Chapter 13
Functionalized Lipid Particulates in Targeted Drug Delivery

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13.1 Introduction

Lipids have been of major interest and importance in field of drug delivery systems. Their application in formulation science has been diverse and promising in different ways. The number of reports published each year on lipid based formulations and lipid based formulations in market establish critical role of lipids in drug delivery. Of considerable importance is the application of lipids in design of particulate systems which are amenable to surface modifications for improved drug/gene delivery. In the past few years, reports on different lipid based particulate systems have increased tremendously with major focus on liposomes and solid lipid nanoparticles (SLNs) [1–7]. Other lipid based particulate systems that are evaluated include nanostructured lipid carriers (NLCs) [6, 8–11], emulsions [12–17], lipid–drug conjugates (LDCs) and recently reported lipid nano-particulates in the form of LeciPlex [18, 19], and polymer-lipid hybrid nanoparticles (PLN) [20–22]. One of the various reasons responsible for success of lipids in formulation of particulate systems is their bio-compatible and biodegradable nature [2, 19, 23]. Thus, lipid based particulate systems can suitably be employed for delivery through not only noninvasive routes, such as oral and topical, but also through parenteral routes which are very demanding with reference to delivery system design.

Lipid particulate systems, over the years, have undergone stupendous modifications. Initially lipid particulate system of micron size were reported which were followed by lipid particulates in nanosize range. The nanosize range of particles made them capable of targeting tumors better, primarily because of enhanced

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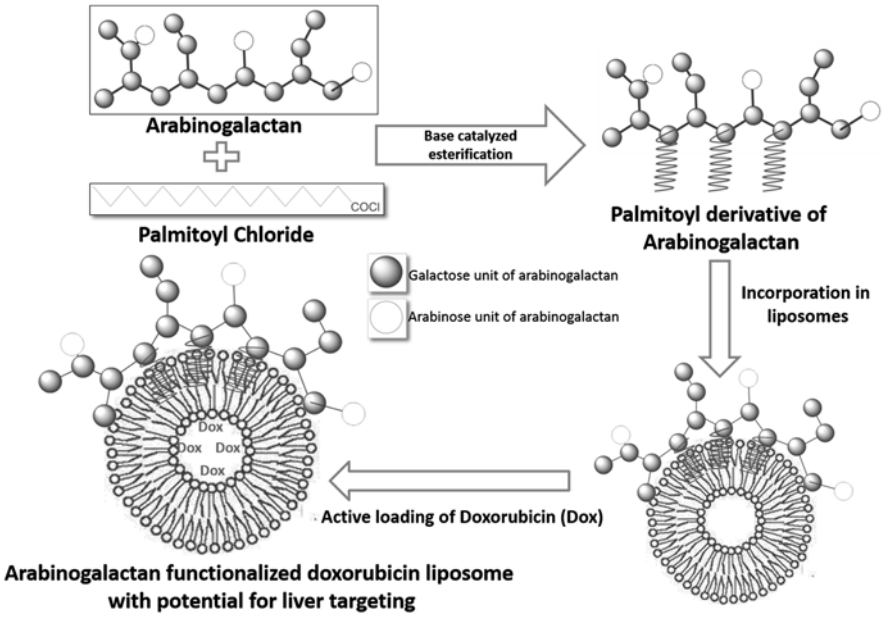

Fig. 13.1 Representation of one of the functionalized liposomal delivery systems [36]

permeation and retention (EPR) effect [24–31]. Subsequently the use of pH and/or temperature sensitive lipids to suit drug release in tumor environment made them more pronounced tool for tumor drug delivery [32, 33]. Another worthwhile modification was to render surface of lipid nanosystems more hydrophilic using different agents such as polyethylene glycols (PEGs) [33–35], which increased the systemic circulation time, resulting in prolonged drug release in plasma. Another significant achievement in lipid based particulate systems is conjugation of surface lipids to ligands that conferred ability to selectively target certain organs/tissue mass, and has been of great use in treatment of various infectious diseases and cancer in particular as depicted in Fig. 13.1 [3, 23, 25, 33, 36–45].

The chapter throws light on some key functional lipids employed in drug delivery and their final fate in human body. The chapter also focuses upon various lipid based particulate systems, their feasibility to functionalization and consequently their role in drug delivery.

13.2 Lipids as Excipients

The classification of lipids is very diverse. Lipids can be classified on the basis of their HLB values; their chemical structure such as glycerides, fatty acids, and so on; on the basis of their fatty acid chain length; depending on the charge they possess or even on the basis of their uses. The lipids discussed here are the ones predominantly employed in pharmaceutical formulations.

13.2.1 Phospholipid

Phospholipids comprise a huge family of lipids that have been instrumental in giving birth to a very important class of novel formulations known today, the vesicular drug delivery systems, better known as liposomes. Phospholipids are biocompatible as they are the key component of human cell membrane [46–51]. They are known to permeate skin as well as other cell membranes very effectively owing to structural similarity to cell membrane components [52–54]. Most phospholipids generally consist of a diglyceride, a phosphate group and a polar head group such as ethanolamine or choline, attached to phosphate moiety. This imparts them, both hydrophilic and hydrophobic regions in same molecule enabling them to act as efficient stabilizers at liquid–solid or liquid–immiscible liquid interface. Phospholipids have been extensively used as emulsifiers and stabilizers for a plethora of particulate systems such as nano-emulsions, micro-emulsions, polymeric and lipidic nanoparticles, and so on.

13.2.2 Liquid and Solid Lipids

Lipids comprise fatty acids, their monoglycerides, diglycerides, or triglycerides with C-chain length of 10 and more; waxes; sterols (cholesterol); and partial glycerides. Generally, short and medium chain glycerides fall in category of liquid lipids while long chain glycerides constitute most of solid lipids. Various solid lipids reported widely in recent literature reports include glyceryl behenate, glyceryl monostearate, glyceryl distearate, glyceryl dilaurate, glyceryl palmitostearate, cetyl palmitate, tristearin, tripalmitin, trimyristin, trilaurin, SOFTISAN® 142, long chain fatty acids like stearic acid, palmitic acid, decanoic acid, behenic acid, to name a few [1, 55–59]. Lipids help in better permeation of drug with poor permeability. Lipids also aid in improving solubilization of drug in gastrointestinal tract (GIT) by increase in micellization of drug by fatty acids liberated upon lipid digestion by pancreatic lipase and other enzymes [60–64]. Lipids also offer improvement in stability of photo- or pH dependent unstable drug [6, 19]. Importantly, both solid as well as liquid lipids, are amenable to chemical modifications imparting target specificity to them [23]. Some of these can be directly conjugated to active drug molecules to render a lipidic prodrug with better physicochemical properties which releases active molecule on reaching the desired site of action [65–67].

13.2.3 Lipids for Functionalization

Numerous reports are available on capability of functionalized lipids to improve drug delivery. Functionalization makes them either more target specific, or renders them a charge, or makes them capable of yielding pH/temperature-sensitive

nanoparticles or nanoparticles with greater plasma circulation half-life or a combination of all above mentioned attributes [68–74]. Attempts have been directed to conjugate hydrophilic molecules such as PEGs to lipids thus rendering stealth properties to the surface of nanoparticles fabricated from them. Conjugation of lipids to molecules possessing charge (+ve/−ve) renders them either a cationic or anionic charge, for instance dipalmitoyl phosphoglycerol (DPPG) imparts negative [75] and didodecyldimethylammonium bromide (DDAB) imparts positive charge [76] respectively. Various ligands based on immunoglobulins, carbohydrates, proteins, vitamins, and so on have been conjugated to lipids mentioned in greater detail later, to make them target specific. Another example of functionalizing lipids is of cholesteryl hemisuccinate molecule which is cholesterol ester of hemisuccinic acid which upon incorporation has been reported to impart pH sensitivity to the fabricated liposomes [77]. Functionalization of lipids has revolutionized the area of drug delivery, as elucidated, in the next sections of the chapter.

13.3 Fate of Lipid Particulate Systems

The major route of administration for a functionalized lipid based particulate system is the intravenous route, though a few functionalized lipid systems improve oral and transdermal absorption of actives. As is well known for metabolism of ingested lipids, and as is true for any lipid systems, whether functionalized or not, after oral delivery, lipids are acted upon by enzymes, especially pancreatic lipases, and the triglycerides are broken down to glycerol and free fatty acids or monoglycerides. After absorption in GIT, these free fatty acids are again reformed to triglycerides that are instrumental in formation of chylomicrons and lipoproteins. The lipoproteins are again responsible for utilization and excretion of cholesterol and also in formation of bile salts. The excess fatty acids is utilized for energy production inside the mitochondria of cells after their uptake as they enter TCA cycle.

Final fate of lipids even when administered intravenously as functionalized nanoparticles remains the same with a few differences. The functionalized lipid owing to target specificity reaches the target cells, helps in internalization of particles via receptor mediated endocytosis or carrier mediated uptake. However, once inside the cell, the biochemical pathway for the lipids remains the same as for lipids ingested by any other route, except that the targeted lipid is initially acted upon by enzymes to break the bond between the targeting ligand and lipid.

In addition to conferring targeting ability, lipid ligand employed for functionalization in lipid particulate system may influence in vivo course. It can render the surface of lipid nanosystems hydrophilic or charged depending on the property of ligand associated with lipid. This is beneficial with respect to reduced opsonization and RES uptake thus resulting in slower clearance and improved circulation times. PEGylation of lipids significantly improves circulation half-life of lipid nanosystems. The complex process of opsonization determines the eventual fate of the lipid

particulate system like its rate of clearance from the blood stream, volume distribution, organ distribution and its elimination from the body. PEGylation is an obligatory requirement to prolong circulation for delivery systems functionalized via antibodies, due to their known interaction with reticuloendothelial system leading to faster clearance of antibody functionalized delivery system from the circulation. Functionalization can change the pharmacokinetic and pharmacodynamics of the lipid based delivery system having a negative or positive effect on the therapeutic efficacy of the system [78].

13.4 Formulation Considerations for Functionalized Lipid Nanosystems

As in case of any formulation, the major concern for functionalized lipid nanosystem from point of view of formulation scientist is its ease to scalability and commercialization. Till the early 1990s, there were no high hopes about the viability of nanoparticulate based delivery systems in market, though the scenario started to change from late 1995, after introduction of Doxorubicin and Amphotericin B loaded liposomes (Doxil®, Caelyx®; and Ambisome®). Since then, various nanoparticulate systems, and predominantly lipid based nanosystems, have been introduced in market.

Today, functionalized lipid nanoparticles find themselves in similar situation as nanoparticles did two decades ago. But their future will depend on the ease of functionalization provided if those techniques can be scaled up without compromising the yield.

Tremendous efforts have been observed recently to effect functionalization of lipids to render delivery systems target specific. Ligands specific to receptors (targets) have been conjugated to lipids by either strong covalent bonds or weak van der Waal forces or hydrogen bonding. Covalent linkages generally employ esterification, amidation or sulfonation reactions between lipids and the ligand. Lipids used possess an acid, amine, sulfate, or alcohol functional group which is accordingly conjugated to its counterpart functional group/s on ligands. One important consideration during functionalization is that the ligand after being coupled to lipid should not show reduction in its affinity for target site. The alternative method of coupling ligands to fabricated lipid nanosystem as reported by many scientists is incubation or lyophilization of ligand together with the nanosystem. In such cases, the association of ligand to surface of lipid nanocarriers has been confirmed by various techniques, but predominantly by measurement of surface charge (zeta potential).

Literature also mentions use of linkers to couple ligand and lipid together. Linkers which are reported include pyridylditiopropionoylamino-PEG, hydrazide-PEG, maleimide-PEG, *p*-nitrophenylcarbonyl-PEG-PE, pyridylthiopropionoylamino-PEG-distearoylphosphatidylethanolamine (PDP-PEG-DSPE) to name a few [79, 80]. It is hypothesized that the linker allows the ligand to be extended from the

Table 13.1 Different ligands used for targeting

Category	Ligands	Receptor target	References
Immunoglobulins	Monoclonal antibodies	Overexpressed antigens on tumor cells	[79, 83–90]
	IgG	Overexpressed antigens on tumor cells	[90]
Carbohydrates	Sugars like galactose, mannose, fucose	Carbohydrate receptor	[91]
	Arabinogalactan	ASGPR (asialoglycoprotein receptor), a type of carbohydrate receptor	[36]
	Pullulan	ASGPR (asialoglycoprotein receptor), a type of carbohydrate receptor	[92, 93]
Proteins	Glycoproteins like transferrin, lectins	Transferrin receptor	[93, 94]
	RGD (arginine-glycine-aspartic acid oligopeptide)	αvβ3 integrins overexpressed mainly on endothelial cells	[95]
	Cell penetrating peptides such as TAT peptides	Interacts directly with lipid bilayer of cells to ensure faster cell internalization	[96]
Vitamins	folate or folic acid	Folate receptor	[37, 38, 70]
	Biotin	Biotin receptors overexpressed on tumor cells	[71]
Amino acids	Glutamic and aspartic acids	Amino acid receptors	[40]
	Arginine	Amino acid receptors	[97]
Actives/chemicals	Haloperidol, anisamide	Sigma receptors	[98, 99]
Positively charged lipids	Cetyltrimethyl ammonium bromide, Lipofectamine, didodecyldimethyl ammonium bromide	Negatively charged phospholipids overexpressed on tumor cells	[70]

surface for better interaction with receptors; however, ligand–lipid conjugates without linkers have also been observed to be equally effective in targeting [81].

Once the lipid–ligand conjugate is prepared, it is then incorporated in suitable amounts in the formula to ensure target specific lipid nanosystem is designed. Different percentages of conjugate are used in preparation of system and based on further in vitro experimentation; the right combination could be selected for further in vivo studies.

The various ligands which have been coupled to lipids as reported in literature are mentioned in Table 13.1 [25, 33, 82]

13.5 Route of Administration

The major concern with functionalized lipid particulates is toxicity. They should be safe at doses to be administered and for the route that they will be employed for. Most functionalized lipid particulates are designed to be administered by parenteral route (mainly intravenous delivery) in order to utilize their target specificity. Lipid particulates wherein surface is rendered more hydrophilic (by association with PEGs or gangliosides) or cationic (use of cationic lipids) are employed for intravenous delivery to improve plasma circulation time by minimizing opsonization and RES uptake. Positively charged lipid based delivery system are also known to improve oral absorption.

Functionalized lipid particulates have been employed for improving drug delivery through other routes including transdermal, pulmonary, vaginal, rectal, nasal, and ocular as reported in scientific literature (Table 13.2).

13.6 Applications in Drug Delivery

13.6.1 Liposomes

Liposomes are the most researched and preferred vesicular drug delivery system due to its versatility, safety, and in vivo advantage. Functionalization of liposomes is achieved via many techniques. The primary and the simplest technique is adsorption. In this technique, the preformed liposomal dispersion is incubated with the solution of targeting ligand. This approach of functionalization is not preferred as it is nonspecific and in most of the cases the rate of desorption is fast during storage as well as in vivo. Other technique of functionalization employs covalent link formation between targeting ligand and appropriate component of liposomes. This is usually achieved via chemical attachment of long carbon chain to functional groups like hydroxyl, carboxyl present on the targeting ligand. An important consideration

Table 13.2 Different routes of administration for functionalized lipid based nanoparticles

Type	Reported route/possible route of administration
Liposomes	
Stealth, galactosylated, mannosylated immunoliposomes, arabinogalactan associated liposomes, haloperidol anchored liposomes, folate conjugated liposomes	For improved intravenous and pulmonary delivery
Solid lipid nanoparticles	
Biotinylated	Improved delivery through oral and ocular epithelium
Galactosylated	Targeting via intravenous route
Nanostructured lipid carriers	
Squalene associated NLCs	Application to skin to achieve better percutaneous absorption
Transferrin associated NLCs	Targeting via intravenous delivery
Cholesterol rich NLCs	Intravenous delivery to target brain
Lipid drug conjugates	Oral/Parenteral route
Nano- and micro-emulsions	Oral/Intravenous route
Mucoadhesive nano-emulsions	Intranasal delivery for brain targeting
LeciPlex, Invasomes, Ethosomes, Transfersomes	Mainly evaluated for topical delivery
Polymer–lipid hybrid nanoparticles	For intravenous delivery
e.g., Lecithmer, Lipomer	

for chemical modification is that the groups chosen for chemical modification do not alter the receptor ligand interaction significantly. Another technique for functionalization involves chemical modification of preformed liposomes. In this technique, the preformed liposomes are incubated with linkers that covalently bind to the phospholipid head groups present on the surface of liposomes. These linker tagged liposomes after separation are treated with a solution of targeting ligand wherein the free end of the linker covalently attaches itself to the functional group present on targeting ligand, imparting targeting ability to liposomes.

Vodovozova et al. showed improvement in efficacy of a synthetic drug octadecylmerphalan after its incorporation in a liposomal delivery system functionalized by use of lectin specific carbohydrate ligand Sialyl Lewis X using its 3-aminopropyl glycoside derivative [65]. In vivo results confirmed superior therapeutic efficacy of Lectin functionalized liposomes as compared to liposomes devoid of it. Tsuruta et al. successfully loaded doxorubicin in an actively targeted liposomal delivery system using Sialyl Lewis X for preventing stenosis after angioplasty [100]. Study established superior activity of doxorubicin liposomes functionalized with Sialyl Lewis X as rats treated with functionalized liposomes had larger lumen area as compared to those treated with Doxorubicin liposomes. Kawakami et al. investigated the effect of glycosylation using galactose, mannose, and fucose on the clearance of liposomes. They concluded that galactose coated liposomes were taken up by the asialoglycoprotein receptor of the parenchymal cells of liver, mannose coated

liposomes were taken up by the mannose receptor on the non-parenchymal cells of the liver and fucose coated liposomes were taken up by the fucose receptor on the non-parenchymal cells of the liver. A higher molar concentration of galactose coated liposomes were also taken up by the non-parenchymal cells of the liver [91]. Shah et al. successfully fabricated actively targeted liposomal delivery system using asialoglycoprotein receptor specific arabinogalactan as the targeting ligand using covalent link to lipid component [36]. For further reading on use of carbohydrate mediated liposomal targeting, readers are referred to an excellent review by Malcolm N. Jones [101].

Wolff and Gregoriadis successfully fabricated monoclonal anti-Thy1IgG1 coated immunoliposomes for targeting to AKR-A cells [90]. Debs et al. successfully fabricated anti-Thy 1.1 monoclonal antibody MRCOX7 conjugated liposomes which demonstrated enhanced uptake in lymph nodes that express high levels of target antigen [84]. Koning et al. showed high intracellular delivery of cytotoxic agent by developing immunoliposomes using monoclonal antibody against rat colon carcinoma containing 5-fluorodeoxyuridine as the cytotoxic agent [86]. Suzuki et al. developed long circulating immunoliposome containing doxorubicin using murine monoclonal antibody HBJ127 that recognizes a peptide epitope of gp125 which is expressed on almost all human cancer cells [88, 89]. Mercadal et al. fabricated My-10 monoclonal antibody coated immunoliposome against CD34 antigen using carboxyfluorescein as marker compound [102]. Yang et al. formulated PEGylated immunoliposome loaded with paclitaxel using Herceptin as targeting ligand for cells overexpressing human epidermal growth factor receptor 2 [103]. Lukyanov et al. modified commercially available doxorubicin loaded long circulating liposomes Doxil® with monoclonal nucleosome specific 2C5 antibody that identifies tumors via surface bound nucleosomes [85, 87]. Biswas et al. reported enhanced suppression of tumor in vivo by surface functionalization of doxorubicin loaded long circulating liposome Doxil® using a cell penetrating peptide Octa-arginine [97].

Kitagawa and Kasamaki improved the intradermal delivery of retinoic acid using positively charged liposomes functionalized with 1,2-dioleoyl-3-trimethylammonium propane as cationic surfactant [104]. Knudsen et al. demonstrated improved delivery of calcipotriol by formulating and altering the fluid state of liposomes composed of dipalmitoyl phosphocholine and dilauroyl phosphocholine [105]. Geusens et al. developed ultradeformable cationic liposomes composed of cationic lipid 1,2-dioleoyl-3-trimethylammonium propane and the edge activator sodium cholate for delivery if siRNA into human primary melanocytes [106].

13.6.2 Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs)

SLNs and NLCs have been reported to a good extent for functionalization and targeting. Modifications are reported either by functionalizing the lipid that is employed for SLN/NLC preparation or the surfactant that is employed in its colloidal

stabilization. Xu et al. designed hepatoma targeted SLN for effective delivery of docetaxel wherein galactosylated-DOPE was utilized for site-specific delivery [23]. The authors report the potential of galactosylated SLNs in effective treatment of locally advanced as well as metastatic hepatocellular carcinoma. In comparison to non galactosylated SLNs, galactosylated-SLNs demonstrated no significant difference in mean particle size, zeta potential, drug loading, and entrapment efficiency. However, remarkable difference was noted in in vitro cytotoxicity and biodistribution of docetaxel when loaded in targeted SLN as compared to normal SLN. Galactosylated SLNs were superior to nongalactosylated SLN as well as marketed Taxotere formulation for improved delivery of Docetaxel.

Alukda et al. fabricated functionalized SLNs with coats of PLL (polylysine) and heparin, as a delivery template, loaded with vaginal microbicide (tenofovir) for prevention of HIV transmission [39]. The authors reported improved therapeutic efficacy of functionalized SLN as compared to nonfunctionalized SLN with no cytotoxicity to vaginal epithelium.

Yet another interesting example is of Kashanian et al. who functionalized SLN with *N*-glutaryl phosphatidylethanolamine and employed in the design of SLN to render the SLN pH sensitive, for enhancement in drug release in acidic pH of tumor [40]. When studied under different pH conditions, pH sensitive SLNs exhibited higher in vitro drug release at acidic pH.

NLCs were designed as a modification of SLN to improve their drug loading capacity, improve their colloidal stability, and decrease drug leakage during shelf life of product. They comprise both liquid and solid lipids unlike their SLN counterparts that make them comparatively more versatile as a drug delivery vehicle. NLCs have also been reported to be amenable to functionalization. Yang et al. prepared hyaluronic acid coated NLCs for targeted delivery of Paclitaxel to CD44 overexpressed on tumor cells. The study reports that functionalized NLCs were superior to Taxol in vitro as well as in vivo. Earlier Chen et al. had also published their work for targeted delivery of Paclitaxel. They conjugated Stearyl-2-amino-2-deoxyglucose (2-DG), a glucosamine derivative serving as a broad tumor targeting ligand, to glyceryl monostearate and oleic acid NLCs, for improved delivery of Paclitaxel to tumor cells.

Folate and transferrin have also been anchored to lipid nanoparticles for establishing tumor specific delivery of etoposide [94]. As mentioned earlier, choice of excipients can also render delivery system like SLN/NLCs target specific. Goppert and Muller discovered that SLN stabilized by Tween 80 adsorbs such plasma proteins as may be required for efficient targeting to brain, whereas Poloxamer 188 stabilized SLN adsorb proteins which imparted ability to prolong plasma circulation time [107]. Several reports have been published about binding of Tween 80 to apolipoproteins in plasma and thus capable of targeting and traversing blood–brain barrier. Incorporation of such functional surfactants confers target specificity to delivery system. Similarly, Rezazadeh and coworkers designed tumor targeted NLCs composed of cholesterol, known for targeting LDL receptors overexpressed on tumor cells [108, 109].

13.6.3 Nano- and Micro-emulsions

Not many reports are available with respect to functionalization of emulsions involving chemical reactions. However, use of specific surfactants possessing charge, negative or positive, in fabrication of charged nanoglobules of emulsions has been examined for improving bioavailability of actives as well as in gene delivery.

13.6.4 Miscellaneous Functionalized Lipid Based Systems

PLNs are a class of lipid nanocarriers that have surfaced up about a decade ago and have demonstrated capabilities to functionalization. PLN was viewed as an attempt to combine advantages and avoid disadvantages of polymeric nanoparticles and liposomes. Though dependent on method of preparation, general PLN description mentions presence of a polymer core coated with lecithin (or other lipid) which in turn can be conjugated/anchored to a ligand. Liu Y. et al. demonstrated that PLN composed of PLGA as polymer and mix of lipids, including PEGylated as well as folate conjugated PEGylated phospholipid [110]. The folate and PEG functionalized PLN when loaded with Docetaxel released ~18 % which was at surface of targeted PLN within first 12 h as would be required to exert immediate action on cancer cells. Thereafter, however, release was considerably slow, with only ~60 % Docetaxel released at the end of 72 h and sustained release continued for 168 h. The in vitro release study results correlated well with results of in vitro cytotoxicity study as well as cell uptake study, demonstrating ability of functionalized PLN to improve drug uptake possibly due to receptor mediated endocytosis.

Selection of polymer also in its own way contributes to functionalization of PLNs. Wu and coworkers demonstrated that use of soybean oil based anionic polymer provided adequate loading of cationic drugs such as Doxorubicin HCl due to ionic complexation in PLN in comparison to their SLN counterparts which lack in polymer content [22].

However, one of the interesting studies on PLN was reported by Clawson et al. who reported fabrication of PLN with pH triggered erosion of PEG shell covering PLN coat. The group designed PLN system for effective treatment of cancer with ability to erode the coat of PEG in response to low pH at tumor site. The targeting lipid (succinate ester based PEGylated lipid) used in design of PLN was synthesized by reacting 1,2-dipalmitoyl-*sn*-glycero-3-phospho(ethylene glycol) with methoxy polyethylene glycol. At neutral pH (physiological pH), the PEG surface coat will be more stable to hydrolysis whereas, in acidic tumor environment, due to erosion of PEG coat, the system will destabilize and show better fusion with cells at tumor site. However, it was worth noting that more the amount of synthesized functional lipid

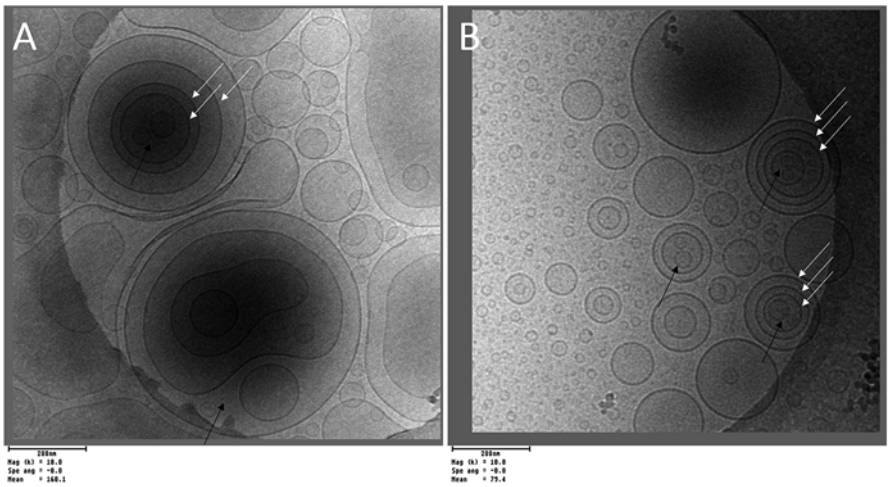

Fig. 13.2 CryoTEM images of LeciPlex system comprising cetyl trimethyl ammonium bromide (**a**) and didodecyl dimethyl ammonium bromide (**b**) as charged lipids. These are multivesicular (*black arrow*) multilamellar (*white arrow*) vesicles having a unique structure. They are unlike the classical multilamellar vesicles as each lamella is well differentiated giving an appearance of concentric rings

used in fabricating the PLN system, more stable was the developed PLN system to aggregation and destabilization, possibly due to greater number of ester bonds which need to be hydrolysed. Thus, PLN with 15 mol% functional lipid were not stable at pH below 6.0, with 20 mol %, stability was lost at pH 5.0, with 30 mol %, particles retained stability until pH dropped to 4.0, with 40 mol %, PLN do not destabilize until pH drops to 3.0 and with 50 mol %, PLN system was stable over entire pH ranges evaluated (pH 7.4–3.0).

A great emphasis has been placed on lipid based nanosystems which are amenable to surface functionalization that aids in rendering them target/site specific. However, several novel lipid based nanosystems can be considered functionally distinct from the others owing to their potential in improving delivery of actives. These include various deformable/flexible vesicular systems like LeciPlex, transfersomes, invasomes, and ethosomes. Though all of these are vesicular in nature, the systems do possess notable differences imparting them certain specific characteristics.

LeciPlex is a novel vesicular system that combines advantages of cationic nanoparticles with those of vesicular systems (Fig. 13.2). The distinct advantage of LeciPlex system is ease of formulation and its amenability to scale-up. It employs a single step fabrication involving addition of aqueous phase to solution of phospholipid and cationic agent in a biocompatible solvent leading to formation of vesicular system under simple agitation [18, 19]. LeciPlex are cationic vesicles, and

therefore capable of loading both hydrophilic as well as lipophilic drugs, and their cationic nature confers better colloidal stability to the system. Besides, the cationic charge allows them capability to target cancer cells overexpressing negatively charged molecules at their surface [111]. Also, the agents that impart positive charge to the vesicles possess selective cytotoxicity to various cancer cell lines. This makes LeciPlex a useful addition to drug delivery systems for cancer treatment. LeciPlex has been implicated in improving oral bioavailability of actives with different polarities, such as Repaglinide and Quercetin. We have established the utility of LeciPlex in condensing DNA for gene delivery. Besides oral delivery, LeciPlex holds a lot of potential for effective topical delivery, the experimentation for which are under way.

Deformable liposomes, ultradeformable liposomes, ethosomes, and invasomes are all, another important sect of functionalized vesicular carriers that have been mainly explored for dermal/transdermal delivery. Transferosomes consist of flexible bilayers and are the first generation of elastic liposomes [112, 113]. These are reported to enhance skin permeation of loaded actives through intact skin under influence of transdermal hydration and osmotic gradients, when applied under non-occluded conditions [113, 114]. The innovative addition in engineering Transferosomes as a different vesicular carrier than conventional liposomes has been addition of an edge activator. The edge activator is a molecule that provides desired flexibility and deformability to the lipid bilayers to improve its skin permeation wherein the vesicle is able to move into skin layers in intact form, thus improving flux of loaded actives [115]. Examples of edge activators include molecules such as sodium cholate, Span 80, Tween 80, and dipotassium glycyrrhizinate [116–118]. The second generation of deformable liposomes, named as proultraflexible liposomes, was reported by Jain et al., following the proliposomal approach known to enhance stability of vesicles. The formulation demonstrated better permeation of loaded active, levonorgestrel and better stability than proliposomal formulation [119]. Numerous reports are available that enlighten ability of deformable liposomes or their likes, such as ultradeformable liposomes or cationic ultradeformable liposome employing a cationic lipid [120] to improve not only skin permeation of actives, such as diclofenac, bleomycin [121], diclofenac [112], 5-FU [122], but also delivery of vaccines and genetic material like siRNA [106, 123].

Ethosomes, comprising phospholipids, a high ethanol content and water, are another specialized vesicular systems that are able to permeate into deeper layers of skin, as has been reported by few confocal laser scanning microscopy studies, due to their high malleability have improved systemic delivery of few actives. Ethosomes have reportedly improved skin permeation of various loaded agents including ketotifen, 5-aminolevulinic acid, and rhodamine red, to name a few [113, 124–129].

Yet another interesting vesicular carrier class comprises invasomes reported by Fahr A. et al. which in addition to phospholipids consists of mixture of terpenes that

act as permeation enhancers. The group reported fabrication of phospholipid based invasomes comprising 3.3 % ethanol and 1 % mixture of terpenes with size less than 150 nm, significantly improved Temoporfin deposition in stratum corneum as compared to conventional liposomes [130].

13.7 Marketed/Potential Lipid Particulates

There are many lipid particulates which have either entered clinical trials or have been successfully launched commercially [131]. In case of liposomal products, currently, there are 53 under therapeutic investigation and 8 liposomal products available commercially, most in comparison to any other lipid based system. There are 19 and 9 emulsion based products being available for therapeutic investigation and commercial use, respectively [132]. Table 13.3 mentions list of few of such functionalized lipid nanoparticulates which are successful or have promise to reach the market.

13.8 Summary and Conclusion

Functionalization of lipid particulates make them more promising for their intended use, such as enhanced permeation, increased target specificity, improved lipophilicity, and so on. A number of reports have been available recently on importance of functionalization of lipid nanosystems, as described earlier in the chapter. Task of functionalizing particulates renders excellent opportunity for formulation scientists and chemists to work together and lay foundation of successful novel drug delivery systems. Functionalized lipid nanoparticulates are expected to face the same challenge that was faced by lipid nanoparticulates a few years back with respect to their commercial feasibility. The efforts of multiple formulation scientists made the lipid based nanosystems a commercial success and it can be expected that functionalized lipid nanoparticles also reach market in increasing number and probability in near future.

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Table 13.3 Formulations under investigation/approved for functionalized lipid based nanoparticles

Name of product	Type of lipid particulate	Company/alliance	Status	Approved indications by FDA
Doxil (USA)	PEGylated doxorubicin HCl liposomes	Ortho Biotech	Available commercially	Metastatic ovarian cancer, AIDS related Kaposi's sarcoma
Caelyx (Outside USA)		Schering-Plough		
ThermoDox	Lyso thermo-sensitive liposomal doxorubicin	Celsion	Phase 2 and 3 clinical trial	Metastatic colorectal cancer and hepatocellular carcinoma
Lipoplatin™	Stealth liposome composed of SoyPC/DPPG/CHOL loaded with cisplatin	Regulon Inc.	Phase 3 clinical trial	Pancreatic cancer, lung cancer
EndoTAG-1	Positively charged liposomal paclitaxel	Medigene and SynCore Biotechnology	Phase 2 clinical trial	Pancreatic cancer and triple negative breast cancer
MBP-426	Oxaliplatin-encapsulated transferrin (Tf)-conjugated *N*-glutaryl phosphatidyl-ethanolamine (NGPE)-liposome	Mebiopharm Co., Ltd	Phase 2 clinical trial	Advanced or metastatic solid tumors, gastric adenocarcinoma, gastroesophageal adenocarcinoma, esophageal adenocarcinoma
SPI-077	Stealth liposome composed of SoyHPC/CHOL/DSPE-PEG loaded with Cisplatin	Sequus Pharmaceuticals	Phase 1/2 clinical trial	Head and neck cancer, lung cancer
C225-ILS-DOX	Anti-EGFR-immunoliposomes loaded with doxorubicin	University Hospital, Basel, Switzerland	Phase 1 clinical trial	Advanced solid tumors
MCC-465	Immunoliposome-encapsulated doxorubicin tagged with polyethylene glycol (PEG) and the F(ab′)2 fragment of human monoclonal antibody GAH	National Cancer Center, Tokyo	Phase 1 clinical trial	Stomach cancer
SGT53-01	Anti-transferrin receptor scFv tagged liposomal p53 DNA and docetaxel	SynerGene Therapeutics, Inc.	Phase 1 clinical trial	Treat solid tumors
S-CKD602	Stealth liposome containing camptothecin analog	Alza Co.	Phase 1	Several cancer types

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