Liposomes for Advanced Drug Delivery

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Abstract Liposomes are sphere-shaped vesicles consisting of one or more phospholipid bilayers. The liposomal drug delivery systems were utilized for delivery of compounds for different diseases. These systems improve the stability as well as cellular uptake of drugs. Site-specific delivery to the target site reduced the site effects. This chapter summarizes the recent advances in liposomal drug delivery systems (i) therapeutic applications-based chemotherapy; (ii) chemotherapy in combination to gene therapy and immunotherapy; (iii) theranostic applications for precise detection and simultaneous treatment of critical diseases and heavy metal toxicity; (iv) stimuli-triggered liposomes. This chapter gives a detailed account on aforementioned applications which might be beneficial to pharmaceutical scientists and industries to develop safe and effective liposomal systems.

Keywords Liposomes · Delivery system · Gene therapy · Theranostic · Stimuli

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1 Introduction

Liposomes are lipoidal carriers constituted of an aqueous core which is surrounded by lipid bilayers. Numerous therapeutic applications of liposomes have been manifested in clinical practices. They range from diagnostic and therapeutic applications to recently employed theranostic applications [1]. The first ever clinical applications of liposomes were the delivery of chemotherapeutic moieties to the diseased sites. Conventional techniques employed for the liposomal formulation lead to inadequate drug delivery to the target sites and lead to side effects thereby obtruding few limitations on the drug dose and frequency. To surmount these hurdles, attempts have been made to develop safe and effective liposomes. Subsequent to their approval as carriers for small molecule therapeutics (i.e., chemotherapeutic drugs), they were inquired with respect to their capability to administer macromolecules like nucleic-acid-based molecules (plasmid DNA, antisense oligonucleotides, and small interfering RNA) to diseased organs. These macromolecules are hydrophilic, high-molecular weight, highly charged molecules which do not have the ability to cross the cell membranes via passive diffusion. Besides, degradation by the enzymes and systemic clearance, non-specificity for the diseased tissues, and inadequate cellular uptake substantially restrict the clinical applicability. Due to these restrictions, it has always been a challenge to deliver nucleic-acid-based agents using liposomes as carrier system. Cationic lipids like 1,2-bis(oleoyloxy)-3-(trimethylammonio) propane (DOTAP) and 3β[N′,N′-dimethylamino-ethane]-carbomoyl] cholesterol (DC-CHOL) have been used for the development of cationic liposomes. The interaction between these cationic liposomes and anionic nucleic-acid-based agents leads to the formation of "lipoplex." These lipoplexes fuse with the plasma membrane, thereby entering the cell and release the nucleic acids from endosomes followed by internalization [2]. Liposomes have evidenced effective immunological adjuvants for protein and peptide antigens [3]. Both humoral and cellular responses are evoked by them for many diseases including cancers. Surface of liposomes can be functionalized by anchoring ligands or antibodies with an aim to attain specific delivery to the diseased site. Likewise, chemical groups could be anchored to the liposomal surface which will be responsive to different stimuli. On the basis of their physiological properties, these smart liposomes could lead to trigger the drug release. The stimuli are of two types: (a) Internal stimuli, e.g., enzymatic activity, pH alterations, or presence of reductants and (b) external stimuli, ultrasound, light, alterations in temperature, or presence of magnetic field. The drug release from liposomes which is triggered by external stimuli renders an enhanced accuracy pertaining to the site of release and hence a better regulation on the drug dose and its delivery [4, 5]. The development of pH-sensitive liposomes endues liposomes with added benefits in comparison with the conventional adjuvants by allowing the evasion of the peptide antigen from endosomes into the cytoplasm and therefore permits the linkage of antigen with MHC-I complex (i.e., major histocompatibility complex), that hastens a cytotoxic T-lymphocyte response. Besides encapsulation, direct modification of liposomes with

an antigen can evoke an immunologic activity. The capability of liposomes to encapsulate a broad range of diagnostic and therapeutic materials has grabbed the attention of researchers in employing them as nano-delivery systems of theranostic applicability. Diagnostic and therapeutic compounds exert a major function in the early detection and treatment of diseases like cancer, diabetes, Parkinson's, and gastrointestinal disorders. This novel strategy integrates both agents (i.e., diagnostic and therapeutic) into one system with an aim to concurrently detect and treat a disease. To attain these objectives, stable and effective theranostic systems are necessitated to be formulated with targetability and free from any encumbrance between the therapeutic and imaging compounds which are used in the developed system. Amidst the various types of nanosystems inquired till date, liposomes persist as one of the most potential carriers because of their high carrying capability and the good encapsulation abilities to entrap both diagnostic and therapeutic agents for clinical utilities [6, 7]. The application potential of liposomes is summarized in Fig. 1.

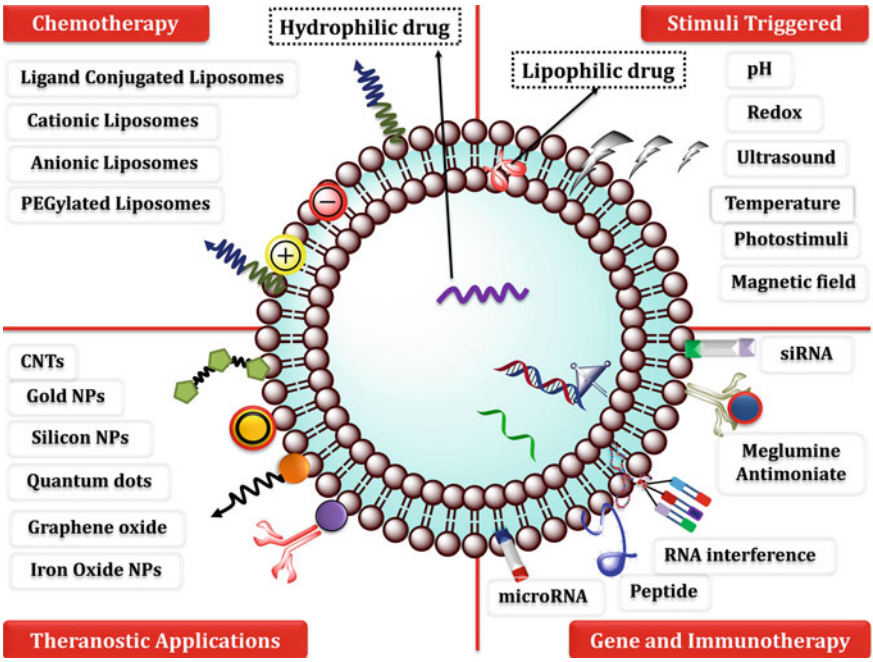

Fig. 1 Application potential of liposomes

2 Applications of Liposomes in Chemotherapy

2.1 In Cancer

Cancer is a deadly disease caused by an uncontrolled cell division and loss of cell growth; these abnormal cells are termed as tumor cells. Cancer can develop in almost every site of the body and affects the normal functions of the body. Chemotherapy is one of the conventional approaches for the treatment of cancer. It helps in improving survival of cancer patients, but there are number of adverse effects noticed in conventional therapeutic approaches. Moreover, cancer patients also face mental and physical disturbances during or after the course of chemotherapy [8]. Chemotherapy leads to adverse effects such as destroying of rapidly dividing normal cells, i.e., bone marrow cells, hair follicle cells, as well as cell linings of the gastrointestinal tract. It can also cause fatigue, nausea, constipation, diarrhea, mouth sores, decreased appetite, and skin and nail problems [9]. To overcome these problems, the chemotherapy requires the selection of suitable chemotherapeutic agents, understanding of tumor patient characteristics and treatment cycles [10]. Chemotherapy is becoming advance, specifically using target drug delivery systems which destroy the tumor cells selectively without affecting the normal cells. This revolution in cancer treatment offers improved efficacy and tolerability for better outcomes [11, 12].

2.1.1 Colon Cancer

Liposomes of 5 Fluorouracil (5FU) were prepared, where folic acid (FA) was used as targeting ligand for colorectal cancer [13]. In vitro cytotoxicity and in vivo tumor inhibition studies were performed to evaluate the 5FU loaded liposomes. The outcomes from these studies showed that collapsing membrane potential increases the cytochrome c activity as well as caspases activity. The molecular-targeted therapy (MTT) studies were performed which exhibited higher cytotoxicity activity as compared to the free drug with liposomal formulation [14]. The developed FA conjugated liposomes were observed to trigger necrosis in HT-29 cells, but in case of HeLa cells, FA-liposomes stimulated the apoptotic pathway by collapse of membrane potential. The in vivo results exhibited that targeted liposomes decreased the tumor volume more efficiently as compared to free drug therapy. From these results, it can be concluded that folic-acid-targeted liposomes is a potential drug delivery system for the treatment of colorectal cancer [15, 16]. The Eudragit S-100 encapsulated chitosan-coated liposomes containing prednisolone were formulated for targeting colon cancer. The liposomes were prepared by lipid film hydration technique using soya phosphatidylcholine (PC) and cholesterol in optimum ratio. The coated and uncoated liposomes were evaluated for in-vitro, ex vivo, and in vivo studies. The in vitro drug release study was carried out using the pH gradient technique. The ex vivo study was performed using excised tissues from male albino rats. In vivo

characterization was done for the comparative study of histopathology and myeloperoxidase (MPO) activity. The ex vivo studies displayed higher tissue-drug entrapment in cancer cells as compared to the normal cells of the colon. The in vivo histopathological studies exhibited a remarkable reduction in colonic inflammation using Eudragit-encapsulated chitosan-coated liposomes (ECLs) in rats. The reduction in healing process was further confirmed using MPO assay in ECLs treated groups. Further, a site-specific release was noticed along with a higher accumulation of drug-encapsulated formulations in colon cancer tissues [17].

2.1.2 Breast Cancer

Matrix metalloproteinases (MMPs) is a potential target for breast cancer. The liposomal system was prepared by conjugating a MMP inhibitor, epigallocatechin gallate (EGCG) and paclitaxel (PTX). In this system, PTX exhibited higher entrapment as compared to EGCG. The in vitro efficacy was assessed by inducing the apoptosis process and reduced cell invasion. The cytotoxicity and caspase 3 activity express the apoptosis process. The MMP-2 and 9 invasion assays revealed cell invasion. The co-loaded liposomal formulation showed better results than the free drug. However, this synergistic outcome of co-loaded liposomes of PTX/EGCG combination was a potential carrier for the treatment of breast cancer [18]. The pH-sensitive folate-coated DOX-loaded liposomes (SpHL-DOX-Fol) were formulated for delivery of doxorubicin (DOX) to breast cancer. The formulation assessed for antitumor activity using both in vitro and in vivo *studies* in a 4T1 breast cancer model system. A higher tumor uptake was showed using radiolabelled SpHL-Fol (99mTc-SpHL-Fol) as compared to the non-folate-coated liposomes (99mTc-SpHL). The antitumor activity of formulations arrests the cellular growth and reduces pulmonary metastasis. Thus, pH-sensitive liposomal system can be considered as a novel drug delivery system to increase the DOX tumor delivery as well as reduce the dose-limiting toxicity [19].

2.1.3 Prostate Cancer (PCa)

The mitomycin C lipophilic prodrug (MLP)-based product Promitil® was explored in clinical trials. The folate-conjugated liposomes was prepared using doxorubicin, and MLP and their antitumor potential were investigated in PSMA-expressing human prostate cancer cell line (LNCaP). It has been revealed that the folate-targeted liposomes displayed more interaction with PSMA over-expressing cells as compared to simple liposomes. The folate-modified liposomes enhanced the cytotoxicity in PCa [20]. It has been investigated that combination of two drugs/agents is beneficial as compared to single drug/agent for chemotherapy. The combination of paclitaxel and imatinib increased the cytotoxic and antiangiogenic potential synergistically. Both drugs were loaded into folate-targeted liposomes, and anticancer activity was determined using the PC-3 cells. The viability of PC-3 cells and VEGF gene expression was found to decrease as compared to the non-targeted liposomes and free paclitaxel [21, 22].

2.1.4 Brain Cancer

A dual-functionalized liposomal system was prepared for the efficient transport across BBB for targeting of brain cancer [23, 24]. The surface of liposomes was modified with transferrin (Tf), which used as receptor for targeting. Translocation of doxorubicin (Dox) and erlotinib (Erlo) was improved into glioblastoma cells of brain using cell-penetrating peptide PFVYLI. The liposomes were evaluated for in-vitro cytotoxicity and haemolytic studies. The cellular uptake studies assessed effective internalization of drug in U87 brain endothelial and glial cells of brain. The dual-functionalized liposomes displayed higher apoptosis in U87 cells of brain [25]. The paclitaxel (PTX)-loaded liposomal system were developed. The liposomes were modified with microenvironment acid-cleavable folic acid (FA) and cell penetration peptide dNP2 for the delivery in glioma cells. The in vitro BBB model significantly increases transmission across BBB by the modification of peptide in liposomal system. The acid-cleavable folate-conjugated liposomes showed a pH-sensitive cleavage of FA at pH 6.8. It leads to an improvement of cellular uptake by the glioma cells of brain. The liposomal system enhanced the antitumor effect and improved accumulation of drug in glioma cells in mice as compared to free drug [26].

2.1.5 Lung Cancer

A novel co-delivery system (L-PTX-PSur) of paclitaxel (PTX) and survivin siRNA (Sur) was developed which specifically delivered the drug to lung cancer cells. Protamine was selected to condense siRNA into the “core” of the delivery system. Furthermore, carbamate-linked cationic lipid was entrapped into the core of drug delivery system. The liposomes with this protamine facilitated the entry of Sur into the NCI-H460 cells and displayed a better encapsulation efficiency. The in vitro studies on the NCI-H460 lung cancer cells exhibited that L-PTX-P Sur has more advantages over the control groups. It demonstrated highest cellular uptake, lowest cell viability, and apoptosis. The expression of surviving protein was reduced substantially by the liposomal formulations in NCI-H460 cells using western blot. The down-regulation of survivin protein could lower the growth of cancer cells and provide PTX more effective with low doses [27]. The docetaxel (DTX) liposome system was prepared by surface modification with CD133 aptamers and intended to target lung cancer. The liposomes were prepared by the thin-film hydration method. The in-vitro study displayed a slower drug release profile. In cytotoxicity study, CD133 aptamers-modified DTX LP significantly reduced the cell proliferation and increased the therapeutic efficiency. The in vivo antitumor activity indicated that the CD133-DTX LP exhibits a higher antitumor activity in A549 tumor mice and reduces the systemic toxicity [28].

2.2 Applications in Other Diseases

2.2.1 Tuberculosis

Liposomes are potential vehicles for the delivery of anti-tuberculosis drugs. The pH-dependent liposomes of isoniazid from isonicotinic acid (4-hydroxybenzylidene) hydrazide were developed. The liposomes were prepared by thin-film hydration method. The in vitro release studies of drug from liposomes were assessed in media of different pH using a dialysis method. It can be concluded that pH-dependent release characteristics of liposomal carrier was used to minimize the leakage of drug from liposomes which might be a potential target drug delivery in tuberculosis [29].

2.2.2 Antifungal

The itraconazole (ITZ)-loaded deformable liposomes (DL) were developed using hydroxypropyl-β-cyclodexterin (HPβCD) (DL-CD) to enhance antifungal activity. These liposomes were reported as realistic vesicles for the delivery of drug into the different skin layers. The liposomal carrier was exhibited higher concentration of ITZ in stratum corneum as well as deeper skin layers as compared to conventional liposomes. It can be concluded that deformable liposomal system in the presence of HPβCD was emerging carrier for effective cutaneous delivery of ITZ for antifungal action [30].

3 Chemotherapy in Combination to Gene Therapy and Immunotherapy

Gene therapy is widely used as an innovative treatment strategy in many diseases including the deadly disease of cancer to prevent the overall deaths. It introduces new genes into a cancer cell and thus reduces the cancer growths or kills the cancer cells. In immunotherapy, genetically modified cells are used along with the viral particles to stimulate the immune system and target the cancer cells. Immunotherapy has been employed to prevent metastatic growth of cancer by improving antigen-specific immune responses. Combination therapy of chemotherapeutic drugs and/or other biomolecules signifies a promising approach that may progress the anticancer effects by synergistic activities. It helps not only in the treatment of cancer but also in many other diseases [31–34]. Sun et al. demonstrated that the combination therapy of anticancer agent and siRNA improves the anticancer effects synergistically in hepatocellular carcinoma (HCC). They developed PEI-modified liposomal system by thin-film hydration method and co-delivery of both sorafenib (SF) and siRNA to target antiapoptotic gene, i.e., GPC3 gene (siGPC3) and cyclin D1 gene, respectively, in HCC

[35]. Another study investigated the pH-sensitive carboxymethyl chitosan-modified liposomes (CMCS-SiSf-CL) assembled with sorafenib (Sf) and Cy3-siRNA. The results demonstrated the co-delivery and penetration into two-dimensional cultured HepG2 cells, three-dimensional cultured HepG2 tumor spheroids and tumor regions of H22 tumor-bearing mice. These liposomes displayed higher vascular endothelial growth factor down regulating effect and trigger apoptosis. Therefore, the CMCS-SiSf-CL system may be a novel co-delivery system and offer an emerging platform for HCC therapy [36]. Zuo et al. prepared novel liposomes which delivered the combination of 7-O-geranylquercetin (GQ) and survivin siRNA or interleukin-10 siRNA (siIL-10) and enhanced the anti-proliferation and pro-apoptosis effects in MCF-7 cells. Further, it decreased the level of survivin and increased the level of caspase-7. This combination gene therapy not only inhibited cancer growth but also down-regulated the expression of survivin and up-regulated the expression of caspase-7 in cancer cells. Moreover, the combination of GQ and siIL-10 slowed down the cancer growth, reduced the level of IL-10, and elevated the level of TNF-α. These results displayed a fruitful effect of the combination therapy to enhance the pro-apoptosis action for the treatment of breast cancer [37]. A topoisomerase inhibitor, i.e., SN38 (prodrug), was combined with a survivin siRNA and co-delivered by transferring-targeted liposomes (Tf)-L-SN38/P/siRNA. It was conjugated with the help of a cell penetrating peptide TAT through a polyethylene glycol (PEG) linker to prepare TAT-PEG-SN38. This prepared TAT-PEG-SN38 was amphiphilic in nature and enhanced the cellular uptake of the liposomes. Moreover, protamine was comprised in the core of the liposomal system to form an electrostatic complex with siRNA. This liposomal combination system was evaluated as a promising therapeutic approach for cancer targeting [38]. Yan et al., demonstrated that the DESI2 (recombinant plasmid/pro-apoptotic gene) and endostatin (antiangiogenic inhibitor) was encapsulated with cholesterol cationic liposomes, and this combined gene therapy more significantly inhibited the cancer growth as compared to the mono therapy. It improved the anticancer activity by inducing apoptosis, inhibiting angiogenesis, and act as a DNA lesions accumulator [39]. A cationic liposomal co-delivery of XY-4 (Aurora-A kinase inhibitor) and Bcl-xl targeted siRNA was developed as an injectable for melanoma cancer therapy. The anticancer ability and mechanisms of these formulations were studied both in vitro and in vivo and it displayed an enhanced anticancer effect on B16 melanoma cells by the activation of mitochondrial apoptosis pathway. Moreover, the intratumoral injection of this liposomal system significantly reduced the cancer growth that was observed in B16 melanoma in vivo xenograft model. The results suggested these formulations as a potential combination strategy for melanoma therapy [40]. Xu et al., prepared dual-therapeutic-loaded GE11 peptide-conjugated liposomes to improve the therapeutic efficacies for the treatment of laryngeal cancer. GE11 is an EGFR-targeting ligand used in the liposomal formulations containing docetaxel and siRNA against the ABCG2 gene that regulates multidrug resistance in many cancers [41]. Liposome-encapsulated DTX/ABCG2-siRNA was targeted against the Hep-2 laryngeal cancer cells. It improved the antitumor and apoptotic effects and may be effective for the treatment laryngeal cancer [42]. Thermal-responsive liposomes (TRL) were prepared using the combination of indocyanine

Table 1 Chemotherapy in combination to gene and immune therapy for cancer and other diseases

Drugs/Biomolecules/Therapeutic agents/	Immune agents/Genetic materials	Diseases	References
Docetaxel	Small interfering RNA (siRNA)	Glioblastoma	[45]
Pemetrexed	RNA interference	Malignant pleural mesothelioma	[46]
X-396 (anaplastic lymphoma kinase inhibitor)	siRNA	Neuroblastoma	[47]
Camptothecin	Anticancer siRNA (siPlk1)	Cancer	[48]
Allopurinol	Meglumine antimoniate	Canine visceral leishmaniasis	[49]
SiRNA	Peptide derived from rabies virus glycoprotein	Neurodegenerative protein misfolding diseases	[50]

green (ICG) and polyinosinic:polycytidylic acid (poly I:C). Poly I:C is a water-soluble immune stimulatory agent used to provide immune response. This novel system is not only intended to provide primary treatment to cancer but also for the prevention of cancer metastasis. The poly I:C- and ICG-containing TRLs (piTRLs) analyzed both in vitro and in vivo and the results showed the potential application of a piTRL with laser irradiation for immuno-photothermal therapy against the metastatic cancers [43]. Yang et al. developed liposome-based nanocapsules with surface endoglin aptamer and encapsulated it using an interferon-inducible protein-10. They tried to target vascular endothelial cells in tumor vasculature of the mouse and observed the significant action against cytotoxic T lymphocytes in melanoma cancer immune therapy [44] (Table 1).

4 Theranostic Applications

"Theranostics" is a new approach merging both diagnosis and treatment in a single delivery system like liposomes. These theranostic liposomes (TLs) contain both drug and diagnosis agents and precisely monitor the treatment efficiency along with the treatment. These systems were utilized for various diseases such as cancer, tuberculosis, and Parkinson's [51]. TLs were developed for the effective management of mycobacterial infections. These targeted TLs improved the therapeutic efficacy of drugs by site-specific delivery to the target and decreased the adverse effects. Folate-modified PEGylated liposomes encapsulating rifampicin and ofloxacin were prepared for in vivo imaging and treatment of mycobacterial infections. The formulation was evaluated for various parameters like physicochemical properties, in vitro

drug release, mycobacterial activity, in vivo blood-kinetics, bio-distribution, and bio-efficacy and stability. The vesicle size was found to be 160.6 nm with excellent anti-mycobacterial activity and considerable colloidal stability (up to 120 days). Entrapment efficiency was found to be 66.89 (±10.9)% and 40.61 (±8.7)% for rifampicin and ofloxacin, respectively. The in vitro drug release studies showed a slow biphasic pattern with longer terminal half-life of 19.13 h. The results of bio-distribution studies revealed higher localization of drugs in organs like spleen, liver, and kidneys one hour post-injection. The cellular uptake of TLs was assessed using scintigraphic in murine model of TB infection. Results demonstrated higher uptake at 2 h [52]. The TLs integrated with superparamagnetic iron oxide nanoparticles (SPIONs) and quantum dots (QDs) as well as cilengitide in a single system were developed for guiding surgical resection of glioma using magnetic targeting (MT). Encapsulation of SPIONs and QDs into TLs was detected by TEM and X-ray photoelectron spectroscopy. The size, zeta potential, and entrapment efficiency of cilengitide were found to be 100 ± 1.24 nm, −17.10 ± 0.11 mV, and ∼88.9%, respectively. In vitro drug release studies revealed a biphasic release pattern (initially rapid followed by sustained). Moreover, uptake of TLs is significantly increased by C6 cells under MT. The in vivo dual-imaging displayed negative-contrast enhancement effect on glioma [53]. Resveratrol (herbal neuroprotective agent) plays crucial roles in the treatment of Parkinson's disease (PD). However, the use of resveratrol is limited due to their poor penetration across the blood–brain barrier (BBB). Differential diagnosis of PD is also one of challenges in neurology. Herein, liposomes modified with a Fe_3O_4 (magnetic targeting) was developed for treatment of PD. The factional anisotropy (FA) values and T2 relaxation time of formulation were observed by magnetic resonance imaging in rats which showed good therapeutic effects. The formulation showed sustained and slow drug release and better stability. The results of in vivo studies displayed higher drug accumulation in target under the external magnetic field. Therefore, the Fe_3O_4 modified liposomal system offers a potential platform for the treatment of cerebral disease due to better penetration of drug across the BBB [54]. In another study, doxorubicin and grapheme nanosheets containing liposomes were developed using thin-film hydration method for the treatment of cancer. The GNSs have good optical properties, like photoluminescence which helps in tracking of the formulation, high absorption in ultraviolet region which can be utilized in photothermal therapy. The formulation was characterized for various parameters such as in vitro drug release, cytotoxicity, and cellular uptake. MCF-7 cells were utilized for cytotoxicity and cellular uptake studies. The formulation demonstrated higher cytotoxicity as compared to free forms of both [55]. Stimuli-responsive drug delivery systems selectively delivered the drug to the target site in presence of stimuli (external or internal). Theranostic liposomal systems were developed for simultaneous diagnosis and treatment. Reactive oxygen species (ROS)-responsive liposomes were developed which release drug upon ROS treatment. These liposomes showed sustained drug release in response to higher H_2O_2 concentration as well as displayed higher cytotoxicity as compared to unmodified counterpart [56]. Prostate-specific membrane antigen (PSMA) is a potential bio-marker for prostate cancer. Lipopolymer-modified liposomes were developed for theranostic delivery to

PSMA-expressing ($PSMA^+$) LNCaP cells. Lipopolymer was prepared using PSMA ligand, polyethylene glycol, and palmitate. Surface of preformed liposomes was modified with lipopolymer by post-insertion technique [57]. Doxorubicin and radiolabelled with ^{99m}Tc radionuclide were loaded into liposomes. Formulation of ^{99m}Tc-labeled lipopolymer-modified liposomal formulation increased the cellular uptake more than threefold in LNCaP cells compared to ^{99m}Tc-labeled plain liposomes. The results of cytotoxicity assay demonstrated that lipopolymer-modified formulation was more cytotoxic to LNCaP cells ($p < 0.05$), but not effective to PSMA-negative PC3 cells. The IC_{50} values of these liposomes were decreased upto ~five fold in case of LNCaP as compared to plain drug-loaded liposomes. These results suggested that PSMA ligand-based theranostic liposomes offer a potential platform for prostate cancer [58]. The folate-conjugated doxorubicin (Dox) and poly(9,9-dioctylfluorene-2,7-diyl-co-benzothiadiazole) (PFBT) as a fluorescent probe-loaded TLs were prepared and characterized. Liposomes were developed by thin-film hydration method using the active loading technique. The size and zeta potential of TLs were found to be 127.30 ± 3.20 (nm) and -25.00 ± 2.00 (mV), respectively. This carrier system showed extended drug release at 24 h under the mild hyperthermia as compared to Dox-Lip-FA. The IC50 value was reduced from 28.3 ± 3.7 (μg/mL) [in case of Dox-Lip-FA (37 °C)] to 16.8 ± 4.5 (μg/mL) in case of PFBT-Dox-Lip-FA. The results of cellular uptake study demonstrated higher drug accumulation inside the target. In vivo studies supported that distribution of PFBT-Dox-Lip-FA properly detected by PFBT. The growth of tumor-bearing mice was also reduced by PFBT-Dox-Lip-FA [59]. The theranostic applications of liposomes are summarized in Table 2.

5 Stimuli-Triggered Liposomes

Stimuli-sensitive drug delivery system (SSDDS) is a type of drug delivery system, which has a wide range of applications in drug delivery and cancer therapy. SSDDS can promote the effective localization of drug to the tumor site and avoid the side effects [69–71]. Traditional chemotherapeutic drugs are associated with several limitations such as systematic toxicity, low concentration of drug in tumor site, and short half-life. So, there is a need of SSDDS, which can deliver the anticancer drug to tumor site and reduce the side effects. The stimuli-sensitive drug delivery system could be fabricated to stimulate the response of living organ by assembling stimuli-sensitive carrier system to identify the dynamic process of body's biochemical reactions and changes of microenvironment, which leads to sustained or controlled release of drugs. Several reactions such as polymerization, isomerization, protonation, and hydrolysis are responsible for changing the behavior of stimuli-sensitive nanocarriers. It is based on the specific intracellular and extracellular physicochemical environment, which leads to accelerate the release of active components in special physiological environment [72]. In this approach, drug can be incorporated into the liposomes (either in core or in bilayer) by physical encapsulation or chemical bonding. It is a

Table 2 Theranostic application of liposomes

Drugs	Diagnosis agents	Ligands	Disease/Application	References
Doxorubicin hydrochloride	Graphene oxide flake under NIR light irradiation	Folic acid	Phototriggered tissue visualization and tumor regression	[60]
Anticancer siRNA	Quantum dots	Anti-EGF receptor aptamer	Theranostics of triple-negative breast cancer	[61]
Apomorphine	Quantum dots		Brain targeting and bio-imaging (disease like Parkinson)	[62]
Docetaxel (DTX)	Quantum dots	RGD-TPGS	Brain cancer imaging and therapy	[63]
Coenzyme Q10 (CoQ10)	Ultrasound-targeted microbubbles destruction (UTMD)		Diabetic nephropathy	[64]
Paclitaxel (PTX)	Superparamagnetic iron oxide nanoparticles (SPIO NPs)	pH-responsive peptide H7K(R2)2	Cancer	[65]
Indocyanine green	NIR dye [indocyanine green (ICG) and perfluorooctyl bromide (PFOB)]		Cancer	[66]
Doxorubicin	Gold nanorods (near-infrared laser light-activated)		Tumor in lymph nodes	[67]
Rapamycin and indocyanine green	Indocyanine green plus NIR laser	Folate	Cancer	[68]

unique strategy to achieve precise drug delivery in which carrier can show response, depending on the various environmental changes or stimuli. There are two types of stimuli, i.e., endogenous stimuli, which will stimulate on change in pH and redox potential, while exogenous stimuli are those which will be stimulated by changing temperature, magnetic field, light, and ultrasound [73]. The applications of different types of SSDDS are discussed as follows.

5.1 Endogenous Stimuli-Sensitive Drug Delivery Systems

The SSDDS are subtle to particular endogenous stimuli, such as pH of different tissue and organ [74–76], and change in redox potential of cell [77–80]. The main strategy of SSDDS is to deliver the drug directly into the endosome or to escape from lysosome

to cytoplasm while in tissue-level studies, endogenous SSDDS can utilize the change of tumor's microenvironment or pathological conditions like inflammation, infection, and hypoxia to achieve targeted release of drug [81, 82].

5.1.1 pH-Sensitive Drug Delivery System

The pH-sensitive drug delivery system is used to achieve targeted drug release. The change in pH is utilized to control the delivery of drug especially to the body organs such as gastrointestinal tract or tumor tissue and intracellular compartment such as lysosomes and endosomes as well as triggers the release of the drug. These stimuli-responsive nanocarriers could be triggered to environmental changes which are associated with pathological conditions, like inflammation or cancer. Various anticancer drug delivery systems have utilized the slight difference in pH which are existing between normal tissues (about 7.4) and the extracellular environment of solid tumors (about 5.5–7.2). This is mainly due to the irregular angiogenesis in fast-growing tumors, which will lead to the deficiency of both oxygen and nutrients subsequent to the production of acidic metabolites in the tumor interstitial. An important strategy is in which, cell-penetrating peptide on the surface of nanocarrier that can act upon the change in pH and leads to cell internalization. Surface-charge reversal of pH-responsive systems from negative or neutral to positive could promote cell internalization [83]. The pH-sensitive liposomes consisted of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) or 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine experience a transition from a lamellar phase to a fusogenic hexagonal phase at acidic pH. Sawant and Torchilin [84] reported the significant delivery of gene and siRNA via conjugation of DOPE to low-molecular-weight PEI due their fusogenic and buffering properties. The positively charged PEGylated liposomes potentially interact with the endosomal membrane, which facilitates the delivery of bioactives. On the other hand, the pH sensitivity can be considered using anchored polymer chain, causing deterioration of lipid membrane through phase transition in lysosomal acidic environments, which leads to release of payload [84].

5.1.2 Redox-Sensitive Drug Delivery System

These are the systems which use electron-transfer reactions to trigger the drug release. Redox-sensitive liposomal vehicles could be destabilized either by changes in charge or hydrophilicity of the amphiphile with chemical reducing agents. It is also disrupted due to elimination of cross-links to initiate the transition of lipid phase. Redox potential is an activating stimulus for both intracellular drug delivery and tumor targeting. These activating stimuli were generated through the high potential differences between the reducing environment of intracellular space and the more oxidative extracellular environment. Powerful thiolytic reducing agents, like dithiothreitol (DTT), are commonly used for the disruption of disulfide linkages within an

amphiphile, which involve in the activation of redox system. The critical micelles concentration (CMC) of the thiolytically cleaved amphiphile byproduct is usually increased, due to reduction reaction [85]. Fu et al. [86] prepared TAT modified paclitaxel liposomes comprising redox-responsive poly(ethylene glycol). At physiological conditions, and the TAT was protected by PEG which makes liposomes as long circulating. Glutathione was used as exogenous reducing agent which facilitates the detachment of PEG at tumor site. After detachment of PEG, TAT was exposed and shown to improve the cell internalization. It was concluded that the system increased tumor localization both in vitro and in vivo with increased tumor inhibition [83, 86].

5.2 Exogenous Stimuli-Sensitive Drug Delivery Systems

5.2.1 Temperature-Sensitive Drug Delivery System

Temperature-sensitive liposomes can regulate the release of drug and also expresses their function in response to local heating of desired tissues, which are used to accomplish target-selective drug delivery [83]. In case of liposomes, the dipalmitoylphosphatidylcholine (DPPC) displays the gel-to-liquid crystalline transition at about 41 °C, the temperature at which the permeability of the bilayer increases. Distinctive temperature-sensitive liposomes consist of DPPC which was used to achieve targeted release of drug. Drug release occurs at temperature higher than that of gel-to-liquid crystalline transition temperature [87]. Temperature-sensitive liposomes are utilizing the property of polymers which are known to change their water solubility in response to temperature. The lower critical solution temperature (LCST) is the specific temperature at which the temperature-sensitive polymers become water insoluble or experience phase separation. It has wide application in the field of drug delivery system. Temperature-sensitive polymers were used to produce temperature-sensitive liposomes. Liposome surface was modified using poly(*N*-isopropylacrylamide) (pNIPAM) and its copolymer. These polymers are decorated on surface of liposomes, which helps in triggering release of drug in response to temperature higher than LCST. It demonstrated that the destabilization of liposomal membrane occurs when polymer chain becomes hydrophobic at temperatures higher than LCST [88, 89]. Temperature-sensitive functions of liposomes are affected by the physical characteristics of temperature-sensitive polymers and their modification methods. Poly[(2-ethoxy)ethyl vinylether] (pEOEVE) shows LCST at around 40 °C. It comprises similar structure on the side chain as that of biocompatible PEG [90]. pEOEVE polymer forms highly hydrophobic domain which offers temperature sensitivity after liposome modification [91]. PEG-decorated liposomes were modified using block copolymer containing a pEOEOVE chain as a thermosensitive block and octadecylvinylether block. Kono et al. [91] prepared doxorubicin (DOX) containing liposomes, which leads to the triggered release of drugs within few minutes at 45 °C. Intravenous liposomal injection to the colon 26 tumor-bearing

mice with local heating of tumor lesion at 45 °C for 10 min leads to suppression of tumor growth [90, 91].

5.2.2 Magnetic-Field-Sensitive Drug Delivery Systems

Magnetic field is an external stimulus, a non-invasive energy source, which shows an important role in sustained release of drugs from magnetic-field-sensitive nanocarriers [92]. Magnetically sensitive liposomes can incorporate both type of drugs (hydrophilic and hydrophobic) using active targeting approaches for the treatment of several diseases. Magnetic field facilitates the delivery of drugs to target sites and maintain its concentration in blood upto its complete absorption [93].

5.2.3 Light-Sensitive Drug Delivery Systems

In light-sensitive drug delivery system, light is used as a physical stimulus to initiate the drug release. For the initiation of release process, light is used as a trigger to activate the photons. Light having 600–900 nm wavelength range is transmissible deep into biological tissues due to small absorption coefficient and low scattering [94]. Photodynamic therapy (PDT) involves the use of photosensitizing agents that can be stimulated by different intensities, wavelengths, or pulse durations to attain direct cell death or selective release of drug from a carrier systems [95, 96]. Photosensitizer (PS) absorbs light that can act as an energy transducer like energy transfer to molecular oxygen which leads to the formation of reactive oxygen species (ROS) that can consequently react with the liposomes to stimulate drug release or directly act on target tissues to activate apoptotic and necrotic cellular responses [97]. Most of the PSs are hydrophobic, and nanocarriers like liposomes and micelles are extensively used for improving the stabilization and tumor targeting of these agents. PDT was clinically approved modality, which can provide diagnostic evidence, specific targeting, and used in combination with other therapies. In case of light-sensitive liposomes, photo-polymerizable phospholipids like DC8,9PC (1,2-bis(tricosa-10,12-diynoyl)-sn-glycero-3-phospho choline) are widely used [97–99]. The major factors for the determination of photo-sensitive drug release are to determine the lateral phase separation and packing properties of polymerizable lipids in the liposome.

5.2.4 Ultrasound-Sensitive Drug Delivery Systems

Ultrasound (US) triggered drug delivery is used to deliver bioactive to the targeted site. The ultrasound can activate the delivery of drugs through several mechanisms such as microbubble activation, cavitation, increased cell membrane permeability, etc. [100–102]. Local heating was achieved via propagation of longitudinal pressure wave on the tissues and a part of its energy is absorbed by the tissue or drug carrier which increases the temperature of tissue viz drug carrier to release the drug [103].

Furthermore, free radicles are obtained from insonation of certain drugs, which can disrupt the cell membrane and enhance the transmembrane transport [104]. Low-frequency US (20–100 kHz) can be utilized in sonophoresis and transient cavitation-induced drug release from the liposomal drug delivery system [105, 106]. At high intensities, high-frequency US (>1 MHz) can lead to the thermal damage to cells and tissues [106]. Awad et al. [107] developed ultrasound-triggered albumin-conjugated liposomes for breast cancer therapy. In this study, human serum albumin (HAS) has been conjugated to PEGylated liposomes to explore the drug delivery (calcein) to breast cancer cells. Fluorescent microscopy displayed the calcein uptake by two breast cancer cell lines (MDA-MB-231 and MCF-7) which were considerably higher with the HAS-PEG liposomes as compared to non-targeted control liposomes [107].

6 Conclusion

The development of liposomes as carriers for therapeutic molecules is an ever-growing research area. The possibility of manipulating the inherent characteristics of these nanocarriers makes them versatile carriers for a wide range of materials (drugs, proteins, peptides, nucleic acids, and so on) and widens their potential use in many clinical settings. In the field of drug delivery, the liposomes have numerous applications due to their versatile nature. It has ability to encapsulate any type of drugs and other therapeutic agents. This vesicular drug delivery system can be administered by different routes which make them potential tool for the delivery of drug. Due to their unique components, they have ability to deliver the drug at target site. Nowadays, liposomes are showing many applications in the field of diagnosis and even in theranostic areas. Furthermore, the ability of liposomes to co-encapsulate both therapeutic and diagnostic agents paves the way for a novel application of liposomes as theranostic platforms. However, a rational design approach to achieve therapeutic objectives might represent the rate-determining step in the development of more sophisticated lipid-based therapeutics in the future.

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