in medicine.

LIPOSOME APPLICATIONS IN MEDICINE

1. Liposomes and cancer therapy

Cancer is a leading cause of death in many countries. Regrettably, the efficacy of standard treatments for a variety of cancers is not optimal.[17] Many anticancer agents are highly toxic, and a number of cytotoxic chemotherapeutics have short half-lives *in vivo* due to their highly hydrophobic nature, which leads to side effects, noncompliance, and patient inconvenience as a result of difficulties in administration. This, in turn, limits their utility in cancer therapy.[9,17,18] In cancer therapies, a liposomal delivery system is particularly useful. The incorporation of chemotherapeutic agents into liposomes can improve their specificity to cancer cells and tumor tissues through passive or ligand-mediated active targeting, resulting in a minimization of the drug's negative side-effects as well as enhanced anticancer efficacy from the increased accumulation of liposomes within the tumors.

A PEGylated liposomal formulation of doxorubicin (Doxil) was the first FDA-approved nanosized anti-cancer drug delivery system. After the approval of Doxil® in 1995, a number of other cytotoxic agent-containing liposomes were approved for clinical use, with a number of liposomal chemotherapeutic formulations having been approved by the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) for the treatment of various cancers in the past few years alone.[19-22] A summary of liposome-based anticancer drugs is provided in Table 1. Several more anticancer liposome formulations are currently in various stages of clinical trials or awaiting approval (Table 2).

Despite these successes by liposomal anticancer drugs, which have been achieved by an enhanced permeation and retention (EPR) effect, there have been many reports of conventional liposomes that have failed for their lack of selectivity. In these cases, a minimum therapeutic concentration of liposomes is delivered within tumor tissues while the drug accumulates in healthy tissue and mucosa, resulting in treatment failure.[23,24]

As an alternative, the active "ligand-mediated" targeting approach has been thoroughly investigated for its improved intracellular delivery of an encapsulated drug to a tumor tissue. A variety of molecules, including peptides, antibodies, proteins, and charged molecules, and some low molecular weight ligands, and aptamers, have been studied for use in conjunction with liposomes to enhance anticancer treatments. For further improvement, multi-functional liposomes with specific features like targeted delivery functionalities designed using a variety of surface functionalization and modification approaches, sustained and triggered release, will play a vital role in future tumor therapies.[19,25] Table 3 presents therapies using ligand-mediated targeted liposomes in the clinical trial stage.

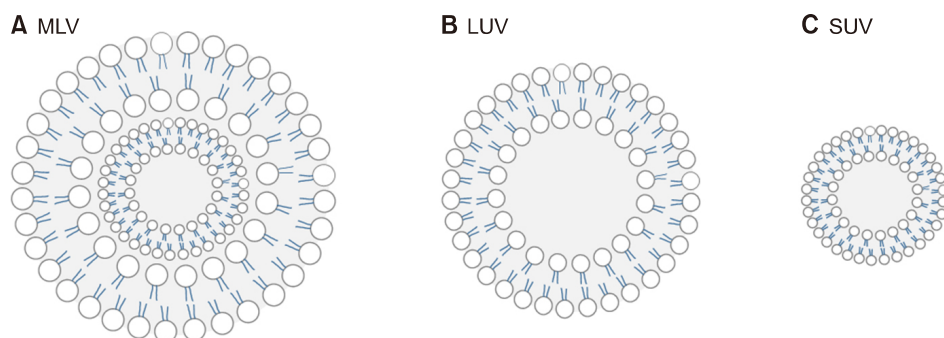**A** MLV   **B** LUV   **C** SUV

FIG. 2. Structure of MLV (A), LUV (B), and SUV (C). MLV: multilamellar vesicles, LUV: large unilamellar vesicles, SUV: small unilamellar vesicles.

TABLE 1. Clinically used liposomal formulation products for cancer therapy

Name	Composition	Active agent	Status	Indications
Doxil/Caelyx	HSPC:Chol:PEG 2000-DSPE	Doxorubicin	1995	Ovarian, breast cancer, and Kaposi's sarcoma
DaunoXome	DSPC:Chol	Daunorubicin	1996	HIV-associated Kaposi's sarcoma (primary)
Myocet	EPC:Chol	Doxorubicin for combination therapy with cyclophosphamide	2000	Metastatic breast cancer
Marqibo	SM:Chol	Vincristine	2012	Acute lymphoblastic leukemia
Lipo-dox	DSPC:Chol:PEG 2000-DSPE	Doxorubicin	2012	Breast and ovarian cancer
Lipusu	PC:Chol	Paclitaxel	2013	Gastric, ovarian and lung cancer
Onivyde	DSPC:MPEG-2000:DSPE	Irinotecan for combination therapy with fluorouracil and leucovorin	2015	Metastatic adenocarcinoma of the pancreas
CPX-351 (Vyxeos[TM])	DSPC:DSPG:Chol	Daunorubicin+cytarabine	2017	Acute myeloid leukemia

TABLE 2. Liposomes in ongoing clinical trials for cancer therapy

Name	Composition	Active agent	Indications
PROMITIL	HSPC:Chol:PEG 2000-DSPE	Mitomycin-C Mitomycin-C lapidated prodrug (MLP)	Solid tumors
ThermoDox	DPPC:MSPC:PEG 2000-DSPE	Doxorubicin Heat-sensitive liposome+RFA	Hepatocellular carcinoma and also recurring chest wall breast cancer
Oncoprex	DOTAP:Chol	TUSC2 plasmid DNA FUS1 (TUSC2)	Non-small cell lung cancer
E7389-LF	HSPC:Chol:PEG 2000-DSPE	Eribulin mesylate (E7389)	Solid tumors
LEM-ETU	DOPC:Chol:cardiopin	Mitoxantrone	Lymphoma and breast cancer
Lipocurc	DMPC:DMPG	Curcumin	Solid tumors
TLD-1	Composition is not available publicly	Doxorubicin	Solid tumors
EndoTAG	DOTAP:DOPC	Paclitaxel	Breast cancer
PTX–LDE	Cholesteryl oleate:Egg-PC:miglyol 812:Chol	Paclitaxel	Epithelial ovarian carcinoma

RFA: Radiofrequency ablation.

TABLE 3. Examples of ligand-targeted liposome formulations undergoing clinical evaluation

Name	Ligand	Bioactive compound	Indications
SGT-53	Anti-transferrin receptor scFv	p53 gene	Solid tumors
Anti-EGFR-IL-DOX	Antibody fragment (Fab') of cetuximab	Doxorubicin	Solid tumors, breast cancer
C225-ILs-dox	Anti-EGFR Fab fragment from mAb C225 (cetuximab)	Doxorubicin	Glioblastoma
Lipovaxin-MM	Single domain antibody (dAb) fragment (VH)	Melanoma antigens+IFNγ	Melanoma vaccine
MBP-426	Transferrin	Oxaliplatin	Gastroesophageal adenocarcinoma
MM-302	Antibody fragment (scFv)	Doxorubicin	Breast cancer
SGT-94	Antibody fragment (scFv)	RB94 plasmid DNA	Solid tumor

2. Liposomes and infectious and vascular diseases

1) Liposomes and fungal infections: Fungal infections are categorized as either superficial or invasive. Invasive fungal infections, while less common, have a higher morbidity and mortality rate, especially in immunocompromised patients.[26,27] Amphotericin B, with a broad antifungal spectrum and minimal risk of resistance, has been considered the gold standard for the treatment of severe systemic fungal infections.[28] Unfortunately, amphotericin B is associated with acute and chronic as well as dose-dependent toxicity. To solve this, amphotericin B encapsulated liposomes were introduced, which reduced its toxicity and allowed for its administration at higher doses.[29]

Three major classes of antifungal drugs exist: (a) polyenes, (b) azoles, and (c) echinocandins.[30] Amphotericin B is a poorly water-soluble polyene produced by Streptomyces nodosus.[31] Encapsulating amphotericin B in a liposome has been useful for overcoming the drug's disadvantages. Research in the 1980s-1990s ultimately led to the development and clinical introduction of three lipid formulations of amphotericin B: (a) liposomal amphotericin B (LAmB; AmBisome), (b) amphotericin B lipid complex (ABLC; Abelcet), and (c) amphotericin B colloidal dispersion (ABCD; Amphotec).[32] AmBisome was developed in the 1980s and showed 30-fold reduction in toxicity while retaining the pharmacological effectivity of the active ingredient in pre-

TABLE 4. Clinically used liposomal formulation products for fungal infection

Name	Composition	Active agent	Status	Indications
Abelcet or ABLC	DMPC:DMPG	Amphotericin B	1995	Invasive severe fungal infections
Amphotec	Cholesteryl sulphate	Amphotericin B	1996	Severe fungal infections
AmBisome or L-AmB	HSPC:DSPG:Chol	Amphotericin B	1997	Presumed fungal infections

TABLE 5. Examples of liposome formulations undergoing clinical evaluation

Name	Active agent	Composition	Indication and approval (year)	Administration route
Arikayce	Amikacin	DPPC:Chol	Lung infections, 2018	Inhalation
ARD-3100, 3150	Ciprofloxacin	Egg-PC:Chol	Bacterial infections	Inhalation

TABLE 6. Clinically used liposomal vaccine products for infection

Name	Applications	Composition	Character	Approval (year)
Epaxal	Hepatitis A	DOPC:DOPE	Inactivated hepatitis A virus (strain RGSB)	1993
Inflexal	Influenza	DOPC:DOPE	Inactivated hemagglutinin of influenza virus strains A and B	1997
Mosquirix	Malaria	AS101=suspension of MPL+QS21, adjuvant	RTS, S/AS101	2015

MPL: 3'-o-desacyl-4'-monophosphoryl lipid A, QS21: Quillaja saponaria 21.

clinical models.[33] Table 4 presents liposomal formulation products currently in clinical use to treat fungal infections.

The current generation of commercially available AmB lipid formulations are expensive and require parenteral administration, resulting in longer hospital stays and increased healthcare costs. There is an urgent need to develop an orally available AmB formulation that decreases the systemic toxicity of the drug, avoids infusion-related adverse events, improves patient compliance, and reduces the costs associated with current commercial AmB formulations.[29,34]

2) Liposomes and bacterial infections: Among the most serious problems in medicine today is the increase in drug-resistant bacterial pathogens, against which conventional therapies have only limited effectiveness.[35] Biofilms are clusters of microorganisms and are a common cause of chronic, nosocomial, and hospital acquired infections like implantable medical devise. Biofilms are associated with high-level resistance to antimicrobials, frequent treatment failure, and increased morbidity and mortality. Both Staphylococcus aureus and Pseudomonas aeruginosa are known as major pathogens. Once a biofilm is formed, bacteria become up to 1000x more resistant to antibiotic treatment than their planktonic type.[36] Localized delivery of antimicrobials is a promising tactic to treat challenging infections like biofilms and intracellular infections such as Salmonellosis.[37-39] Many research results have shown that liposome encapsulation improves the efficacy of antibacterial drugs against a broad range of pathogens both *in vitro* and *in vivo*.

Liposome-encapsulated amikacin, marketed under the name Arikace, is clinically approved for the treatment of Mycobacterium avium complex lung disease.[40] Arikace is a novel formulation of inhaled liposomal amikacin for the treatment of patients suffering from chronic Pseudomonas aeruginosa biofilms.[41] Table 5 presents liposomal formulation products for bacterial infections in use or in clinical trials.

3) Liposomes and vaccine formulation: Vaccination is the most cost effective prophylactic strategy against many types of diseases like as pathogenic infections (viral, bacterial, fungal or parasitic origin), cancerous lesions, and even rheumatoid arthritis.[42] Gregoriadis and Allison were the first to report on the ability of liposomes to induce immune responses to incorporate as vaccine adjuvants or associated antigens.[43] Since then, liposomes (natural, anionic, and cationic lipid), archaeosomes (polar glycerolipid plus lipid), and virosomes (viral extract and lipid) have become important vaccine systems, and interest in liposome-based vaccines has markedly increased.

Epaxal, Inflexal, and Mosquirix are now clinically approved liposome-based vaccine products.[42,44] Table 6 presents details associated with these three approved drugs. All three are classified as virosomes, which are liposomes comprised of a phosphatidylcholine membrane vesicle that incorporates a virus-derived protein. All three vaccines are safe, well-tolerated, and effective at generating an immune response. Advantages of particulate vaccines are greater protection of antigens from enzymatic degradation by encapsulation and also can deliver molecular adjuvants with antigen to antigen presenting cells (APC), thus promoting cellular and humoral immune responses.[45]

Traditionally, vaccines have relied on the use of whole killed or live attenuated pathogens. Today, research is focused on the development of subunit vaccines that are better defined, easier to produce, and safer. Liposomes are promising delivery systems for subunit vaccines composed of fragment of a pathogen which can trigger immune response.[43] 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) based cationic liposomes have been extensively studied as potential vaccine delivery systems and adjuvants. Recently, combining liposomes with immuno-stimulating ligands has been studied as a potential next-step in the development of novel adjuvant systems.

3. Liposomes and angiogenesis in vascular disease

Vascular disease including atherosclerosis, thrombosis, stroke, coronary artery disease (CAD), and peripheral artery disease (PAD) is a leading cause of death and disability worldwide.[46] Inflammation plays a main role in the initiation and progression of both PAD and CAD. CAD and PAD are common vascular diseases which are characterized by reduced blood supply caused by a buildup of plaque in the peripheral or coronary arteries, leading to an ischemic state, i.e., a deficient nutrient and oxygen supply to the head, organs and limbs.[47] One potential approach to overcoming this disease is the promotion of angiogenesis in ischemic tissues via the delivery of angiogenic factors. Angiogenic factors promote the generation of new blood vessels and increase blood flow in damaged tissue in the ischemic state.[48] Successful angiogenesis is highly dependent on delivery of vascular endothelial growth factor (VEGF) to an ischemic site.

Clinical trials of VEGF growth factor in patients failed to obtain a significant therapeutic effect and this was due to the short *in vivo* half-life and lack of specificity to the target site.[49] Therefore, targeted delivery and then sustained release of growth factors in ischemic sites is a grand challenge. Inspired by the targeted nanocarriers in cancer therapy, various types of nanopaticles have been studied as potential therapies for ischemic lesions, including graphene oxide,[49] silica nanoparticles,[50] polyglutamic acid (PGA) polypeptides,[51] ONO-1301 containing lipid nanoparticles,[52] polylactic-co-glycolic acid (PLGA) nanoparticles,[53] and ironoxide nanoparticles.[54]

Nam et al.[55] injected VEGF protein-loaded Alexa Fluor 750-labeled liposome into the ischemic hind limb mouse model. The liposomes were delivered intravenously to target ischemic sites and achieved VEGF-influenced angiogenesis at ischemic sites. In our lab, when PEGylated liposomes loaded with angiogenic peptide from the VEGF sequence origin was used in a cerebral ischemia rat model, with vascular density increasing in response to angiogenic therapy.[56] Furthermore, ^{99m}Tc-radiolabeled PEGylated liposomes loaded with angiogenic peptides as a theranostic agent were administered to hind limb ischemia in an animal model. The liposomal agent improved ischemic limb perfusion and promoted angiogenic responses.[57] This agent was also applied to aischemia/reperfusion heart in-

jury in an animal model. This liposome accumulated in the ischemic myocardium and induced its own protective effect against ischemia/reperfusion injury.[58]

4. Liposomes, theranostic applications, and diagnostic techniques

Theranostics is the combination of the terms "Therapeutics" and "Diagnostics," and refers to technologies that include both diagnostic and therapeutic functions in one complex. Theranostics have attracted a great deal of attention and theranostic nano-carrier systems are considered to be a critical component of the next generation of medicine. Well-made theranostic nanoparticles allow for the monitoring of real-time drug delivery, accurate diagnosis and assessment of biological signals, easier determination of responses to a therapy, an increase in the use of minimally invasive procedures that rely on precision guidance, and better decisions concerning the end point of therapy, all of which promote the development of individualized medicine.[59,60]

The development of new imaging modalities has led to the design of various diagnostic and theranostic nano-delivery systems. Today, imaging techniques including optical fluorescence imaging, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET) and single photon emission computed tomography (SPECT) are in wide use. Nuclear imaging is high sensitive, capable of imaging an entire body, without risk of issues associated with tissue penetration, and highly accurate. High sensitivity is particularly important in therapeutic nanomedicines. Unlike MRI and CT scans where gram quantity of a contrast medium is required, nuclear imaging is achieved with injections of micrograms or less.[61] Nuclear imaging modalities are limited in that their spatial resolution in the range of below 10 mm, which is lower than optical or MR imaging. The majority of radionuclide imaging is performed by SPECT or PET.[62,63]

Flexibility of liposomes in surface functionalization provide opportunities for labeling with various imaging probes which can co-deliver therapeutic drugs that allow them to act as both therapeutic and imaging agents (multifunctional system). In the initial stage, liposome systems mainly relied on the EPR effect, known as passive targeting. FDA-approved liposomes were non-targeted liposomes which reduced side effects and improved patient tolerability over conventional anti-cancer drugs, but largely failed to improve therapeutic outcomes and patient survival rates. These outcomes are considered due to the heterogeneity in EPR conditions individually. To improve the effectiveness of therapies that rely on the EPR effects, upgraded formulations and protocols will be needed. Image-based or assisted nanomedicine is considered to be crucial for addressing the EPR's heterogeneities, ligand expression, or tumor environments. Clinically available technology, such as CTs and MRIs, might be used to image the EPR effect in patients and provide clinicians with valuable information (e.g., vessel density, vessel permeability, fluid

pressure) for medication regimens and treatment planning. Imaging the EPR variation across patients may also provide a means of identifying patients likely to respond best to liposomal therapy.

Unfortunately, EPR imaging is currently only in the developmental stage. Until now, only a few studies have reported or clinically analyzed the EPR effect in patients. Børresen et al.[64] recently investigated the correlation between the degree of the EPR effect (^{64}Cu-liposome) and tumor neoangiogenesis (^{68}Ga-RGD), fluid pressure, glycolytic activity (^{18}F-FDG) and diffusivity (diffusion-weighted MRI) to identify potential biomarkers suitable for prediction of the EPR effect in cancer models. The researchers determined that ^{64}Cu-liposome and ^{68}Ga-RGD uptake were moderately correlated, and the authors ultimately concluded only that ^{68}Ga-RGD does not qualify as a surrogate marker, and that ^{18}F-FDG (metabolic activity) and ^{64}Cu-liposome uptake were not correlated. Lee et al.[65] introduced ^{64}Cu-MM-DX-929 (untargeted, no-drug PEGylated liposome) as a universal companion diagnostic agent to prospectively select patients for liposomal therapeutics. ^{64}Cu-MM-DX-929 was injected into an animal cancer model, then tumor deposition and intra-tumoral distributions were evaluated. The mouse received a median tumor deposition score and a liposomal drug or free drug (MM-302, PLD, Liposomal irinotecan, and doxorubicin) and was treated to identify an improved treatment response. These researchers ultimately recommended a quantitative PET for longitudinal imaging as providing the best estimation of liposomal drug delivery in tumors.

Moving beyond EPR effect-based strategies, active targeting is one means of delivering drugs or theranostics to a lesion site while avoiding normal tissue. Active targeting strategies include targeting a tumor cell surface receptor or targeting a tumor micro-environment, and stimuli-response strategies that rely on changes in pH, temperature, redox, enzyme, light, and ultrasound to trigger drug release. Releasing the encapsulated drug in the target area is particularly difficult as a result of a limit in the liposome system. The aforementioned stimuli-response strategies were designed to overcome this issue.

Antibodies and their fragments are among the most studied targeting agents in preclinical and clinical trials.

Antibody conjugated liposomes, called immunoliposomes, will doubtlessly play a pivotal role in precision cancer diagnosis and treatment in the future. Current clinical trials of immunoliposomes are shown in Table 3. Among these is MM-302, a dox-loaded immunoliposome (ILs-Dox) that targets the human epithermal growth factor receptor-2 (HER-2) currently in Phase I clinical trials to determine the maximum tolerable dose. In this study, Lee et al.[66] administered ^{64}Cu-labeled MM-302 and employed hybrid PET/CT to visualize tumor accumulation and therapeutic response in HER2-positive metastatic breast cancer patients. High accumulated activity tumor lesions showed favorable treatment efficacy and were well correlated with preclinical data.

A deeper knowledge of the molecular events associated with cancer, liposome formulation techniques, and image interpretation techniques will facilitate the development of future theranostic systems.

5. Future research directions

Today, liposomal vesicles are among the most effective delivery options for various classes of drugs designed to treat everything from cancer to pain. Table 7 lists FDA-approved liposomal drugs that were not discussed in this paper. While this list in long, serious problems remain for conventional liposomes as they are used in biomedical applications, including the storage stability problems caused by lipid oxidation and hydrolysis, drug leakage from the vesicle, rapid clearance during blood circulation, and the absence of cancer cell-specific targeting.[51] To survive on the market, pharmaceutical drugs must maintain their properties during storage, a problem that has attracted considerable attention by researchers. Liposomes in an aqueous state can be degraded by oxidation and hydrolysis, which can cause structural changes such as liposome sedimentation, aggregation, or fusion. To remediate these problems, researchers have tried freeze-drying, adding surfactants or antioxidants, modifying with chitosan, and changing their composition to improve rigidity.[67] Despite these efforts, the challenge of ensuring stability remains unsolved. Stability can be influenced by pH, size, surface charge, lipid composition, and temperature, and these parameters also impact the drug encapsulation efficiency and

TABLE 7. Clinically used various liposomal drugs

Name	Active agent	Composition	Form	Indications	Approval (year)
Visudyne	Verteporphin	EPG:DMPC	Conventional	Age-related macular degeneration	2000
DepoDur	Morphine sulfate	Chol:triolein:DOPC:DPPG	Depofoam	Pain management	2004
DepoCyt	Cytarabine	Chol:triolein:DOPC:DPPG	Depofoam	Lymphomatous meningitis	2007
Exparel	Bupivacaine	Chol:DPPG:tricaprylin:DEPC	Depofoam	Pain management (anesthesia)	2011
Onpattro (Patisiran)	RNAi for the knockdown of disease-causing TTR protein	Chol:DSPC:Dlin-MC3-DMA: PEG2000-C-DMG	Conventional	Polyneuropathy caused by hATTR amyloidosis	2018

the half-life of blood circulation *in vivo*.

To improve therapeutic efficacy, researches have concentrated on developing multi-functional liposomes, capable of long circulation, increase accumulation at the target site, and increased cellular internalization. Various strategies have been adopted to achieve this, including strategies that rely on passive or active targeting. In passive targeting through EPR, several factors, including lipid composition, charge, size, individual heterogeneity of vascular condition, can influence. In order to improve EPR mediated therapy, gaining EPR relevant biomarkers through physical modalities is useful.

Active targeting strategies include targeting a tumor cell's surface receptors, targeting a tumor's micro-environment, and stimuli-response strategies including pH-, temperature-, redox-, enzyme-, light-, and ultrasound-triggered drug release.[25] At this time, ThermoDox is the only thermosensitive liposomal formulation (encapsulated doxorubicin), and it is currently in phase 3 of its clinical trials,[68] in which it has been provided to patients with hepatocellular carcinoma by radiofrequency ablation. Coupling targeting ligands to surface features such as proteins, low molecular weight ligands including peptides, carbohydrates, or monoclonal antibodies or their fragments has been studied, and VEGF, vascular cell adhesion molecule (VCAM), matrix metalloproteinases (MMP), and integrin have been evaluated as tumor environmental targets.[25] Targeted therapies, however, rely on ligands presented by only a few types of tumors and have not yet overcome the problem of the heterogeneity of tumor cells and their surface markers. One possible direction for future research may be ligands coupling of different natures for more sensitive malignant lesion detection.

In addition to these developments, we will likely see increased focus by researchers on the ability to visually track both the process of a drug as it is injected the process of treatment. A focus on quantification, which is achievable through a variety of imaging instruments, will help practitioners offer precision or personalized treatments.

CONCLUSION

This paper has briefly summarized how and where liposomes are currently applied in the biomedical sciences. Liposomal drug formulations offer a means of overcoming the limitations of conventional therapies and have a wide variety of therapeutic applications, ranging from cancer to pain management. While many obstacles remain to the realization of their full potential, growing interest in the development of liposomal-based drug formulations may spur the development of the next generation of liposomes as drug carriers, and result in significant improvements to the quality of life of patients.

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CONFLICT OF INTEREST STATEMENT

None declared.

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