

LIPOSOMAL DELIVERY SYSTEM

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Abstract

Liposomes are biodegradable and biocompatible lipid bilayer vesicles which are employed as a potential carrier for the delivery of drugs to desired site. It is used for the delivery of both hydrophobic and hydrophilic drugs. Because of the variability in composition and structural properties, liposomes are widely used in biomedical applications. Recently, multifunctional liposomes have been reported for tumor targeting. Liposomal delivery systems are modified by several ways according to their applications. Ligand modified liposomes are used for targeted drug delivery. Stimuli sensitive liposomes are used to obtain the trigger drug release at the site of action. Theranostic liposomal systems are used for diagnosis as well as therapeutic purpose. This chapter highlights the basics and classification of liposomes. It encompasses a detailed account of ligand targeted liposomes (such as folate, transferrin, mannose, hyaluronic acid and asialoglycoprotein), and stimuli-sensitive liposomes (such as pH, temperature, magnetic field, hypoxia and photo-triggered) for tumor targeting. It also includes some marketed liposomal products used for cancer treatment.

Keywords: Liposomes; cancer; ligand; stimuli-sensitive drug delivery systems; theranostic applications

1. INTRODUCTION

Liposomes are vesicular carrier system in which aqueous compartment enclosed by phospholipid bilayer. From last few decades, liposomes were extensively used as carrier for the delivery of bioactives, as they are appropriate to entrap both hydrophobic and hydrophilic drugs (Mu et al. 2017b). Liposomes are associated with some advantages like biodegradability, biocompatibility, low-immunogenicity, increased efficacy and therapeutic index, reduction in toxicity of encapsulated agents, improved pharmacokinetic effects. Liposomes have displayed favorable outcomes in drug delivery. The liposomes formation is occur when phospholipids are hydrated. Phospholipids composed of hydrophilic polar head and hydrophobic non-polar tail. The hydrophobic tail (Fatty acid chains) and hydrophilic polar head (consist of phosphoric acid) are self-organized in the presence of aqueous phase to form vesicular structure (Jain and Jain 2016a; Jain and Jain 2016b; Jain and Jain 2016d; Lasic 1998; Verma et al. 2020). These amphiphiles are employed in the drug delivery, via soaps, detergents, polar lipids, are used to design concentric bilayered structures. The hydration of lipid film leads to swelling of liquid crystalline bilayer. This hydrated lipid sheet get detach during agitation and close together to produce large, multilamellar vesicles (MLVs), which avoid interaction of water with hydrocarbon core of the bilayer at the edges (Vyas & Khar, 2002). On the basis of sizes and charactertics of liposomes they involve different method of preparation. The thin film hydration method is extensively used for the formation of MLVs. In this method, a thin-film (lipid film) is hydrated (using aqueous buffer) above the transition temperature (TT) of lipids. Generally, this method is used to form MLVs which can be sonicated and passed through polycarbonate filters to form small unilamellar vesicles (SUVs). Major limitation associated with this method is its poor entrapment efficiency (5-15%) of hydrophilic drugs. However, reduction of liposomes size leads to reduce amount of entrapped drug. Freeze-drying of SUV dispersion in an aqueous solution of the drug is another method to prepare MLV with high entrapment efficiency (upto 40%). The % EE of MLV can be increased by hydrating lipid in the presence of an organic solvent. Various methods have been described for the formation of large, unilamellar vesicles (LUV), including solvent (ether or ethanol) injection, detergent dialysis, calcium induced fusion, and reverse-phase evaporation (REV) method. The drug which showed lipophilic and hydrophilic properties (amphiphilic nature) on the basis of pH of solution may not be entrapped efficiently. It is due to diffusion of drug in and out of lipid membrane. Therefore, it is challenging to

entrap drug inside the liposomes. However, these types of drugs can be entrapped into perforated liposomes with high efficiency (up to 90%) using the active loading technique (Sharma and Sharma 1997). Moreover, conventional liposomes exhibit some problems like lesser encapsulation efficiency and enhanced blood clearance by reticulo-endothelial system (RES). The recognition of liposomes (by opsonins) as foreign material leads to uptake by macrophages (mononuclear phagocyte system (MPS)) and RES. Several physicochemical properties like net surface charge, size, hydrophobicity, and hydrophilicity of liposomes have an essential role in the clearance of liposomes from circulation. However, the vesicles size (>200 nm) and hydrophobicity of liposomes helps in opsonization and RES uptake. PEGylation is an essential method to avoid such problem of RES uptake. It is also used to develop long circulatory liposomes. Several other PEG alternatives (sialic acid, polyvinyl alcohol, and poly-N-vinylpyrrolidones) are used to form long circulatory liposomes. These agents are served to improve the blood circulation time but poor selectivity for tumor (Saraf et al. 2020b). Thus, targeted liposomes were then designed for selective drug delivery to the targeted site. Several targeting moiety (ligands) like folate, transferrin, mannose, hyaluronic acid, asialoglycoprotein are being used in targeting of liposomes. Recently, several characteristics of tumor microenvironment (TME) (such as acidic pH, raised in temperature, and hypoxia) have been utilized for the designing of stimuli-sensitive drug delivery systems (SDDS). Stimuli-sensitive materials are used to change their features in response to variations in the surroundings. SDDS specially deliver the drug in the presence of stimuli (intrinsic and extrinsic) (Lee and Im 2019). Moreover, antibodies (Ab) and Ab fragments are extensively used targeting agent for liposomes (because of higher specificity for their target antigens). This led to develop novel nanocarriers known as immunoliposomes. They are formed by conjugation of Ab to the surface of liposomes and permit for active targeting via binding to cancer cell-specific receptors (Paszko and Senge 2012; Saraf et al. 2020a). Further, advanced technique such as theranostic liposomes have been developed for diagnosis as well as treatment of cancer. The theranostic liposomes have both diagnostic agent and therapeutic agent in a single nanocarrier which can display the drug localization at particular site, and improve therapeutic effects (Lee and Im 2019). These theranostic liposomes may use diagnostic agents such as quantum dots (QDs) and graphene oxide along with therapeutic agent. The different types of liposomes are represented in figure 1. Advances in nanotechnology have provided an opportunity to designed site specific and individualized therapies for cancer

(Saraf et al. 2016; Vyas et al. 2015). In this chapter, the application potential of several ligands such as folate, transferrin, mannose, hyaluronic acid and asialoglycoprotein functionalized liposomes and SSDD (like pH, temperature, magnetic field, hypoxia and photo- triggered liposomes) have been thoroughly discussed. This chapter also includes the advancement of liposome, and marketed formulation used for cancer treatment. Several liposomal formulation are approved for the treatment of cancer includes Onivyde™, Marqibo®, Visudyne®, Doxil®, and Depocyt®.

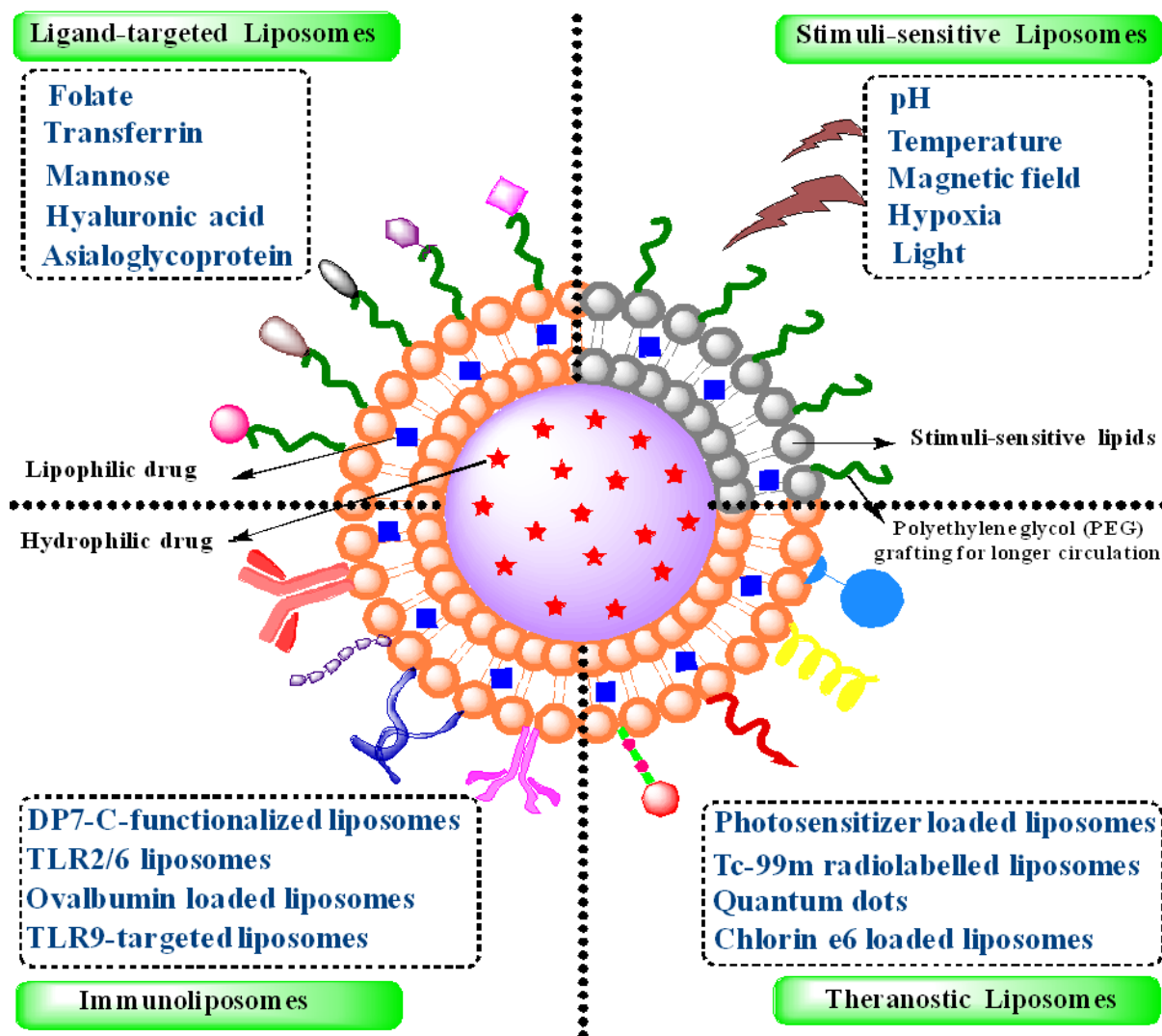


Figure 1: Schematic representation of types of liposomes

2. Classification of liposomes on the basis of size and chemical composition

Liposomes are vesicular carrier system employed for targeting of many solid tumors such as colon, prostate, breast, and brain (Fu et al. 2020; Jain et al. 2019; Lai et al. 2021; Panda et al. 2019; Saraf et al. 2020b). Liposomes are classified on the basis of size (small, intermediate, or large) and lamellarity (uni-, oligo-, and multilamellar vesicles). These

liposomes include MLVs, SUVs, and LUVs etc. The MLVs are 500 to 5,000 nm in size and comprised of different concentric bilayers while SUVs are about 100 nm in size and formed by a single bilayer and LUVs are 200 to 800 nm in size (Jain and Jain 2015; Jain and Jain 2016b; Jain and Jain 2016c). The development of unilamellar vesicles (ULVs) or multilamellar vesicles (MLVs) depends upon the method of preparation. Since ULVs contain a huge aqueous core, and appropriate for the entrapment of water soluble drugs while the MLVs favorably entrap the lipid-soluble drugs. They have the capability to entrap numerous drugs with different solubility features. MLVs are noticed to form more easily at larger hydrodynamic diameters and displayed higher entrapped volume than ULVs. But, ULVs (130 nm) show a much faster release rate than MLVs (250 nm) due to lesser number of phospholipid bilayer that it has to cross before for being released (Akbarzadeh et al. 2013; Sharma and Sharma 1997). Apart from this, multivesicular liposomes (MVLs) are developed and widely used for the treatment of cancer. These multivesicular liposomes (DepoFoam) containing the vesicle having multiple vesicles entrapped within one vesicle. Nanotechnology use multivesicular liposomes (DepoFoam) which permit the delivery of therapeutic agents into the target site. This reduces the frequency of administration, maintains the therapeutic concentration for longer period of time and minimized the side effects. Recently, several DepoFoam drugs have been approved for the treatment of many diseases including cancer for example DepoCyt® has been approved for the effective treatment of lymphomatous meningitis (Salehi et al. 2020).

On the basis of chemical nature of lipids liposomes are classified into cationic, anionic and neutral liposomes. These liposomes are widely used in cancer targeting (Shi et al. 2002). Cationic liposomes are basically positively charged liposomal formulations that have capacity to entrap with negatively charged DNA as well as the cell membranes (Simões et al. 2005). They are generally composed of cationic lipids such as 1,2-dioleoyl-3-triethylammonium-propane (DOTAP), N-[1-(2,3-Dioleyloxy) Propyl]-N,N,N-trimethylammonium chloride (DOTMA), N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC) and dioctadecylamidoglycylspermine (DOGS) etc. It has also been studied that anionic liposomes displayed higher stability in solution due to less aggregation in comparison to the neutrally charged liposomes. These anionic liposomes are fabricated with the help of anionic lipids such as dimyristoyl-phosphatidyl glycerol (DMPG), dipalmitoyl-phosphatidyl glycerol (DPPG), dioleoy-phosphatidyl glycerol (DOPG), 1,2-distearoyl-sn-glycero-3-phosphatidic acid (DSPA), 1,2-distearoyl-sn-glycero-3-

phosphatidylglycerol (DSPG), and 1,2-distearoyl-sn-glycero-3-phosphatidylserine (DSPS) etc. Moreover, it is also described that the anionic liposomes improved the process of endocytosis as compared to both cationic liposomes and neutral liposomes, respectively (Neves et al. 2016). Neutral phospholipids such as phosphatidylcholine (PC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) are used in the preparation of neutral liposomes (Payne et al. 1986; Shafaa 2020).

Liposomes are further classified on the basis of advance modification such as plain, PEGylated, targeted, stimuli-sensitive, immuno and theranostic liposomes. The long-circulating liposomes (stealth liposomes) can stay in the blood or systemic circulation for a longer duration than the non-modified liposomes. But these liposomes showed non-specific distribution to normal cells which cause toxicity and reduce the therapeutic effect of anticancer drug. Thus to avoid this problem, targeted drug delivery system (DDS) have been designed. Recently, advanced liposomes have been developed such as targeted liposomes (folate, mannose, transferrin, hyaluronic acid, and asialoglycoprotein), stimuli-sensitive liposomes (pH, temperature, magnetic field, hypoxia and photo) and immuno-liposomes. Targeted liposomes are fabricated on the basis of ligand-receptor interactions. Several receptors are overexpressed on the surface of cancer cells which are recognized as the marker to target cancer. Moreover, several characteristics of TME such as acidic pH, elevated temperature and hypoxia have been employed using SDDS. Immuno-liposomes are specialized liposomes which are carrying Ab that attached to their surfaces and able to accumulate in the area within the body where an attached Ab recognizes and binds its antigen (Bozzuto and Molinari 2015; Çağdaş et al. 2014). Currently, advanced technology has been used to develop theranostic liposomes which are exploited for the diagnosis and treatment of cancer. Classification of liposomes on the basis of chemical nature of phospholipids, advance modification and applications of liposomes are showed in table 1.

Table 1 Classification of liposomes on the basis of nature of phospholipids, advance modification and applications of liposomes

Type of liposomes	Chemicals and solvents	Method of preparations	Drug/Active agents/Biomolecules	Uses	References
Cationic	1-Palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine (HSPC), DPPC, unsaturated (POPC and DOPC), DOTAP, dimethyldidodecylammonium bromide (DDAB), Cholesterol (Chol)	Thin film hydration	Doxorubicin (DOX)	Breast cancer	(Chowdhury et al. 2020)
	1,2-dioleoyl-3 trimethyl ammonium propane (DOTAP),phosphatidylcholine(PC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(aleimide(polyethyleneglycol)-2000) (Mal-PEG) and Cholesterol (Chol)	Thin-film method	Trastuzumab	Prostate cancer	(Sicard et al. 2020)
	PEG, DC-Chol/ dioleoyl phosphatidylethanolamine (DOPE)	Thin-film hydration	KSP siRNA	Ovarian cancer	(Lee and Ahn 2018)
	DOTAP/DOPE/Chol	Thin-film hydration method	EGFR siRNA	Glioma	(Wei et al. 2016)
Anionic	Cholesterol and Distearoyl phosphatidylcholine (DSPC)	Ultrafiltration centrifugation	siRNA	Pulmonary fibrosis	(Pan et al. 2021)
	Phospholipids and Chol	Thin film hydration method	Antigen	Autoimmune diseases	(Benne et al. 2020)

	Dipalmitoyl phosphatidylcholine (DPPC); 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N, Diethylenetriaminepentaacetic anhydride (DTPA)	Film-hydration method Chol, acid	Paclitaxel (PTX) and Vinorelbine (VNB)	Lung cancer	(Karpuz et al. 2020)
	HSPC, DOTAP, DSPE-PEG2000, Soybean phosphatidylcholine	Film hydration method	Celecoxib (CXB), curcumin (CUR), and DOX	Breast cancer	(Liu et al. 2020)
Neutral	DSPC, stearyl amine (SA)	Film hydration method	Tamoxifen (TMX)	Breast cancer	(Shafaa 2020)
Stealth	DPPC, DSPC, and DSPE-MPEG-2000, Cholesterol	Thin film hydration method	Sirolimus	Prostate cancer	(Nandi et al. 2020)
	Egg-PC, cholesterol and DSPE-PEG2000	Thin film hydration method	quercetin	Cervical cancer	(Saraswat and Maher 2020)
Folate -targeted	Folic acid, 1-Ethyl-(3-dimethylaminopropyl) carbonyldiamine hydrochloride (EDC·HCL), 1-hydroxybenzotriazole (HOBT) and 4-dimethylaminopyridine (DMAP), 1,2-ethanedithiol (EDT)	Ethanol injection- ammonium sulfate gradient method.	DOX and Astragaloside IV	Triple negative breast cancer.	(Yue et al. 2020)
Transferrin targeted	- Transferrin, (DPPC), Chol, DSPE-PEG2000, and polyethylene glycol-distearoyl phosphatidylethanolamine-maleimide (DSPE-PEG2000-MAL)	Film dispersion method	Dioscin	Tumor targeting	(Wang et al. 2020)

Hyaluronic acid-targeted	Hyaluronic acid (HA), DOPE, DOTAP, and DSPE-PEG2000	Film dispersion method	5-fluorouracil (5-FU)	colorectal cancer	(Mansoori et al. 2020)
Mannose -targeted	Soya phosphatidyl choline (Pho), Chol	Film dispersion method	PTX	Colon Cancer	(Xiong et al. 2017)
Asialoglycoprotein-targeted	Asialoglycoprotein, (DSPE-PEG2000), Egg yolk lecithin, ; DSPE-PEG2000-GAL, Chol	Lipid film hydration	norcantharidin	Hepatocellular carcinoma	(Liu et al. 2017b)
pH sensitive	DPPC, DC-Chol, Chol, pH-sensitive octylamine grafted poly aspartic acid (PASP-g-C8)	Reverse phase evaporation method	Cytarabine	Tumor targeting	(Zhao et al. 2020)
	DOTAP, DOPE, DSPE-PEG2000, heparin	Thin-film Hydration method	DOX and Epacadostat	Melanoma	(Chen et al. 2020)
Immuno liposomes	Lipids soy phosphatidylcholine (SPC),DSPEPEG-2000, CHOL	Thin film hydration method	Docetaxel (DTX)	Prostate cancer	(Eloy et al. 2020)
	SPC, cholesterol, and DSPE-PEG2000, (2'-Benzothiazolyl)-7-diethylaminocoumarin	Film dispersion/post-insertion method.	Calatase and programmed death ligand 1 monoclonal antibodies (aPDL1s)	Melanoma immunotherapy	(Hei et al. 2020)

3. Application of liposomes in cancer therapy

Recently, several strategies such as targeted, stimuli-sensitive, theranostic and immunoliposomes are developed for effective treatment of cancer. Several applications of liposomes in cancer therapy have been discussed below (Mu et al. 2017a).

3.1 Ligand targeted liposomes

Generally liposomes undergo passive and active targeting. The higher accumulation of drug inside the tumor cells (because of EPR effects) occur in passive targeting. Additionally, active targeting approaches (ligand-appended liposomes) have been employed as a third generation of liposomes. Functionalization of liposomes can provide a site specific delivery like cell organelles (because of high affinity for particular receptor) (Jain and Jain 2016d; Saraf et al. 2020a).

3.1.1 Folate-targeted liposomes

The folate receptor (FR) is overexpressed on the tumor cells, such as ovary, uterus, testis, colon, kidneys and lung cancer. Folic acid conjugates linked via γ -carboxyl group to drug molecules and enter into the folate overexpressing cancer cells. Receptor mediated endocytosis has been taken place because of high affinity of folate conjugates for cancer cell surface. Folic acid functionalization helps the targeted delivery of bioactive and diagnostic agents to cancer cells in the presence of normal cells. Folate mediated targeting is better approach for cancer targeting (Liu et al. 2016; Raikwar et al. 2018). Park et al. (2021) developed folate-targeted pH-sensitive liposomes loaded with DTX and doxycycline (anti-calpain-2) for non-small cell lungs cancer (NSCLC). The poor diagnosis of NSCLC is due to tumor-associated macrophages (TAMs). Additionally, calpain-2 (CAPN2) is overexpressed in NSCLC and is involved in tumor growth. This study is used to improve antitumor efficacy and reduce side effects associated with the treatment. Cytotoxicity assay demonstrated that the FR-mediated targeting (FR β) in M2 TAMs and NSCLC cells effectively suppressed tumor growth. This combinational therapy showed synergistic anticancer effects by suppressing CAPN2 expression (Park et al. 2021). Some methods such as non-specific contrast agents, and other treatment approaches are inadequate in the treatment and early prognosis of tumor. Thus, there is necessity to fabricate more specific, targeted approach to overwhelm the limitation of non-targeted DDS. The folate conjugated PTX and VNB encapsulated Tc-99m radiolabelled liposomes were formulated and evaluated. *In vivo* therapeutic efficacy of liposomes was determined by bio-distribution study. *In vitro* study was performed in LLC1 cell lines to determine

tumor growth inhibition. Active and passive targeted liposomes possess higher cellular uptake and co-drug loaded liposomes display higher cytotoxicity than free drug combination (in LLC1 cells). Outcomes of bio-distribution studies in tumor-bearing C57BL/6 mice indicated that greater liposomes were observed in cancerous tissue (compared to non-cancerous tissue). Additionally, histopathological assay demonstrated that the FR-targeted liposomes not only suppress tumor growth but also inhibited lungs metastasis. Hence, these liposomes have immense potential in cancer prognosis and therapy (Karpuz et al. 2021). In another study, diacid metabolite of norcantharidin (DM-NCTD)-loaded folic acid-functionalized, polyethylene glycolated (DM-NCTD/FA-PEG) liposomes was prepared as clinically effective approach for hepatocellular carcinoma (HCC). *In vitro* cytotoxicity study of DM-NCTD/FA-PEG liposomes displayed higher cytotoxicity on H22 (hepatoma cells) compared to non-targeted PEG liposomes. Additionally bio-distribution study of DM-NCTD liposomes showed improved tumor targeting efficiency. The DM-NCTD/FA-PEG liposomes exhibit higher efficiency to inhibit tumor (in H22 tumor-bearing mice) compared to free DM-NCTD or DM-NCTD/PEG liposomes, *in vivo*. Thus, FR-targeted liposomes could have immense potential in HCC patients (Liu et al. 2016).

3.1.2 Transferrin targeted liposomes

Transferrin receptors (TfR) are overexpressed on cancer cells which are used for tumor targeting. Several hurdles like blood brain barrier (BBB) complexity and multidrug resistance (MDR) are related with the treatment of gliomas (brain tumor). To overcome above challenges, Tf-functionalized vincristine and tetradrine (TET) entrapped liposomes were prepared (Sawant and Torchilin 2012). *In vitro* assay of Tf-functionalized liposomes displayed the improvement in transport ability across BBB, enhanced cellular uptake, inhibited the MDR, and blocked the invasion of tumor cell and vasculogenic mimicry (VM) channels. It also induces apoptosis of C6 cells and C6/ADR cells via up-regulating caspases proteins. *In vivo* studies suggested that Tf-functionalized liposomes could enhance localization of drug into brain tumor tissues and increase treatment efficiency. Hence, the developed liposomes have higher therapeutic effects in gliomas (Song et al. 2017). D-alpha-tocopheryl PEG 1000 succinate mono-ester (TPGS) liposomes bearing DTX and QDs were formulated and evaluated for diagnosis and treatment of brain tumor (brain theranostic). TPGS-liposomes were characterized for their particle size, polydispersity index, morphology, drug entrapment efficiency and *in vitro* release study.

The size of liposomes was found to be below 200 nm with higher entrapment efficiency (71%). Outcomes of animal study demonstrated that the TfR-targeted liposomes considered as an effective brain targeting strategy. Targeting efficacy of these liposomes is because of its higher permeability and smaller size (nano-size) (Sonali et al. 2016). In another study, Tf and rabies virus glycoprotein (RVG) peptide conjugated liposomes were developed for brain tumor therapy. These liposomes have homogenous particle size and capability to prevent enzymatic degradation of plasmid DNA. RVG-Tf conjugated liposomes are exhibiting higher ability to transfect cells than that to non-functionalized liposomes. *In vitro* studies (triple co-culture BBB model) of these liposomes revealed the efficient translocation ability and higher permeability through BBB. Thus, the alterations of liposomes with brain-targeting ligands used as potential strategy for gene delivery (dos Santos Rodrigues et al. 2020). Fernandes et al. (2020) developed Tf-conjugated DTX bearing liposomes for prostate cancer (PC) treatment. The anticancer effects of Tf-conjugated DTX bearing liposomes (LIP-DTX-TF) were evaluated on PC cell lines. MTT assay (in PC-3 and PNT2 cell lines) showed more cytotoxicity of LIP-DTX-TF to PC-3 cells than the conventional formulation. Thus, these liposomal systems have promising potential for PC treatment (Fernandes et al. 2021).

3.1.3 Mannose receptors targeted liposomes

Mannose receptor (MR) is highly expressed on tumor cell surface. It plays an important role in the transportation of glucose (energy source for metabolically active cancer cells) into the cancer cells. Mannose-functionalized nanocarriers are used for cancer cell targeting. It may facilitate receptor-mediated intracellular transport of therapeutics. The MR is overexpressed especially in resistant colon cancer cells (HCT8/ADR). Hence, MR-targeted liposomes can enhance the transport of chemotherapeutic drug intracellularly. Kang et al. (2017) prepared mannose-functionalized DOX and dihydroartemisinin (DHA) bearing liposomes for effective targeting of cancer cells. The co-delivery of DOX (anti-cancer effect) and DHA (anti-MDR effect) in HCT8/ADR cells could overcome the MDR effect and provide promising approach for anti-MDR colon cancer therapy (Kang et al. 2017). MR is responsible for tumor growth, invasion, proliferation, metastasis in the TME, and overexpressed in TAMs. Chlorogenic acid (CHA) has been identified as a potent immunomodulators which induced polarization of TAMs from an M2 (pro-tumorigenic) to an M1 (anti-tumorigenic) phenotype. MR-targeted CHA-entrapped liposomes have been used for targeting TAMs through MR. Results of *in vitro* and *in vivo* studies determined

the anticancer effects of CHA-entrapped mannosylated liposomes. They also exhibit a desirable particle size, stability, and targetability. Additionally, CHA-entrapped mannosylated liposomes displayed higher suppression in tumor growth (in G422 gliomas). Hence, CHA-entrapped mannosylated liposomes could provide a promising potential for TAMs-targeted cancer immunotherapy (Ye et al. 2020). In another study, targeted mannose-6-phosphate (M6P)-functionalized liposomes bearing anti-cancer drugs have been formulated for cancer treatment. The behaviour of M6P-functionalized and non-functionalized liposomes was compared in MCF-7 cells and HDF normal cells. They also prepared calcein-entrapped liposomes and displayed the higher uptake of M6P-functionalized liposomes in MCF-7 cells as compared to HDF cells. Hence, the developed liposomes have higher strength and selectivity toward tumor cell (Minnelli et al. 2018).

3.1.4 Hyaluronic acid targeted liposomes

Hyaluronic acid (HA) is a glycosaminoglycan which is used for cancer cell targeting. HA-functionalized nanocarriers have been used to target CD44 receptor and improve tumor targeting (Prajapati et al. 2019a; Prajapati et al. 2019b). Combination of phototherapy and chemotherapy was used to enhance the tumor targeting. The HA hexadecylamine (HA-C16) (HA derivative) was functionalized on the surface of liposomes and biocompatible magnetic nanoparticles and anticancer drug (DTX) was entrapped into it. These liposomes show enhanced therapeutic efficacy ($IC_{50} = 0.69 \pm 0.1036 \mu\text{g/mL}$) compared to radiation and DTX monotherapy. Glioblastoma multiforme (GBM) is a type of brain malignancy. The distinction between tumor and healthy brain cells are remains challenging for GBM, thus there is necessity to develop an effective approach to target specific GBM cells. The developed HA-conjugated liposomes (HALMPs) are showing potentiality for targeting GBM cells over other brain cells (because of higher CD44 receptor expression). Thus, HA-conjugated liposomes show targeted delivery of bioactives through CD44 receptor in GBM cells (Hayward et al. 2016). In another study, HA-functionalized liposomes containing DOX and PTX were developed to improved cancer chemotherapy. This combination displayed synergistic effect and reduced drug resistance. The *in vitro* study exhibited that the release of both drugs over 72 h were higher in pH 5.5 phosphate buffer than pH 7.4 (PBS). *In vitro* MTT assay showed highest cytotoxicity and synergistic effects in comparison to free drug or single drug loaded liposomes. Additionally cell uptake study suggested that higher internalization ability of HA-DOX-Lip compared to HA-

unconjugated DOX-Lip and free DOX. Hence, the HA-conjugated dual-drug loaded liposomes exhibits promising potential for targeted tumor therapy (Song et al. 2019).

3.1.5 Asialoglycoprotein receptor (ASGPR) targeted liposomes

ASGPR is overexpressed in HCC which are widely used in targeted DDS. The use of natural ligands (asialofetuin), and synthetic ligand with galactosylated or lactosylated residues, such as galactosylated cholesterol and galactosylated polymers have achieved significant targeting efficacy to the liver (Cho et al. 2001). Lactoferrin (Lf) is a unique targeting ligand for HCC cells because of its specific binding with ASGPR. In one study, lactoferrin (Lf)-modified DOX-loaded PEGylated liposomes (Lf-PLS) were developed and its targeting efficacy and antitumor effect to HCC was determined. The confocal laser scanning microscopy (CLSM) showed that the Tf-PLS was considerably higher uptaken in ASGPR-positive cells but not in ASGPR-negative cells as compared to non-functionalized PEGylated liposomes (PLS). *In vitro* cytotoxicity study indicated that the DOX-loaded Lf-PLS displayed stronger anti-proliferative effects on HCC cells. Outcomes of this study suggested that the DOX-loaded Lf-PLS have promising potential in HCC-targeting therapy (Wei et al. 2015). Targeted liposomes were designed using polymeric cholesteryl arabinogalactan to target HCC. The *in vitro* study displayed increased cytotoxicity and specificity towards HepG2 cell lines. These liposomes are specifically localized into liver in comparison to other organ like kidney and heart. These *in vitro* and *in vivo* studies showed that the prepared liposomes have high stability and entrapment efficiency due to presence of cholesteryl arabinogalactan. Thus, these targeted liposomes have promising potential in the treatment of HCC (Pathak et al. 2016). In another study, ASGPR targeted liposomes were developed to determine the anticancer effect and selectivity of co-delivery of DOX and vimentin siRNA to HCC. The aim of this study was to develop galacosylated liposomes bearing drug, known as gal-DOX/vimentin siRNA liposomes (Gal-DOX/siRNA-L). Additionally, animal study of these liposomes demonstrated that the efficient delivery of drugs to the liver. All findings showed that the Gal-DOX-siRNA-L could be used as effective carrier for the delivery of bioactives and increase transfection efficacy (Oh et al. 2016).

3.2 Stimuli-sensitive liposomes

Liposomes which show trigger response to various stimuli (pH, temperature, magnetic field, hypoxia, and light) for the delivery of drug to the site of action are known as stimuli-sensitive liposomes. Stimuli-sensitive liposomes are used to attain cancer specific targeting

with higher therapeutic effects and lower side effects. The key biomarker for the designing of SDDS is the specific features of TME like lower pH, higher temperature, hypoxia, and overexpression of proteolytic enzymes (Torchilin 2007). The TME responsive strategies can be exploited to target cancer cells. SDDS can elicit the release of bioactive as a result of destabilization caused by several stimuli (such as pH, temperature and light) (Trédan et al. 2007). Thus, SDDS have wide application in cancer therapeutics. These DDS have several benefits such as higher accumulation, improved pharmacokinetic parameters enhance cell uptake and drug release selectively in cancerous tissues in response to the stimuli (Yang et al. 2016). SDDS have been designed to remain their structural integrity of nanocarrier(s) throughout the circulation and triggered the release of bioactive in response to particular stimuli at the target site. These nanocarrier(s) shows faster changes within the TME like disruption and accumulation of system leading to triggered release of bioactive (Arias et al. 2011; Deshpande et al. 2013). The fabrication of SDDS and efficacy of these systems was confirmed by preclinical animal experiments (Xiao et al. 2015; Yang et al. 2016). Various stimuli-sensitive DDS have been discussed below-

3.2.1 pH-sensitive liposomes

The pH-sensitive liposomes respond to change in the pH of the surrounding of disease tissue like cancer cells (Goenka et al. 2014). The pH of TME is different from the pH of normal cells. Thus, the pH-sensitive liposomes have been employed as favorable strategy for enhancing the localization of drug at tumor site. They show selective release of bioactive in response to pH. They further modified with ligand to enhance the targetability and enhance the therapeutic efficacy. These liposomes showed pH-dependent disruption of lipid membrane (at acidic pH of endosomes). Therefore, the encapsulated bioactive is internalized into the cell and shows its pharmacological action (Paliwal et al. 2015). Metastasis is a most common hurdle in cancer chemotherapy and leads to failure in the melanoma therapy. Indoleamine-2,3 dioxygenase (IDO) overexpressed in the TME lead to the immune escape. The combinational approach (chemotherapeutic agent with IDO inhibitor) were used which was effective for melanoma treatment. Thus, heparin-functionalized pH-sensitive DOX and epacadostat (EPA) bearing liposomes were formulated. Heparin functionalization increases the stability of liposomes, induced the cell uptake and avoids the interaction of B16F10 cells to platelets and extracellular matrix (ECM). *In vitro* studies of pH-sensitive liposomes showed improved cytotoxicity and apoptosis. Systematic administration of these liposomes exhibited optimum anti-metastasis

activity on the melanoma. Results of *in vitro* and *in vivo* study suggested that the higher efficiency of heparin-functionalized pH-sensitive liposomes. Reduction in tumor cell invasion, migration, and induction of anti-metastatic immunity showed by DC maturation, CD8⁺ cytotoxic T lymphocytes (CTLs) activation confirmed the therapeutic efficacy of pH-sensitive liposomes. Thus, heparin-functionalized pH-sensitive DOX and EPA loaded liposomes exhibits higher anti-tumor immunity and applicable for the treatment of lungs metastasis in melanoma (Chen et al. 2020). Folate-targeted DOX and imatinib (IM) loaded pH-sensitive liposomes (FPL-DOX/IM) were formulated and evaluated for anti-tumor efficacy. This study triggered the release of bioactive at acidic pH and reversed the drug resistance. Outcomes demonstrated that the FPL-DOX/IM could provide an immense potential against MDR tumor (Chen et al. 2018). Han et al. (2018) prepared D- α -tocopheryl poly(2-ethyl-2-oxazoline) succinate (TPOS)-modified DTX liposomes. TPOS have ability to prepare pH-responsive nanocarrier(s) and employed for cancer targeting. Results of *in vitro* suggested that the TPOS-modified DTX liposomes had showed slower drug release (at pH 7.4), while the higher release (86.92% $\pm$ 1.69%) at pH 6.4. Cell uptake and flow cytometry study displayed that the TPOS modified coumarin-6 liposomes (TPOS-C6-L) had higher cellular uptake (at pH 6.4 than pH 7.4). *In vitro* cytotoxicity study revealed that cytotoxicity of TPOS-DTX-L (at pH 7.4) was lower than pH 6.8 after 48h of incubation. The apoptosis assay displayed that the PEG-DTX-L had no remarkable improvement with decreased pH (from 7.4 to 6.8), while TPOS-DTX-L significantly induced apoptosis in HeLa cells with decreased pH (from 7.4 to 6.8). Hence, TPOS-C6-L could be employed as an effective carrier for the designing of pH-sensitive DDS (Han et al. 2019).

3.2.2 Temperature sensitive liposomes

Temperature is an external stimuli used to elicit the release of bioactive from temperature-sensitive liposomes (TSLs). TSL are classified into two types: traditional liposomes containing the temperature-sensitive component and liposomes modified with temperature sensitive polymers. These liposomes show an increase in permeation of membrane at its gel-to-sol crystalline phase TT. These systems show stability at physiological temperature (37°C) but show a temperature-elicited drug delivery above 37°C under the influence of stimulus (Wang et al. 2017). At hyperthermic temperature (above 40°C), these liposomes showed rapid release of entrapped drug. Ruiz et al. (2020) developed DOX-loaded TSLs for targeted delivery of drug to the tumor. These liposomes show a rapid release of DOX

in response to mild hyperthermia (HT). The thermosensitivity of TSLs can be affected by DOX crystal loading. The characteristics of DOX crystals were altered through different active loading methods (ammonium sulphate, ammonium EDTA and magnesium sulphate). These studies revealed that the ammonium sulphate or ammonium EDTA loading methods had similar encapsulation efficiency (%EE) into TSLs, while low DOX loading was observed in metal complexation method. Additionally, cryogenic transmission electron microscopy (cryo-TEM) presented a variation in the nature of DOX crystal formed inside TSLs. *In vitro* DOX uptake through CT26 and PC-3 cells demonstrated that the use of optimum lipid:DOX ratio showed controlled release characteristics in combination with mild HT. Results of animal study indicated that the reduction in tumor growth was found to be highest in TSLs with HT. Hence, developed DOX-loaded TSLs exhibited a pronounced potential in cancer treatment (Ruiz et al. 2020). The TMX and IM loaded TSLs were formulated and evaluated for breast cancer treatment. These liposomes undergo several characterizations like particle size, zeta-potential, and drug release below and above the liposomal TT. The % EE of developed liposomes (for TMX and IM) was found to be more than 70% (above TT of lipids i.e 39.4°C). The uptake study demonstrated the faster uptake of TSLs by breast cancer cells (MCF-7 and MDA-MB-231). Hence, the combinational therapy shows synergistic anticancer effect against breast cancer cells (MCF-7, MDA-MB-231). Finding indicated that the TSLs have promising potential for the delivery of TMX and IM for breast cancer treatment (Jose et al. 2019). The DOX-entrapped TSLs were prepared for the site-specific delivery of DOX into brain tumor. These liposomes quickly release the encapsulated bioactive inside the tumor in response to HT (>40°C). HT plays a significant role in the transient opening of the BBB when combined with TSLs. Thus, HT could achieve effective concentration of drug inside the brain. DOX-entrapped TSLs was intravenously infused over 30 min at a fixed dose (0.94 mg/kg) in anesthetized beagles (age 17 months). Fluorescence microscopy showed an efficient delivery of DOX across the BBB. Additionally, histopathology in haematoxylin & eosin (H & E) stained samples display the localized damage near the probe. Survival studies described that this approach is safe with no normal tissue toxicity (Bredlau et al. 2018). Zhang et al. (2014) prepared DTX loaded TSLs for targeted delivery of drug to tumor. *In vitro* drug release study exhibited higher DTX release at 42°C than 37°C, which could be due to temperature-responsive drug release from the liposomes. Thus, it could be a favorable strategy to deliver drug at tumor site (Zhang et al. 2014).

3.2.3 Magnetic field sensitive liposomes

Magnetic field (MF) is a significant stimulus which plays a vital role in sustained release of drugs from magnetic field sensitive nanocarriers (Jhaveri 2016). The MF is applied locally to increase the accumulation of drug in the tumor, or by varying the MF to induce tumor heating to attain better anticancer efficacy. MF is an external stimulus which facilitates the transfer of anticancer drug to target sites and maintain its concentration in blood up to its complete absorption (Arias et al. 2011). Anilkumar et al. (2020) prepared liposomal nanocomposites comprising citric acid-modified iron oxide magnetic nanoparticles (CMNPs) for dual magneto-photothermal cancer therapy. It is prompted by modifying MF (AMF) and near-infrared (NIR) lasers. These CMNPs were entrapped into the cationic liposomes led to form nanometric magnetic liposomes (MLs) for concurrent magnetic hyperthermia (MH) in the presence of AMF and photothermia (PT) induced by NIR laser exposure. The liposomes with 121nm size and 87% EE were found to be best for designing of MLs. After the endocytosis of MLs shows inducible thermal effects which led to enhance cytotoxicity of cancer cells in the presence of AMF-NIR lasers. Therefore, developed systems have favorable potential for bimodal MH-PT dual magneto-photothermal cancer therapy (TS et al. 2020). Curcumin loaded PEGylated ML were formulated and evaluated for anti-tumor efficacy. This study enhances the solubility of lipophilic drug and provides controlled release of therapeutic agents. *In vitro* drug release study showed that the carriers displayed no significant release at 37°C, while it showed rapid release at 45°C (due to inductive magnetic heating). Moreover, it would show (3 times higher) curcumin release under high frequency MF (HFMF) exposure, than without HFMF exposure at 45°C. Florescence microscopy of PEGylated ML displayed efficient internalization into the MCF-7 cells. Hence, Cur-loaded PEGylated MLs can be used as potential approach for cancer treatment (Hardiansyah et al. 2017).

2.2.4 Hypoxia-sensitive liposomes

Tumors are hypoxic in nature which is lack of blood circulations which restricts the drug entry deep inside the tumor cells. This hypoxic condition of tumor causes drug resistance in chemotherapy, radiotherapy, and phototherapy. It is reported in many literature that the hypoxia-responsive liposomes may enhance the delivery of bioactives. Hypoxia-responsive liposomes were prepared and evaluated by entrapment of nitroimidazole derivative into the liposomal membrane. At hypoxia condition of tumor, these derivative displayed reductive metabolism which facilitates a triggered release of bioactive due

liposomal degradation. CLSM and NIR imaging confirmed the oxygen-dependent drug (DOX) release. The *in vivo* fluorescence microscopy displayed the selective release of drug to hypoxic tumor site. Thus, hypoxia-responsive liposomes revealed a selective cytotoxicity in hypoxic cells. Finding suggested that the hypoxia-responsive liposomes provide a better strategy for cancer treatment (Li et al. 2019). Lui et al. (2017) developed hypoxia-responsive ionizable liposomes for the effective transport of siRNA and anti-cancer drugs into the brain tumor. These liposomes selectively increase cell uptake (siRNA) under hypoxic condition and low pH to treat gliomas. To achieve the above objectives, malate dehydrogenase lipid molecules were synthesized. The synthesized molecules have nitroimidazole groups which are responsible for the hypoxia mediated specificity and sensitivity. The *in vivo* studies (histological and microscopic examinations) of brain tissue revealed that the liposomes effectively delivered siRNA into gliomas. Thus, hypoxia-responsive ionizable liposomes provide a wide platform for the delivery of siRNA into the tumor (Liu et al. 2017a).

3.2.5 Light sensitive liposomes

Light is also an extrinsic stimulus which is used for the delivery of bioactives. Light play a significant role in the activation/inactivation of several biochemical processes which has been known as effective strategy for various biomedical applications. Various factors like wavelength, intensity, pulse duration and cycle of activated light were employed in biomedical research. Several light radiations such as UV, visible light, and NIR were clinically utilized for the delivery of drugs. The NIR showed deep penetration of bioactive in tumor tissues (Zhu and Torchilin 2013). Some photosensitizing agents such as chlorins, porphyrin derivatives, phthalocyanines and porphycenes were employed for prognosis and treatment of tumor. Number of reports displayed that light-triggered drug release is a potential strategy for delivering chemotherapeutic agent to tumor cell. Liposomes loaded with calcein (fluorescence marker), talaporfin sodium (TPS, a water soluble photosensitizer) and gemcitabine (chemotherapeutic drug) were developed and characterized for effective cancer treatment. These liposomes showed site specific drug release upon irradiation with a NIR-laser. Liposomes consists of phospholipids (DSPC/DOPE/cholesterol/PEG2000-DSPE in a molar ratio of 85/10/5/5) and demonstrated higher drug release (70% within 10 min) upon IR irradiation, but no drug release in the absence of NIR-laser irradiation. DOPE is responsible for NIR-laser-triggered drug release, the quantity of DOPE can also affects the leakage of drug in

absence of NIR-laser-irradiation at 37°C (body temperature). Thus, DOPE plays an essential role in the effective delivery of bioactive into the tumor tissue. Gemcitabine is an anticancer drug which is entrapped into the liposomes to established NIR-triggered drug release. The *in vitro* cytotoxicity assay showed that the NIR-laser with liposomes (entrapped with gemcitabine and TPS) provide highest cytotoxic effects against EMT6/P cells. Outcomes of this study suggested that the light-sensitive liposomes may offer a wide platform for the treatment of cancer (Fuse et al. 2018). Yang et al. (2019) prepared tirapazamine (TPZ) and lipophilic IR780 entrapped liposomes combining with photodynamic therapy (PDT) for synergistic anticancer effect. This PDT employed as non-invasive and effective treatment approach for cancer therapy. The application of PDT is restricted because of its poor selectivity to tumor and hypoxia-induced resistance. Thus, to enhance the effectiveness of PDT, they combine TPZ and lipophilic IR780 entrapped liposomes with PDT. The hydrophilic chemotherapeutic drug (TPZ) was entrapped into the core and lipophilic IR780 was entrapped into the lipid bilayer. IR780 helps to produce ROS and hypoxic microenvironment in local site. This led to release TPZ radicles, causes DNA double strand break. These liposomes cause cell death (by inducing ROS) in response to laser irradiation. *In vivo* study showed that the TPZ and lipophilic IR780 loaded liposomes have higher anticancer efficacy through combining PDT with chemotherapy. Thus, TPZ and IR780 bearing liposomes have immense potential to combine PDT with hypoxia-activated chemotherapy (Yang et al. 2019).

3.3 Theranostic liposomes

Theranostics liposomes have been designed to combine multiple imaging agents with bioactives to improve therapeutic effects in advance stage of cancer. The liposomes with higher entrapment efficiency (for hydrophilic and lipophilic agents) are formed to co-deliver different diagnostic and therapeutic agents (Kelkar and Reineke 2011). Thus, this strategy can enhance the imaging capacity along with the cancer treatment. Karpuz et al. (2020) developed folate-targeted PTX and VNB encapsulated theranostic liposomes for diagnosis and treatment of NSCLC. The particle size, zeta potential and %EE of these liposomes was found to be around 150-180 nm, -10mV, and 15% (PTX) and 20% (VNB), respectively. The uptake study suggested that the uptake of drug for targeted liposomes was greater than the untargeted liposomes (in H1299 cells). The targeted liposomes displayed higher cellular internalization rate for H1299 cells than A549 cells. The liposomes showed good biocompatible profile on cancerous cells. Anticancer efficacy of

dual-drug loaded liposomes was higher than single drug encapsulated liposomes. Thus, these theranostic nanosized, dual-drug loaded liposomes provide a wide platform for NSCLC treatment (Karpuz et al. 2020). In another study, photosensitizer (verteporfin (VP)) loaded theranostic liposomes have been developed. These liposomes formed by DPPC and the triblock copolymer (F127), and functionalized with 5(6)-carboxyfluorescein (CF) due to its photodynamic potential. Additionally, the cell uptake study showed that VP has 6.0 ns, monoexponential decay by time-resolved fluorescence microscopy. This photoactive approach was used to reduce 98% viability in T98G cells. Thus, it could be concluded that novel theranostic liposomes have immense potential in cancer treatment (de Oliveira et al. 2020). Karpuz et al. (2021) developed folate targeted, dual drug loaded, Tc-99m radio labeled liposomes for *in vivo* and *ex vivo* assessment. The dual drug loaded liposomes exhibited higher cytotoxicity profiles than blank formulation in LLC1 cells. Bio-distribution study was carried out in NSCLC tumor-bearing C57BL/6 mice. The cell uptake assay demonstrated that the radiolabeled, folate targeted, dual drug loaded liposomes (Fol-Lipo/PCX/VNB) showed higher uptake of drug (compared to non-targeted liposomes). Histopathological study displayed that the targeted liposomes could not only suppress the tumor growth but also reduce lung metastasis (Karpuz et al. 2021). In one study, biomimetic system (DOX-QDs-Lip@M,) has been synthesized for the treatment of breast carcinoma. The DOX and quaternary QDs (as imaging agent) were encapsulated into liposomes. These liposomes were prepared by fusing and coating the isolated macrophages on the surface of liposome. This strategy improved blood circulation time of biomimetic system, leads to target tumor cell. Additionally, naturally formed bio-film can stabilize the liposomes structure, and inhibit drug leakage. Thus, the biomimetic liposomes could improve diagnosis and cancer treatment (Liang et al. 2020). Zhang et al. (2018) developed multifunctional hypoxia-induce theranostic liposomes. They were loaded with photosensitizer (Chlorin e6 (Ce6)), hypoxia-activated prodrug (Tirapazamine (TPZ)), and gene probe for synergistic PDT. The Ce6-mediated PDT upon irradiation with a laser induces hypoxia, leads to disrupt the liposomes and induced the antitumor effects of TPZ for enhanced cancer cell killing. The gene probe helps to detect oncogenic biomarker which improves diagnosis. The results of *in vitro* and *in vivo* assay displayed that the developed system showed enhance anti-cancer activity as compared to conventional PDT. This study concluded that the hypoxia-responsive multifunctional liposomal combined PDT can be used as synergetic approach in cancer diagnosis and treatment (Zhang et al.

2018). Saesoo et al. (2017) prepared superparamagnetic iron oxide nanoparticles (SPIONs) conjugated with rituximab (RTX) containing liposomes for brain targeting. The BBB restrict the entry of bioactives into the CNS in brain. To overcome this problem, targeted nanocarriers are used to transport drug specifically into cancer cells. The liposomes having theranostic property enhance the transport of drug to target site. The results of TEM and VSM (Vibrating sample Magnetometer) study indicated that the SPIONs loaded liposomes are spherical in shape and having super paramagnetic property. The BBB model was used to determine targeting and therapeutic efficiency of liposomes. This study concluded that the liposome could use as effective DDS for the delivery of RTX into brain (Saesoo et al. 2018). The chemiluminescent liposomes have also been formulated as a theranostic carrier for the detection of tumor cell under oxidative stress conditions. Hydrogen peroxide (H_2O_2) is one of the fundamental key of oxidative stress and a marker of ROS related diseases (cancer). However, particular exposure and scavenging of overproduced H_2O_2 provide an effective treatment for ROS-associated diseases. This property helps to identify malignant tumor cells utilizing chemiluminescent peroxyate response. Thus, novel contrast agent existing for H_2O_2 (peroxyate) liposomes were developed, which identify H_2O_2 through chemiluminescence response. These liposomes are formed by bis (2, 4, 6-trichlorophenyl) oxalate (TCPO) and curcumin as fluorophore. Here curcumin is used for selective tumor damage and act as photosensitizer which leads to cell death. The cell uptake study suggested that the liposomes improve extravasation into the membrane and enhance the bioavailability of curcumin (Mohammadi et al. 2019).

3.4 Immunoliposomes

Liposomes are modified with antibody (Ab) where Ab fragments have been widely used as targeting moieties called as immunoliposomes. These immunoliposomes have high specificity for their target antigens. Active targeting was achieved by binding of liposomes to the tumor cell-specific receptors. Moreover, these Ab conjugated liposomes can enhance the sensitivity of cancer cells to other chemotherapeutics (e.g. through the use of siRNAs). These liposomes employed for tumor targeting by modifying normal immune system. The system comprises of two types of immunity, innate and acquired immunity. Innate immunity includes immune system elements that provides immediate host defense. This immunity includes cells such as neutrophils, monocytes, and macrophages. The acquired immunity involves B and T lymphocytes, and seen in higher animals. Acquired immunity is far slower to grow than innate immunity but

retains memory for specific antigens. Both innate and acquired immunity have been examined for immunomodulatory response (Jain and Jain 2014; Jain and Jain 2015; Jain and Jain 2016d). Zhang et al. (2020) prepared DP7-C-functionalized liposomes to improve immune response and provide anticancer effect of vaccine (a neoantigen-based mRNA). In this study, cholesterol modified cationic peptide DP7 (having transmembrane structure and immune-adjuvant property) has been used. The DOTAP (DOTAP/DP7-C) altered liposomes were utilized for efficient mRNA delivery. The liposomal system was mainly used to enhance the transport of mRNA encoding individualized neoantigens to dendritic cells (DCs) and activate the DCs. This system has dual properties, immunoadjuvant and as a carrier to transport mRNA. *In vitro* study suggested that the liposomal system could deliver mRNA to different type of DCs. It also promotes the development of antigen-specific lymphocyte reactions that were better than DOTAP/LL2 neoantigen-encoding mRNA complex group. Thus, these liposomes could provide a promising platform for delivery of mRNA (Zhang et al. 2020). In another study, c(RGDyK)-modified liposome were developed for targeted spatial delivery into gliomas cells. This study enhanced brain penetration and anti-glioma efficacy. The integrin $\alpha v \beta 3$ receptors are overexpressed on capillary endothelial cells and in glioma cells. This receptor has been employed to target tumor cell via c(RGDyK)-modified liposomes for targeted spatial delivery into glioma cells. The c(RGDyK)-modified liposome encapsulated with AL3810 which bring hypothermia on multiple dosing. This anti-c(RGDyK)-modified liposomes activated IgG and IgM antibody with suitable complements (C3b and C5b-9) cause complement dependent opsonization. This system has some benefits like targetability, higher blood circulation and anti-glioma efficacy. Thus, the c(RGDyK)-modified liposome have promising potential for cancer therapy (Li et al. 2020). Jacobberger-Foissac et al. (2020) reported three vaccine alternatives, with a peptide from HA flu virus protein (as CD4 epitope), HPV16 E7 oncoprotein (as CD8 epitope) and TLR4, TLR2/6 (as adjuvant) in this system. TLR4 liposomes induce a potent Th1-oriented anticancer immunity that leads to suppress tumor growth and increase the survivability of mice. Thus, TLR4 liposomes were considered as a better treatment against the HPV-transformed orthotopic lung tumor mouse model. Moreover, TLR2/6 liposomes induce weaker Th1-immune response which was not sufficient for the induction of prolonged anticancer activity. Therefore, this study provides a wide platform for strategical development of vaccines (Jacobberger-Foissac et al. 2020). In another study, PEGylated liposomes (PEG-Lip) have been developed to transfer

antigens to splenic marginal zone B (MZ-B cells). MZ-B cells are produced from spleen and responsible for efficient trapping of blood borne pathogens and for induced primary antibody response. The blank PEG-Lip and antigen-loaded-PEG-Lip were introduced intravenously and induced antigen-specific immunity. The liposomes were functionalized with several PEG derivatives having different functional groups via. (methoxy PEG (CH₃O-PEG), hydroxy PEG (HO-PEG) or polyglycerol (PG), to activate the complement system and deliver a standard antigen, ovalbumin (OVA), to splenic MZ-B cells by *in vitro* and *in vivo*. The *in vitro* study indicated that the modified HO-PEG-liposomes activated the complement system and assisted the preferential association of HO-PEG-liposomes with MZ-B cells. After a single dose immunization system, it provides effective antigen delivery to MZ-B cells and improves earlier immunization system. Hence, this approach could deliver antigen to MZ-B cells that induced humoral immune response (Shimizu et al. 2020). Yoshizaki et al. (2017) prepared pH sensitive polymer modified liposomes (MGlu-HPG-liposomes) for the stimulation of specific immune response. They used cationic lipids to form MGlu-HPG-liposome which activate DCs and improve anticancer effects. The CpG-DNA (a ligand to toll receptor 9 (TLR9)) expressed in endosomes of DCs) was introduced into polymer modified liposomes containing cationic lipids. This ligand used to activate antigen-specific immunity by complexation methods. Here TLR9 receptor is expressing in endosomes of DC. The liposomal solution expressed TLR9 receptor more efficiently than free antigen solution. These liposomal formulations induced cytokine production and promoted *in-vivo* antigen-specific immune activities. They also have good anticancer effects than conventional pH-sensitive polymer-modified liposomes. The outcomes suggested that the MGlu-HPG-liposome could be a better approach to design effective vaccine carriers (Yoshizaki et al. 2017). Liang et al. (2017) developed liposomes-coated gold nanocages (Lipo-AuNCs) for targeted delivery of melanoma antigen peptide TRP2 to promote activation of DCs, and increase tumor explicit T lymphocytes reactions. The *in vivo* study established that the targeted antigen-bearing AuNCs could improve anticancer immune response by inhibiting tumor growth and metastasis (in lungs metastatic models). Hence, Lipo-AuNCs was a favorable nanoplatform for anticancer immunotherapy and *in vivo* imaging (Liang et al. 2017).

4. Marketed liposomal products

Liposomes were first discovered by Bangham in 1960s, but at that time it was not marketed for clinical use. Various researches have been done to market the drug loaded

liposomes in cancer treatment. This leads to approve the first DOX-loaded liposomal formulation (Doxil®). Doxil® is a marketed anticancer drug, comprises of DOX which is entrapped in the liposomes. It was approved in 1995 to treat AIDS-related kaposi's sarcoma, ovarian cancer and breast cancer. Doxil® is consists of high phase-transition-temperature (T_m) phospholipid HSPC, Chol, MPEG-DSPE in a molar ratio of 56:38:5 (Bulbake et al. 2017). Consequently several products have become available for the treatment of cancer and different disease. Additionally, Marqibo is used to treat lymphoblastic leukemia. It is composed of vincristine sulfate liposomal injection, designed to enhance the pharmacokinetics and pharmacodynamic of vincristine. In 2015, Merrimack Pharmaceuticals marketed a newer anticancer product i.e. Onivyde. It is composed of liposomal irinotecan injection for intravenous administration. Irinotecan acts by inhibiting topoisomerase-I enzyme (it allow DNA replication inducing single-strand breaks in DNA) and leads to cell death. It is extensively used in the treatment of pancreatic cancer (Saraf et al. 2020b). Some marketed liposomal DDS are described in table 2.

Table 2 Marketed liposomal formulation for cancer

Marketed Product	Drug used	Application	Manufacturer Company	Year
Doxil	Doxorubicin	Ovarian cancer, AIDS-related Kaposi's sarcoma, multiple myeloma	Sequus Pharmaceuticals, USA	1995
Depocyt	Cytarabine	Lymphomatous meningitis	Sky Pharma Inc., La Jolla, CA, USA	1999
Myocet	Doxorubicin	Breast cancer	Elan Pharmaceuticals, Princeton, NJ, USA	2000
Marqibo	Vincristine	Lymphoblastic leukemia.	Spectrum Pharmaceuticals, USA	2012
Onivyde	Irinotecan	Pancreatic cancer	Merrimack Pharmaceuticals, USA	2015

5. CONCLUSION AND FUTURE PERSPECTIVES

Cancer is caused by several genetic alterations and uncontrolled growth of abnormal cells. There are several types of anticancer agents commonly employed in cancer therapy using liposomes, including DOX, PTX, DTX, gemcitabine (GEM), vincristine (VCR) etc.

Selective receptors present on cancer cells provide an opportunity for targeted DDS. Targeted liposomes are able to identify specific molecular systems on cell surface. This strategy, known as active targeting, is able to enhance the specificity of DDS interaction with target tumor. Liposomes have multi-functional properties based therapeutic strategy for achieving desirable outcomes. The liposomes exhibit potential strategies for the treatment of cancer such as surface modification (via ligands and PEGylation) and stimuli-sensitive characteristics. These advanced liposomes could improve the applicability of liposomes in cancer therapeutics. Additionally, the ligand-modified liposomes selectively target the bioactive to the cancer site and increase the efficiency of bioactives. Moreover, better understanding of molecular biology can help in the selection of existing and novel targeting moiety for cancer treatment. In future, researcher will focus on the fabrication of safe and effective liposomes with improved efficacy for cancer treatment.

Conflict of interest

Authors declares no conflict of interest

Acknowledgements

Sarjana Raikwar (SRF), Pritish Kumar Panda (SRF), Pooja Das Bidla (SRF) and Shivani Saraf (SRF) are highly obliged to Indian Council of Medical Research (ICMR, New Delhi) for rendering funding assistance. Ankit Jain gratefully acknowledges financial support from the DBT-RA program (Govt. of India) in Biotechnology and Life Sciences.

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