

Chapter 6

Surface Engineering: Incorporation of Bioactive Compound

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Abstract Surface engineering facilitates incorporation of various bioactive compounds and provides unique advantages for the specific delivery of imaging and therapeutic agents. Several molecules with imaging, diagnostic, prognostic, sensing, and therapy can be incorporated in the bioformulations with the help of different surface engineering techniques. This chapter reviews drug carriers which were surface engineered for targeted drug delivery at the requisite location. A single or combination of surface engineering has been used for efficient delivery of carriers. The carriers reviewed here were divided into two categories: lipid-based carriers (liposomes and solid lipid nanoparticles) and non-lipid-based carriers (niosomes, polymeric nanoparticles, hydrogels, dendrimers, quantum dots, gold nanoparticles, and mesoporous silica nanoparticles). Various kinds of bioactive compounds along with the involvement of surface engineering techniques in incorporation were also discussed. This chapter focuses on recent advances in the surface engineering of nanocarriers for therapeutic applications.

Keywords Surface engineering · Lipid carriers · Non-lipid carriers · Liposomes · Active targeting · Solid lipid nanoparticles · Niosomes · Polymeric nanoparticles · Hydrogels · Dendrimers · Quantum dots · Gold nanoparticles · Mesoporous silica nanoparticles

6.1 Introduction

Surface engineering plays a pivotal role in preparation of nanocarriers. It involves the utilization of synthetic or natural biomaterials for advancement and amenability of biological function, i.e., targeted drug delivery system. The need for new biological materials and novel technologies arises the requirement of surface engi-

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neering. It helps in construction of molecular design, easy synthesis, and tailoring broad range of applications. Surface engineering has the ability to manipulate biological interfaces and precise the arrangement with the modification of surface according to the need and requirement, for the development of well-controlled bioformulations.

Three most important approaches for surface modifications are: direct deposition, incorporation, and encapsulation. Direct deposition means the filming of a bioactive compound directly on the surface of a nanocarrier. Whereas, in incorporation various bioactive compounds are integrated in between the surface layer of the nanocarrier using suitable surface modification techniques. Though, enclosing a bioactive compound in a nanocarrier, i.e., the addition of a bioactive compound inside a nanocarrier is called encapsulation. Therefore, the encapsulation of a bioactive compound in a nanocarrier means to encapsulate/surround a bioactive compound inside a nanocarrier, i.e., liposomes. Here in this chapter, we focused on the incorporation of bioactive compound in nanocarriers for drug delivery.

In current healthcare practice, especially in chemotherapy drug toxicity is an important factor for consideration. A conventional drug given to a patient affects both normal and diseased tissues. To decrease the adverse effects on normal tissues, targeting of the diseased tissues can be achieved via surface engineered targeted drug carriers. Many drug carriers, i.e., liposomes, solid lipid nanoparticles (SLNs), niosomes, polymeric nanoparticles (PNPs), hydrogels, dendrimers, quantum dots, gold nanoparticles (AuNPs), and mesoporous silica nanoparticles (MSNPs) have been developed, which include surface engineering for controlled release with enhancement of drug potency [1–4].

Here, we have categorized drug carriers into two classes, i.e., lipid-based and non-lipid-based carriers and further describes the surface engineering involved in the incorporation of bioactive compounds in these carriers. The difference between these two carriers is explained as follows. Lipid carriers are made up of lipid molecules and form a vesicle or certain structure to carry the desired biomolecules load. Liposomes and solid lipid nanoparticles are the examples of lipid-based carriers. Liposomes have phospholipid bilayers and contain incorporated drugs. Solid lipid nanoparticles have a solid lipid matrix with drug embedded in the matrix or adsorbed on the surface [2]. Non-lipid nanocarriers are those carriers which are composed of materials other than lipids such as nonionic surfactants, polymers, etc. Niosomes have nonionic surfactant bilayers and contain incorporated drugs. Polymeric nanoparticles are composed of natural or synthetic polymers and hydrogels are gels made of hydrophilic polymers. Dendrimers are synthetic polymeric macromolecules. Quantum dot is composed of a semiconductor material.

Applications of lipid-based nanocarriers have an advantage of least toxicity and drug safety. Lipid-based delivery systems include micelles, liposomes, solid lipid nanoparticles, nanoemulsions, and nanosuspensions. Lipid-based nanocarriers are a class of biocompatible and biodegradable vehicles. It provides advanced delivery of therapeutic and imaging agents, with improved pharmacokinetics. In this category, the most extensively studied drug carrier is liposome. A number of non-lipid-based nanocarrier formulations have also been described in terms of incorporation of

bioactive compound and their formulation with respect to surface engineering. Most of the non-lipid-based carriers are studied to improve the oral, intranasal, and central nervous system delivery of analgesic agents. This part includes niosomes, polymeric nanoparticles, hydrogels, dendrimers, quantum dots, gold nanoparticles, and mesoporous silica nanoparticles.

6.2 Lipid-Based Nanocarriers

6.2.1 Liposomes

Alec Bangham, the father of liposomes described them for the first time in 1965. First they were called as Banghasomes [5] but later were called as liposomes [6]. Phospholipid is amphiphilic in nature and has hydrophilic head with two hydrophobic fatty acid chains. They form multilamellar vesicles (MLVs) on the hydration of phospholipid film and the hydration is done above the phase transition temperature of the phospholipid. Cholesterol is added to lipid mixture to toughen the liposomes. The presence of charge on the liposomes provides physical stability to liposomes. The addition of DOTAP (1,2-dioleoyl-3-trimethylammonium-propane chloride) to lipid mixture provides +ve charge to liposomes. Cationic liposomes are used for the loading of -ve charged nucleic acids. Liposomes can be giant unilamellar vesicles (GUVs), multilamellar vesicles (MLVs), large unilamellar vesicles (LUVs), and small unilamellar vesicles (SUVs). The size range is >1000, >500, >100, and 20–100 nm for GUVs, MLVs, LUVs, and SUVs respectively. LUVs are now used for the drug delivery studies [7, 8].

The extrusion of MLVs through polycarbonate filters generates LUVs. The sonication of MLVs gives SUVs. LUVs are obtained by solvent injection, detergent removal, reverse phase evaporation [7]. The following techniques can be used for the large-scale production of liposomes [7, 9]:

- Microfluidization [10, 11]
- Spray drying [12]
- Freeze drying/lyophilisation [13]
- Super critical reverse phase evaporation (SCRPE) method [14]

Liposomes applications for targeted drug delivery include the incorporation of bioactive compounds, i.e., anticancer, antibiotic, antifungal, and anti-inflammatory drugs and genes where comes the role of surface engineering. Figure 6.1 illustrates the role of surface engineering to formulate different types of liposomes. In general, biomaterials can be incorporated in liposome by active or passive loading. The surface properties of bioactive materials dictate the final application of liposomes.

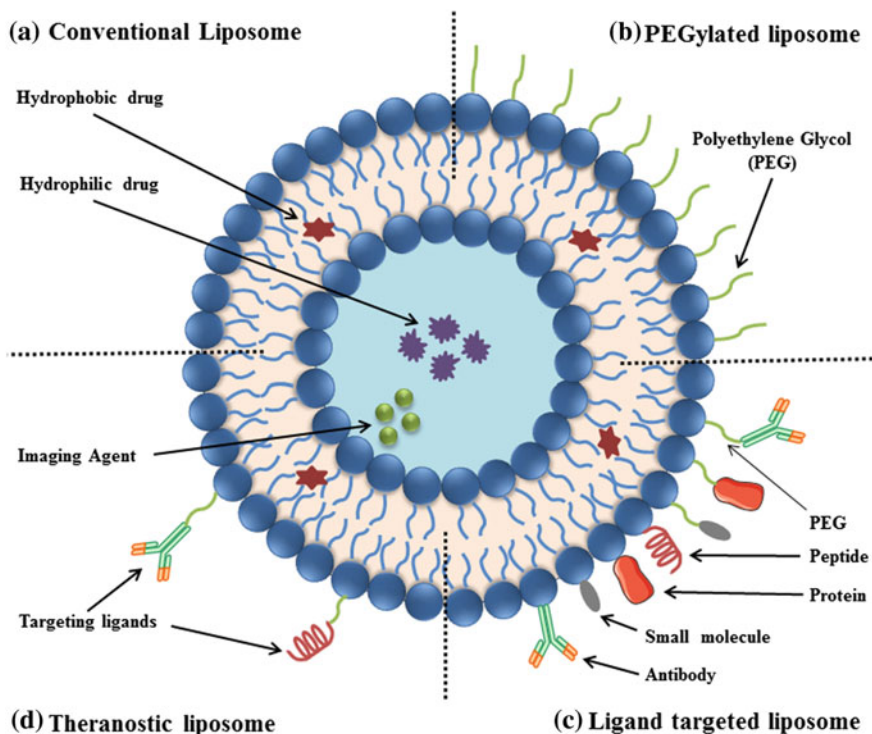


Fig. 6.1 Different types of liposomes. **a** Conventional liposome. **b** PEGylated liposome. **c** Ligand-targeted liposome for specific targeting (ligands may be antibodies, peptides, etc). **d** Theranostic liposome contains an imaging agent such as Gd-DOTA-DSPE for MRI and a therapeutic drug such as paclitaxel which is a hydrophobic anticancer drug

6.2.1.1 Stealth Liposomes/PEGylated Liposomes

Stealth liposomes are the liposomes which show extended blood circulation time while reducing mononuclear phagocyte system uptake. Advancement of liposomal preparation based on lipid composition, size, and charge of the vesicle modulate it into long-circulating liposomes. Inclusion and incorporation of the synthetic polymer poly-(ethylene glycol) (PEG) in liposome composition has developed stealth liposomes (see Fig. 6.1). Further, by synthetic modification of the terminal PEG molecule, stealth liposomes can also be actively targeted with monoclonal antibodies or ligands and incorporation of these active molecules provide high target efficiency and activity. PEG is the universally used polymer for improvement of circulation time among the different polymers investigated to date.

Different surface engineering are involved in the incorporation of PEG on the liposomal surface but the most widely used method is the use of cross-linked lipid, i.e., PEG-distearoyl-phosphatidylethanolamine (DSPE). Poly(ethylene glycol)-linked phospholipids such as DSPE-PEG 2000 or methoxy PEG 2000-DSPE are

commercially available for the preparation of stealth liposomes. DSPE is a synthetic amphiphilic phospholipid. The role of phospholipid portion in poly(ethylene glycol)-linked phospholipid needs to be investigated. Therefore, non-phospholipid-PEGs such as Cholesteryl-PEG (Chol-PEG) are also being developed for the preparation of stealth liposomes [15]. An FDA approved liposomal doxorubicin preparation Doxil[®] contains stealth liposomes composed of cholesterol, hydrogenated soybean phosphatidylcholine (HSPC), and methoxy PEG 2000-DSPE [16].

Other surface modifications involved are: physical adsorption of the polymer onto the surface of the vesicles, incorporating of PEG-lipid conjugate during liposome preparation, or by covalently attaching reactive groups onto the surface of preformed liposomes.

6.2.1.2 Immunoliposomes

Immunoliposomes are liposomes in which antibodies or their fragments are conjugated onto the surface. The surface modification with antibody increases the therapeutic index of many drugs via targeted drug delivery and controlled release of drugs. Two strategies are involved in coupling of antibody to the liposome surface. One involves covalent cross-linking with a liposomal lipid while another involves incorporation of chemically modified antibody into the bilayer by increasing hydrophobicity of the antibody. These approaches can attach a wide variety of antibodies to various types of liposomes.

For covalent cross-linking, first derivatization of the free amino group of phosphatidylethanolamine (PE) with amino reactive bifunctional crosslinking molecules have been employed. This modified PE is combined with the bulk lipid to form liposomes. Later approach requires chemical modification of the antibody molecule with a hydrophobic substituent which increases the hydrophobicity of the antibody and hence, increases the affinity for the liposomal bilayer.

Nowadays, PEGylated antibodies are used for targeted delivery. The lipid is usually cationic such as DOTAP (1,2-dioleoyl-3-trimethylammonium-propane chloride) which is added to lipid mixture to provide +ve charge to liposomes. See Fig. 6.2 for structure of DOTAP. A solution of +ve charged lipid along with a helper neutral lipid such as dioleoylphosphatidylcholine (DOPC) is mixed with gene material/nucleic acid which have -ve charge such as DNA. A +ve charged complex between nucleic acid and lipid is formed which is called as lipoplex. Cationic lipids are usually used in the delivery of genes but anionic lipids such as phosphatidic acid can also be used in the presence of divalent metal ions such as Ca^{2+} [17]. Due to risk of inactivation of whole antibody during the chemical conjugation process, fragment antigen-binding (Fab') or single-chain fragment variable (scFv) are used to get immunoliposomes. The latter are the smallest fragment of an antibody containing the complete antigen-binding site [18]. Immunoliposomes are used for the delivery of genes which include DNA [19, 20], plasmid-DNA [21, 22], and RNA-based therapeutics such as antisense oligonucleotides and small interfering ribonucleic acid (siRNA) [23, 24].

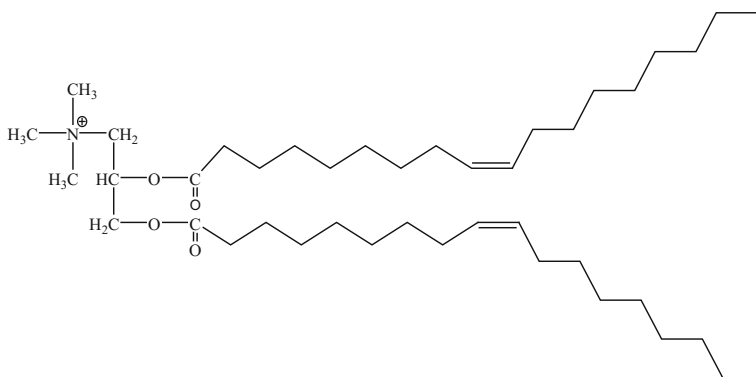


Fig. 6.2 Structure of DOTAP

Immunoliposomes are now extensively used to treat cancer cells in animal models. The targeting of immunoliposomes containing anticancer drugs at the overexpressed receptors in cancer cells resulted in the treatment of cancer. Transferrin receptors (TfRs) are overexpressed in cancers. An improved therapeutic activity was observed using TfR-targeted stealth immunoliposomes against liver cancer [25]. Cationic liposomes are used in the delivery of drugs to brain through blood–brain barrier [26, 27].

6.2.1.3 Multifunctional Liposomes: Theranostic Liposomes

Surface engineering plays a significant role in preparation of multifunctional liposomes and these are the advanced formulations of nanotechnology having simultaneous functions of diagnostics and therapeutics (theranostics) (see Fig. 6.1). Preparation of theranostic liposome requires the engagement of therapeutic, diagnostic, and imaging agent for defined targeted delivery of drugs which are toxic in free form. Theranostic liposomes are formed by correct utilization of surface engineering of the bilayers [28].

Surface engineered multifunctional liposomes containing UCNPs (lanthanide-doped up converting nanoparticles) and doxorubicin were prepared. UCNPs consisting of Er^{3+} and Yb^{3+} co-doped NaGdF_4 upon exposure to an excitation wavelength of 890 nm showed a decrease in emission signals (a quenching effect). This allows the monitoring of drug release and drug loading. It was suggested that these prepared liposomes have a potential as theranostic nanocarriers [29]. A theranostic liposomal drug delivery system was reported with paclitaxel, carboplatin loaded as therapeutic agents and Gd-DTPA-BMA as contrast agent for MRI. These liposomes have prolonged circulation time and 36 times higher T_1 relaxation-rate as compared to a commercial MRI contrast agent (Omniscan). These theranostic liposomes can carry both hydrophilic and hydrophobic drugs for lung cancer treatment with significant reduction in toxicity. The biodistribution of drugs was imaged via

T₁-weighted MRI in real time while drug delivery was achieved by the use of targeted tandem chemotherapy. The study offers new possibilities such as the evaluation of therapeutic response by in vivo imaging via T₁-weighted MRI [30].

6.2.1.4 Active Loading or Remote Loading of Bioactive Compounds in Liposomes

Passive drug loading in liposomes means loading of drug in the process of formation of liposomes. Conventional liposomes usually have low encapsulation efficiency. Active drug loading requires the modification of surface to enhance the encapsulation efficiency and drug loading was done on preformed liposomes which had a pH gradient. This is known as active or remote drug loading. For desired biomedical application of liposomes, covalent immobilization of bioactive compounds onto the surface plays a significant role. Active loading have several advantages, i.e., high specificity and efficiency. The method of surface functionalization varies according to the final application of liposome formulation. By using surface functionalization, several types of liposomes were prepared and each of them have distinct functions.

In this pH gradient was developed by adding amine solution or basic drug to liposomes in pH 4.0 citrate buffer. To generate a pH gradient, the pH of the external medium was raised to pH 8 [31]. This helps in the incorporation of therapeutic agent in liposomes. The loading of amphiphilic amines (catecholamines), amphipathic (amphiphilic) weak base drugs having amine groups, i.e., anthracyclines were incorporated by pH gradient. In liposomes formulation, i.e., Myocet[®], DaunoXome[®], doxorubicin drug was loaded by using pH gradient [31–34]. Ammonium sulfate gradient is another kind of gradient and here liposomes were prepared in 300 mM ammonium sulfate solution (pH 4.0). To create a gradient, the liposomes were dialyzed against pH 7.5 HEPES buffer or diluted in pH 7.5 HEPES buffer [33, 34].

6.2.1.5 Incorporation of Targeting Agents

Targeted delivery is the utmost requirement of today's research for effective chemotherapy. The aim is to provide required drug to the affected tissues. It is called active targeting of drugs. It involves the attachment of a ligand to the surface of drug carrier which detects and binds to the diseased cells [35]. Active targeting of drug leads to enhanced drug level at targeted site [36]. In active targeting, nanocarriers, i.e., liposomes are surface modified with specific targeting ligands. The ligand binds to overexpressed receptors on target cell surface. Commonly used ligands for active targeting of liposomes include antibodies, peptides, and small molecules [37, 38].

Nanocarriers can be incorporated covalently or non-covalently with a targeting ligand due to their flexible surface chemistry [39]. Ligands for active targeting have

been attached directly to lipids or attached covalently to distal end of PEG-chain [40]. Targeting ligand's optimal density on nanocarrier is also an important aspect of surface modification. High targeting ligand density in optimum range may increase binding at the target site. Issues like aggregation or raised cost may arise by increasing the ligand density beyond optimum range [41].

Targeting ligands provide better internalization of nanocarriers in tumor cells [39]. Ligand-mediated endocytosis plays a vital role in delivery of drugs and genetic materials, i.e., siRNA and oligonucleotide [41]. Active drug targeting has an added advantage of declining the nonspecific distribution to undesired tissues [36]. Therefore, active targeting plays an important role in efficacy and safety of treatment. Table 6.1 describes some examples of targeting ligands and their targeting sites along with their category and drug delivered with the site and type of cancer.

Peptides as Targeting Ligand

Peptides are short chains made up of amino acid monomers. Peptides are also used as targeting ligands for active drug targeting. A peptide, vasoactive intestinal peptide (VIP) has been used in active drug targeting [53]. A VIP-receptor is relatively a new target for active drug targeting. VIP-receptors are overexpressed in human breast and lung cancer cells [54, 55].

⁹⁹Tc-HMPAO encapsulated liposomes conjugated with VIP as targeting ligand has been developed for theranostic application. ⁹⁹Tc-HMPAO is a radiopharmaceutical and used as a diagnostic agent in liposomal formulation. Targeted delivery of VIP-conjugated ⁹⁹Tc-HMPAO in breast tumor model has shown significantly better uptake in breast cancer cells as compared to non-targeted liposomes [42]. VIP has shown no adverse effect on normal tissues mainly due to the fact that VIP-conjugated-carriers extravasates out at only leaky vasculature on disease site [56].

SP94 is another targeting peptide, used on a nanocarrier based on surface modified liposome called protocell. In protocell, a nanoporous silica core is encapsulated by lipid bilayer. The targeting peptide resulted in 10,000 folds higher binding affinity for hepatocellular carcinoma as compared to normal hepatocytes, *in vitro* [57].

Folate/Folic Acid as Targeting Ligand

Folate (folic acid) is a small-molecule targeting agent [36]. Folate receptors are overexpressed in various human tumors such as lung, breast, brain, colon, kidney, and ovarian cancers [58–60]. Folic acid exhibits high affinity for folate receptors and demonstrated better internalization in tumor cells [61]. Another important feature is folate-receptor location. They are located on apical-membrane of epithelial cells reducing the chances for entry of targeted nanocarrier into normal cells [58, 62].

Folic acid being a small molecule has several advantages over antibody ligands. The advantages include non-immunogenicity, nontoxicity, low cost, small size, and higher stability in preparation, storage, and circulation [63]. In folate receptor targeting of nanocarriers, off-target effect should also be considered [36].

Doxorubicin loaded folate-targeted liposomes have been formulated demonstrating 45-folds higher uptake as compared to non-targeted liposomes. Surface modified targeted liposomes demonstrated 85-folds higher cytotoxicity than

Table 6.1 Targeting ligands and targets for ligand-targeted liposomes in cancer treatment

Targeting moiety/ligand	Example	Diagnostic/therapeutic agents	Targeting site	Type of cancer	Refs.
Peptide	Vasoactive intestinal peptide (VIP)	⁹⁹ Tc-HMPAO	Vasoactive intestinal peptide receptor (VIP-R)	Breast cancer	[42]
Small molecule	Folate	Doxorubicin	Folate receptors	Asciatic tumor	[43]
Small protein	HER2-specific affibody	Calcein	Human Epidermal Growth Factor Receptor 2 (HER2)	Breast cancer	[44]
	EGFR-specific affibody	Mitoxantrone	Epidermal growth factor receptor (EGFR)	Breast cancer	[45]
Aptamer	sgc8 aptamer	FITC-Dextran	protein tyrosine kinase 7 (PTK7)-receptor	Leukemia	[46]
	AS1411 derived aptamer	Cisplatin	Nucleolin (NCL)	Breast cancer	[47]
Antibody (fragment)	Fab' fragments of cetuximab	Doxorubicin	Epidermal growth factor receptor (EGFR)	Breast cancer	[48]
	Anti-MT1-MMP Fab	Doxorubicin	Matrix metalloproteases (MMPs)	Cancer cells expressing MT1-MMPs i.e. fibrosarcoma	[49]
Antibody	Cetuximab	Oxaplatin	Epidermal growth factor receptor (EGFR)	Colorectal cancer	[50]
	Trastuzumab	Doxorubicin	Human Epidermal Growth Factor Receptor 2 (HER2)	Cancer cells over-expressing HER2 i.e. breast cancer	[51]
	Carbonic anhydrase IX (CA IX) Antibody	Docetaxel	Carbonic anhydrase IX (CA-IX)	Lung cancer	[52]

non-targeted liposomes [43, 64]. Folate conjugated liposomes having 5-fluorouracil or doxorubicin encapsulated have shown improved biodistribution as compared to unconjugated liposomes [65, 66].

Affisomes as Targeting Ligand

Affibody is a small molecule which has been considered as antibody mimics and used as targeting ligand for nanocarriers, i.e., liposomes. Affibodies are small proteins (approximately 8 kDa) having high stability and solubility. Affibodies are expressed in a bacterial system or produced by peptide synthesis. Affibodies have relatively higher binding affinity as compared to corresponding antibodies [67].

A research group has reported formulation of affisomes by surface modification of thermosensitive liposomes for breast cancer treatment. HER2-specific affibody having 8.3-kDa mass was conjugated to thermosensitive liposomes surface [44]. Similarly, EGFR-specific affibody having 14-kDa mass has been conjugated to PEGylated liposomes for the formulation of affisomes targeted at EGFR-expressing cells. Mitoxantrone loaded affisomes has shown increased cytotoxicity against EGFR-expressing cells [45].

Affibodies have different receptor-binding domains than antibodies that may result in altered outcome. Thus, further research is required to establish the advantage of using affibodies as targeting ligands for nanocarriers, i.e., liposomes [67].

Aptamers as Targeting Ligand

Aptamers are RNA or ssDNA oligonucleotides. They show high affinity and recognize target molecules by hydrogen bonding, electrostatic and hydrophobic interactions [68]. Aptamers have several advantages in their application as targeting ligand due to following reasons: smaller size, higher stability, simple synthesis method, easy modification and show high antigen recognition as compared to other ligands, i.e., nucleotide sequence which can be easily altered for better selectivity [69].

sgc8 aptamer-conjugated liposomes have been developed for leukemia. Liposomes were covalently linked to the aptamer using a PEG spacer. Aptamer-conjugated liposomes bound target cells and released the drug within 30 min after cell incubation [46]. A research group has reported aptamer-conjugated multifunctional liposomes encapsulating cisplatin as a therapeutic agent. AS1411 derived aptamer was used as a targeting ligand for cisplatin encapsulated multifunctional liposomes for targeted delivery in breast cancer. AS1411 derived aptamer due to its high binding affinity to nucleolin (NCL) has exhibited anti-proliferative activity in many tumor cell lines such as MCF-7. Therefore, AS1411 derived aptamer is a promising ligand for targeting of breast cancer [47].

Antibody as Targeting Ligand

Monoclonal antibody (mAb) is a promising ligand for active targeting of drugs using a nanocarrier. The surface modification of a nanocarrier can be done with whole antibody or fragment of mAb, enhancing cellular uptake at the diseased site [36]. Use of antibody fragments i.e. Fab (antigen-binding fragment) and ScFv

(single-chain variable fragment) not only reduces immunogenicity but also results in substantial size reduction of ligand favoring development of ligand-targeted nanocarriers [41].

Certain configuration should be maintained for antibody and peptide ligands to have the desired binding affinity. So, a high temperature or organic solvent during preparation should be used with caution [41].

The targeting moiety is attached to PEGylated liposomes to target overexpressed surface receptors, e.g., EGFR, HER 2, carbonic anhydrase IX or receptors in tumor vasculature such as VCAM 1, MT1-MMP.

Epidermal Growth Factor Receptor (EGFR)

EGFR immunoliposomes to target EGFR were prepared. The regression in breast cancer was observed compared to nontargeted liposomes [48]. EGFR targeted liposomes containing two anticancer drugs (oxaliplatin, a platinum derivative and cetuximab, a monoclonal antibody) were prepared. In a colorectal cancer (CRC) xenograft model, an increased drug delivery was observed for targeted formulation as compared to non-targeted liposomes [50].

Human Epidermal Growth Factor Receptor 2 (HER2)

HER2-targeted liposomes containing doxorubicin were developed. The liposomes also contained trastuzumab which targets signaling pathway of HER2. The results showed increased anti-tumorigenesis (inhibition of the development of a neoplasm) with no cardiotoxicity or cardiac dysfunction, while on the other hand conventional anthracycline based cancer therapy is associated with cardiac toxicity [51].

Carbonic Anhydrase IX (CA IX)

CA IX is overexpressed due to hypoxia in cancers such as lung cancer [70]. In a study, anti-carbonic anhydrase immunoliposomes containing docetaxel showed growth inhibition in CA IX positive lung cancer cells as compared to free drug or non-targeted drug containing liposomes. The prepared liposomes have an encapsulation efficiency of ~85% and ~144 nm size [52]. In a review, the potential of targeted CA IX immunoliposomes to deliver anticancer drugs to cancer cells in vivo has been discussed [70].

Vascular Cell Adhesion Molecules (VCAMs)

VCAMs play roles in atherosclerosis (cholesterol or fatty deposition in the arteries) and inflammation. VCAM 1 is overexpressed on cancer vessels [68]. In a study the delivery of anti-inflammatory drugs by liposomes was investigated. Anti-VCAM 1 liposomes were prepared by the coupling of 'Fab' with liposomes containing celecoxib (used to treat inflammation) [71].

Matrix Metalloproteases (MMPs)

Several MMPs (enzymes) particularly membrane-type MT1-MMP are involved in angiogenesis (formation of new blood vessels) [72]. MT1-MMPs are expressed on cancer cells and angiogenic endothelium cells. Doxorubicin liposomes with 'Fab' fragments were prepared. The fragments were used as targeting ligands against

MT1-MMP. An enhanced uptake of the liposomes was observed in HT 1080 cancer cells with highly expressed MT1-MMP compared with non-targeted liposomes [49]. In another study, MMP sensitive liposomes were prepared containing: (a) PEG coating, (b) monoclonal antibody (mAb 2C5) for specific targeting to cancer cells, (c) matrix metalloprotease 2-sensitive bond which cleaves in tumor, and (d) cell penetrating peptide such as TATp [73].

6.2.1.6 Stimuli-Sensitive Surface Engineered Liposomes and Their Benefits in Drug Release

Stimuli or triggers have been used to release drugs. The stimuli may be external such as magnetic field or local such as pH. The latter utilizes the local environment of the target/tumor site while the former use an external stimulus such as heat, ultrasound or light [74].

pH Sensitive Liposomes

These liposomes release their drug loads at the acidic environments of tumors. The tumor tissues and inflamed areas have low pH than normal tissues [75].

Various approaches were tried to get pH-sensitive liposomes. In one approach, a pH-sensitive bond (hydrazone bond; $=C=N-NH_2$) was inserted between PEG and bilayer. Such liposomes are stable at neutral pH but release their contents in the acidic environments [75]. In a second approach, fusogenic peptides such as glutamic acid-alanine-leucine-alanine (GALA) were incorporated in liposomes. Such liposomes do not fuse to cells at pH 7.4 but at lower pH fuse into cells and release their contents [76, 77]. In another approach, poly 2-propylacrylic acid (PPAA) based cationic (DOTAP) liposomes were prepared for the delivery of antisense oligonucleotides. PPAA is pH-sensitive hydrophobic polymer. The aim was to silence genes in A2780 human ovarian cancer cells. It was hoped that this system could also be used for the delivery of plasmid-DNA, aptamer, or siRNA [78].

Liposomes containing overexpressed hyaluronic acid (HA) receptors and doxorubicin were reported. These liposomes were also pH-sensitive. In vitro studies showed a higher release in mild acidic conditions than at pH 7.4. Further enhanced antitumor activity was observed in cancer cells with high levels of CD44 than non-targeted liposomes [79].

Temperature Sensitive Liposomes

Thermosensitive liposomes are stable at body temperature but release their contents when temperature is raised. The presence of lysolipids increase permeability in the bilayers. Doxorubicin liposomes containing a lysolipid (10%) were prepared. An increased drug release was observed on producing mild hyperthermia (39–40 °C) [80].

These can be prepared by use of a temperature sensitive polymer. In a study, doxorubicin loaded liposomes containing both temperature sensitive polymer poly (*N*-isopropylacrylamide) pNiPAAm and pH sensitive polymer 2-propylacrylic acid

(PAA) were prepared. The drug was released in the acidic environment of tumors. The drug release was further enhanced by the use of heat which was generated by focussed ultrasound at the specific (cancer) site [81]. Temperature sensitive liposomes containing gadolinium (Gd-DTPA) for MRI and an aptamer (TSLs-AS1411) for specific targeting against cancer cells were developed. At 42 °C, liposomes released gadolinium at the cancer site which was easily detected by MRI. It was suggested that these liposomes could be used for the early detection of cancer [82]. In a study, paclitaxel loaded temperature sensitive liposomes were prepared. The particle size was ~100 nm and encapsulation efficiency was ~95%. The phase transition temperature of the liposomes was 42 °C which was determined by differential scanning calorimetry. At 42 °C, the drug release was higher than at 37 °C [83].

Magnetic Liposomes

These become magnetized on the application of an external magnetic field. This produces instability in the bilayers resulting in the release of drug. In a study, liposomes containing magnetite (Fe_3O_4 , ferrihydrite) and doxorubicin showed enhanced release of the drug in the tumor due to the application of an external magnetic field of 0.4 T strength [84]. Folate receptor targeted magnetic liposomes loaded with doxorubicin were prepared. The site-specific drug release was observed on the application of alternating magnetic field due to the presence of magnetized particles and hyperthermia produced [85]. Super magnetic cationic liposomes containing anticancer drug paclitaxel showed enhanced drug delivery to the brain of rats. The vesicles were of uniform diameter (20 nm). It was concluded that cationic magnetic liposomes are potential vehicles for the delivery of drugs to the brain [27].

6.2.2 Solid Lipid Nanoparticles (SLNs)

The nanoparticle has a solid lipid matrix. The drug is in the matrix or adsorbed on the surface of the nanoparticle [2]. For hydrophilic drugs, cold homogenization technique is usually used to get SLNs. This technique is also suitable for thermosensitive drugs by dispersing of lipids in the cold aqueous dispersion medium. Hot homogenization technique is used for lipophilic and insoluble drugs. High pressure homogenization at a temperature above the lipids melting point gives o/w emulsion, which gives SLNs after cooling at room temperature [86]. They can also be prepared by supercritical fluid (SCF) technology. SLNs are carriers for drugs, peptides (i.e., cyclosporine A—a cyclic peptide which is an immunosuppressant), proteins (i.e., bovine or human serum albumin BSA/HAS), and antigens (i.e., hepatitis B surface antigen). They are usually administered by IV route and can also be delivered by other routes, i.e., oral, pulmonary, and nasal and transdermal [87].

Their surface can be PEGylated to increase blood circulation time. PEGylated SLNs (having stearic acid lipid core) formulations of methotrexate (MTX) has been

reported. The formulations were further evaluated with the help of ^{99m}Tc radionuclide. The kinetic studies showed longer blood circulation times for the formulations. SPECT imaging showed efficient tumor uptake of SLNs. The characterization of these nano particles was also done. The particle size found was about 130 nm (via TEM and AFM) and zeta potential was about -34 mV [88]. In another study, paclitaxel (PTX) loaded SLNs (with trimyristin as a solid lipid core and pegylated phospholipid as a stabilizer) were evaluated for biodistribution in organs such as kidneys, heart, and lungs. It was found that the prepared PEGylated SLNs (containing paclitaxel) are similar to Taxol[®] (a commercial preparation for paclitaxel) because both showed similar biodistribution and circulation times [89].

SLNs have limitations which include low drug entrapment efficiency and possibility of drug degradation during hot dispersion technique. A new generation of SLNs is also known as nanostructured lipid carriers (NLCs) which are free from low drug entrapment efficiency. These have solid matrix with oil nano-compartments containing drug surrounded by a lipid shell. Due to the presence of the nano-compartments, NLCs have high drug loading capacity [2]. Docetaxel-loaded NLCs were produced where the anticancer drug was held in core. These NLCs showed more cytotoxicity than free docetaxel [90].

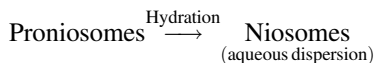
SLNs have several advantages. They can be called as alternate drug carriers because they can be used in place of liposomes. They can be sterilized by autoclaving or by γ irradiation. Surface modifications can be made to make SLNs site-specific. The ease of scale up to industrial scale production (particularly by hot dispersion technique) is due to the availability of the homogenization production lines for the production of emulsions in pharmaceutical industry. The cost of excipients is low. They can be lyophilized and also spray dried [86].

6.3 Non-lipid Nanocarriers

6.3.1 Niosomes

Niosomes are nonionic surfactant vesicles and, like liposomes, have bilayers. The nonionic surfactant has a polar head and a nonpolar chain (amphiphile). They are formed by the hydration of a nonionic surfactants film. Cholesterol (as rigidizing/stabilizing agent for the bilayer) is added to the nonionic surfactant. The surfactants are biodegradable, inexpensive, and relatively nontoxic. Niosomes are used as an alternate to liposomes as a drug delivery system. Like liposomes, they are multilamellar vesicles (MLVs), large unilamellar vesicles (LUVs), and small unilamellar vesicles (SUVs) and their methods of preparations are similar to those in liposomes. The preparation methods include thin film hydration, ether/alcohol injection, and reverse phase evaporation. The size reduction by extrusion is used to get LUVs.

Proniosomes as a dry product have been prepared which on hydration gives niosomes.



There are some differences between niosomes and liposomes. A phospholipid has two nonpolar chains while nonionic surfactant has one nonpolar chain. Liposomes may be charged or neutral while niosomes are uncharged. Phospholipids can undergo oxidation and hydrolysis. Nonionic surfactants are comparatively chemically stable [86, 91–93].

The surface charge is important for the physical stability of niosomes. A charge is produced at the surface of neutral niosomes by the addition of $-ve$ charged dicetyl phosphate, dihexadecyl phosphate or by the $+ve$ charged stearylamine and cetyl pyridinium chloride. The surface charge is measured by zeta potential. As a rough rule, a value greater than 30 mV indicates electrostatic stability while 5–15 mV values indicate limited flocculation and 3–5 mV indicate maximum aggregation. Cationic niosomes containing DC-Cholesterol ($+ve$ charged cholesterol) showed good physical stability and were used for the delivery of genes. A complex is formed between cationic niosomes and $-ve$ charged genes, which helps in the gene transfer [92, 94].

Niosomes have been used for the treatment of various diseases [92]. They are readily taken up by the RES system. They have been successfully used to treat leishmaniasis where parasites attack liver and spleen. Niosomes containing sodium stibogluconate (an antileishmaniasis agent) successfully treated leishmaniasis in mice [95]. Metformin (an oral hypoglycaemic agent) loaded niosomes using Span 40 and Span 60 nonionic surfactants were prepared for oral administration. The best sustained release formulation contained cholesterol and surfactant in equal molar ratio. It was suggested that the sustained release metformin niosomes are useful for the treatment of Type II diabetes [92]. Isoniazid containing niosomes were prepared for the treatment of tuberculosis. The $-ve$ charge was given to niosomes by the use of dicetylphosphate. The drug uptake (about 62%) by macrophage J744 A was observed. The formulations showed decreased drug toxicity and better patient compliance [96]. Niosomes containing paclitaxel (anticancer drug) were prepared for oral administration. The formulations were slow released and formulation with span 40 showed stability against trypsin, chymotrypsin, and pepsin (GIT enzymes) [97]. Niosomes have been used as a topical drug delivery system because of increased drug penetration, sustained drug release, and increased drug stability. In a review, the role of niosomes in dermatology has been discussed [98].

All surface modifications done on liposomes to achieve the goal of targeted drug delivery can be easily done on niosomes. PEGylation of niosomes increase their circulation times. Niosomes containing PEG 4400 and glucose conjugates were used for targeting overexpressed glucose receptors in a carcinoma model. Improved targeting of encapsulated paramagnetic agent such as gadolinium was observed using MRI [99]. Niosomes containing anticancer mitoxantrone and pH sensitive PEG-PMMI-CholC6 copolymer were prepared. The niosomes were more cytotoxic against cancer cell lines (ovarian and breast) than conventional niosomes [100].

6.3.2 Polymeric Nanoparticles

The nanoparticle has a polymeric matrix. The drug is dissolved or dispersed in the matrix or adsorbed on the surface of polymeric nanoparticles (PNPs) [2]. See Fig. 6.3 schematic differences between solid lipid nanoparticle (SLN), polymeric nanoparticle (PNP), nanocapsule and nanostructured lipid carrier (NLC). Solid lipid nanoparticle (SLN) and polymeric nanoparticle (PNP) have different matrix, i.e., lipid solid and polymeric matrix. Similarly, nanocapsule and nanostructured lipid carrier (NLC) have different composition of shell, i.e., polymeric and lipid shell. They are made of natural hydrophilic polymer (proteins such as albumin, gelatin, lectins, and polysaccharides such as chitosan, dextran, and alginate) or synthetic hydrophobic polymers. The synthetic polymers are either pre-polymerized polymer such as poly lactic acid (PLA), poly E-caprolactone (PECL) or a polymer which is further polymerized during the process of nanoparticle formation. The latter compounds include poly isobutylcyanoacrylate (PICA) which is a monomer [86].

Since few synthetic polymers are nontoxic, this limits their use as drug carriers. Poly alkyl cyanoacrylate (PACA) is a biodegradable and nontoxic monomer [86].

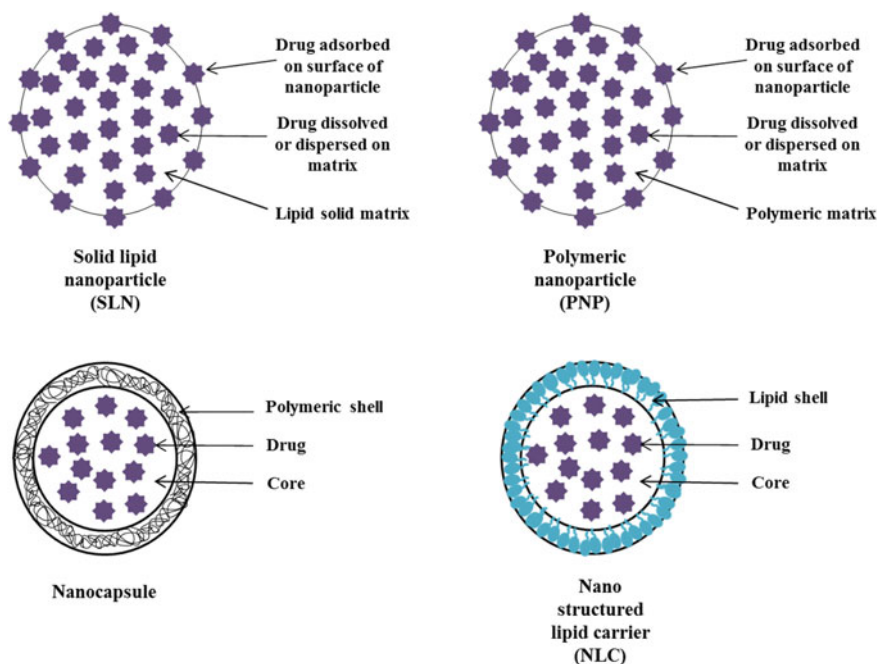


Fig. 6.3 Schematic differences between solid lipid nanoparticle (SLN), polymeric nanoparticle (PNP), nanocapsule and nanostructured lipid carrier (NLC). Solid lipid nanoparticle (SLN) and polymeric nanoparticle (PNP) have different matrix i.e. lipid solid and polymeric matrix. Similarly, nanocapsule and nanostructured lipid carrier (NLC) have different composition of shell i.e. polymeric and lipid shell

Depending upon the polymerization medium, PACA monomers can be polymerized to give various types of nanocarriers such as nanoparticles, nanospheres, and nanocapsules. These can carry a variety of drugs. PACA first appeared in 1979. A review on PACA nanoparticles was published 33 years later (in 2012) by Georgi Yordanov to show their importance as drug carriers. It was suggested to prepare multifunctional PACA-based nanoparticles (for improved drug action) containing a drug, a contrast agent for biodistribution imaging study and a drug stabilizer. These nanoparticles should be decorated with targeting moieties for active drug targeting at the overexpressed receptors [101]. Polysaccharide conjugated PACA nanoparticles have been formulated in aqueous-medium through redox-radical-emulsion polymerization. Drugs, i.e., doxorubicin can be loaded on surface of such nanoparticles by adsorption [102]. PACA nanospheres have been prepared by zwitterionic polymerization. PACA nanospheres were covalently attached with PEG-chains. Polymerization was done at low pH in the presence of PEG or methoxy PEG [103]. Further clinical trials should be initiated for already developed PEGylated nanoparticles particularly for oral and topical use because of lesser danger of systemic toxicity by these routes [101].

PACA nanoparticles containing doxorubicin showed better drug uptake by cancer cells than free drug. It was also found that these doxorubicin containing PACA nanoparticles were also effective in multidrug resistant (MDR) cancer [104]. Stealth/PEG-coated injectable PACA nanoparticles can be prepared by the use of poly (MePEG cyanoacrylate-co-alkyl cyanoacrylate) copolymer [105]. By modifying PACA nanoparticles surface with polysorbate 80 surfactant, the particles can be targeted into brain because these surface modified particles first adsorb apolipoproteins from plasma and then cross brain endothelium through endothelial cells [101]. Nanoparticle-mediated drug delivery to brain is based on coating of nanoparticles with polysorbates, i.e., polysorbate-80. Drugs may be adsorbed or chemically attached to the nanoparticle surface [101]. Another research group has used polylactic acid (PLA) instead of PACA and reported formulation of PLA (Polylactic acid) nanoparticles coated with polysorbate-80 for targeted delivery to brain. Suspension of surfactant-free nanoparticles (SFNPs) was prepared by modified nano-precipitation method. Suspension was prepared by dissolving PLA in acetonitrile and then transferring the solution slowly into 50% ethanol. Suspension was slowly transferred to water with shaking. Surplus water and organic solvents were removed in a vacuum evaporator. Addition of FITC-dextran was done in formulation of SFNPs and incubation was done for 24 h. After this step, Polysorbate-80 was added into formulation and incubation was done for another 24 h. The weight ratio between Polysorbate-80, FITC-dextran, and nanoparticles was 10:1:10. The experimentation involved preparation of two controls, FITC-dextran-loaded nanoparticles and polysorbate-coated nanoparticles. The controls were prepared in similar way as PLA-nanoparticles, only without addition of Polysorbate-80 and FITC-dextran, respectively [106].

Inhalable effervescent doxorubicin containing nanoparticles based on polybutyl cyanoacrylate (monomer) have been reported for curing lung cancer in mice and it

was suggested that this noninvasive route for the administration of anticancer drugs might be useful in the treatment of lung cancer [107].

Polyethylene glycol 100 stearate (PEG-100S) coated SLNs containing levothyroxine (a drug to treat hypothyroidism) were reported with development of an oral drug delivery system for hydrophobic drugs. Electron microscopy showed that the coated SLNs were spherical while photon correlation spectroscopy showed a size of about 187 nm. Differential Scanning Calorimetry (DSC) showed that the nanoparticles were in amorphous state, a state that is appropriate for drug delivery. A reduction in zeta potential from -40 to -23 mV occurred due to PEG coating. A controlled drug release from these nanoparticles was observed due to surface modification by PEG. A comparison of drug release from PEG-coated SLNs and uncoated SLNs has been illustrated in in vitro drug release plot. The plot has shown slow drug release from PEG-coated SLNs due to covering effect of coating layer. PEG coating reduces burst release effect because levothyroxine adsorbed on lipid surface was slowly released from PEG-coated SLNs. In fact, coating layer of PEG provides resistance against diffusion of levothyroxine leading to reduced drug release [108].

6.3.3 Hydrogels

Natural or synthetic hydrophilic polymers in water form a three-dimensional cross-linked mesh or network which can entrap drugs, proteins, peptides, monoclonal antibodies, etc. Due to high affinity for water absorption, they have soft consistency. The cross-links help to keep their physical integrity in the presence of water [109]. Physical integrity of hydrogels is maintained by physical and/or chemical cross-links. Physical crosslinks include tie-points and junctions; while, chemical cross-links includes entanglements and crystallites. Chemical crosslinks provide integrity and network structure to hydrogels [109, 110]. Hydrogels, particularly stimuli-sensitive, (pH and enzyme sensitive) are used as an oral drug delivery system because of the controlled release of entrapped drug to a specific site within GIT [109]. Recently, multi-responsive hydrogels have also been developed which release their contents on the application of more than one stimulus (temperature, electric or magnetic field, pH, etc). Hydrogels are finding use in targeted drug delivery, controlled drug release and soft contact lens, wound repair, targeted cell growth/tissue engineering [111]. By the use of osteoblasts (cells from which bone develops) and alginate hydrogel, an improved bone formation was observed [112].

Hydrogels may be classified on the basis of charge as neutral, amphiphilic, cationic, or anionic. They are also classified on the basis of source such as synthetic, natural, or hybrid made from both natural and synthetic polymers. Another classification is based on the bonding such as covalent bonding or non-covalent bonding. The latter are usually weak attraction forces like hydrogen bonding. pH-responsive hydrogels may be cationic or anionic hydrogels [109].

Natural polymers, alginate, and chitosan have been extensively used for the oral delivery of drugs using hydrogels. Alginate, a linear polysaccharide (anionic

polymer from sea weed) forms a mesh or gel due to reaction between -ve charged G blocks of the polymer (the polymer is made of alternate G blocks and M Blocks) and Ca^{2+} ions. Chitosan (cationic polymer) has amino groups on the polymer which are pH sensitive. At low pH, groups are protonated and are dissolved easily. Due to pH response, chitosan hydrogels have been used for the delivery of drugs to stomach [109].

Paclitaxel-loaded chitosan-based temperature-sensitive hydrogel system was developed for the local treatment of cancer by Biosyntech Inc., Canada. The system consists of chitosan-solution neutralized with b-glycerophosphate, liquid at room temperature but becomes gel at the body temperature. Hydrogel containing paclitaxel was formulated by pouring chitosan-solution on sterilized paclitaxel powder and stirring for 4 h. The mixture was then mixed with glycerophosphate solution. The hydrogel formulated contained 6.4% (w/v) paclitaxel. One intratumoral injection of the hydrogel showed controlled drug delivery for a period of over 30 days and was effective as four Taxol[®] IV injections against cancer cells (EMT-6) in mice [113]. In another study insulin containing chitosan hydrogels were developed. The sustained release of insulin was observed over a period of 21 days. The gel forming material chitosan/glycerol-phosphate was solution at room temperature, but once injected inside the body by injection it becomes gel [114].

β -Cyclodextrin (β -CD, a biopolymer) based multifunctional supramolecular (supramolecular chemistry is based on weak interaction forces to hold groups of molecules together) hydrogel was prepared where β -CD acts as a host by forming a cavity where guest molecule PNIPAAm binds. It contained β -CD dimmers and PNIPAAm whose chains were modified with light-sensitive azobenzene groups. β -CD is commonly used as a host molecule in supramolecular hydrogel formation while other compounds such as crown ethers can also be used for this purpose [111, 115]. Poly(*N*-isopropyl acrylamide) (PNIPAAm or pNIPAAm or PNIPAm) is a temperature-sensitive polymer which is hydrophilic below 32 °C [116, 117]. See Fig. 6.4 for temperature sensitive hydrogel.

6.3.4 Dendrimers

Dendrimers are synthetic polymeric macromolecules that have three main parts: a small central core to which are attached branches or shells and the outermost shell/part is composed of surface groups. Overall, dendrimers have tree-like structure.

Polyamidoamine or PAMAM dendrimers are the most common dendrimers and have also been extensively studied. These were first introduced in 1978 by Fritz Vogtle. Their generations (G0 to G10) are now commercially available such as Starburst[®] dendrimers. They have alkyl-diamine internal core such as ethylene diamine (2 carbon core) and 1, 4-diaminobutane (4 carbon core) and with repetitive amidoamine branches or tertiary amine branches and primary amine surface groups. They are becoming spherical and by fourth generation they are three-dimensional

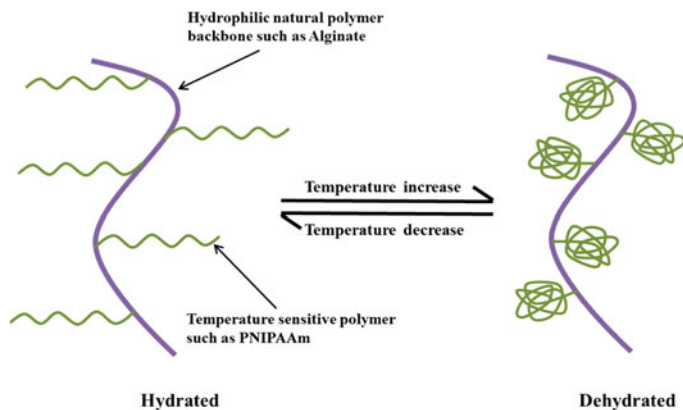


Fig. 6.4 Temperature sensitive hydrogel where a temperature sensitive polymer i.e. PNIPAAm (poly(*N*-isopropyl acrylamide) is grafted on hydrophilic natural polymer i.e. alginate backbone. On heating at >32 °C, the hydrogel collapses due to phase transition of PNIPAAm from swollen to shrunken state

spheres. They are synthesized by repetitive polymerization and after fourth generation their dimensions can be precisely controlled. When we go to next generation, an increase in size, molecular weight, and number of end groups occurs, e.g., number of end groups increases from 4, 8, 16, 32, and 64 respectively when we go from G0 to G1, G2, G3, and G4. The end groups may be same such as amine group. Dendrimers with different functional end groups such as hydroxyl, carboxyl, etc., are also available. A second generation means two shells outside the central core with more surface groups than first generation. Dendrimers, because of the void spaces, has been used as vehicles/carriers for nano drug delivery. Various routes of administration such as oral, parenteral, local have been tried [118–124].

The increased bioavailability of pilocarpine was observed when the drug containing PAMAM dendrimer (with carboxyl or hydroxyl surface groups) eye drops were instilled to the eyes of New Zealand albino rabbit. The study showed the ability of surface modified dendrimers as ocular controlled drug delivery vehicle [125]. Lee et al. [126] conjugated doxorubicin with biodegradable polystyrene dendrimers and found to be effective in the treatment of C-26 colon carcinoma in mice. The long circulation time (half life about 16 h) was achieved through PEGylation. The antitumor effect was similar to equal dose of liposomal doxorubicin injection Doxil[®] [126]. Amphotericin B (AmB) is an antifungal agent but its use is limited due to its poor water solubility and nephrotoxicity. In a study, its solubility increased in the presence of a third-generation PAMAM dendrimer. The study showed that the drug binding inside dendrimers is responsible for its sustained release [127]. Dendrimers have the ability to cross blood–brain barrier. In a review, the latest research opportunities and challenges in the production of various dendrimers (PAMAM, PPI, and PLL) for the diagnosis and treatment of brain tumor have been discussed [128]. A multifunctional dendrimer may contain one

anticancer drug, targeting ligands for overexpressed receptors such as EGFR and a diagnostic agent say for MRI imaging (gadolinium) or gamma imaging (a radionuclide) [121].

6.3.5 *Quantum Dots*

Quantum dot is composed of a semiconductor material. For medical applications, commonly used quantum dots (QD's) are cadmium selenide (CdSe), zinc sulfide (ZnS), cadmium telluride (CdTe), indium phosphide (InP), and indium arsenide (InAs). They are fluorescent, spherical nanoparticles or nanocrystals of 2–10 nm size which emit light strongly than fluorescent compounds (organic dyes such as eosin). The surface of QD's can be PEGylated to increase circulation times. They can be made site-specific by linking suitable ligands [129].

A single quantum dot conjugated to HER2 MAb (monoclonal antibody) has helped in the location of tumors in mice. In vivo, QD's were followed by the use of confocal microscope fitted with a high-speed camera [130, 131]. Quantum dots can be incorporated in nano-drug-carriers such as liposomes but long term toxicity particularly in disease state needs to be addressed [132].

QD's as drug carriers have been reported. QD's surface is coated with an amphiphilic polymer. The hydrophobic compound, i.e., QD capping compound tri-*n*-octylphosphine oxide (TOPO) can be placed between quantum dot internal core and the polymer. The hydrophilic therapeutics, i.e., oligodeoxynucleotide (ODN), siRNA are attached to the polymer surface. To the polymer surface, various ligands (aptamers, antibody, and peptide) can also be attached for site-specific targeting. Targeting moieties can be immobilized on hydrophilic portion of amphiphilic polymer by covalent or non-covalent bond. siRNA and peptide have been conjugated to QDs in parallel manner. Targeting ligands, e.g., peptides were prepared and simultaneously linked to QD-surface.

Small interfering RNA containing QD's have been used to silence genes. The co-transfection of quantum dot and siRNA was done by the use of lipofectamine (a transfection reagent) [133, 134]. In a review, quantum dot preparation, their toxicity, quantum dots as probes and as carriers for targeted delivery have been discussed [135].

6.3.6 *Gold Nanoparticles*

Gold nanoparticles of different sizes can be prepared by reduction of gold salts in presence of suitable stabilizing agents. Stabilizing agent prevents agglomeration of gold nanoparticles. Many research groups have reported formulation of gold nanoparticles with functional moieties. Functional moieties are anchored with thiolinkers in monolayers of gold nanoparticles. Gold sol (colloidal suspension)

containing gold nanoparticles is prepared by the reduction of gold salt such as chloroauric acid (HAuCl_4) by a reducing agent such as sodium borohydride (NaBH_4) in the presence of appropriate stabilizing agent such as a surfactant, cetyl trimethylammonium bromide (CTAB). In the absence of a stabilizing agent, agglomeration of nanoparticle occurs. Since CTAB is toxic, a thiol capping agent dodecanethiol (a capping agent prevents the growth of nanoparticle) is generally used in its place which also attaches thiol ($-\text{SH}$) groups to the surface of gold particles by forming stable Au:S bonds. Gold particles obtained by this method has 1.5–6 nm diameter and are soluble in nonpolar solvents. The reduction with sodium citrate gives particles of 15–150 nm size [136, 137].

Gold nanoparticles can successfully deliver large biomolecules such as nucleic acids, i.e., RNA or DNA, proteins and peptides, etc [136]. A research group has fabricated DNA-binders through anchoring β -cyclodextrin on periphery of modified oligo(ethylenediamino) gold nanoparticles. The modified gold nanoparticles successfully delivered plasmid-DNA into MCF-7 breast tumor cells [138]. Thiol ($-\text{SH}$) modification of nucleic acid strands has been done for grafting on nanoparticles. A research group has reported conjugation of thiolated-siRNA (SH-siRNA) with gold nanoparticles for cellular delivery [139]. A study has demonstrated gold nanoparticles as carriers of insulin. Gold nanoparticles were stabilized with chitosan. Chitosan-coated gold nanoparticles adsorb insulin on the surface and have been effective in oral delivery of insulin [140].

Based on the surface modification requirement, gold nanoparticle formulations vary in shape and size for different therapeutic applications. Depending upon the method of preparation, various gold nano-forms are produced such as nanospheres, nanorods, nanoshells, and nanocages. Gold nanoshells (50–150 nm) usually have silica core with thin layer of gold, however, pure gold nanoshell can also be prepared. Gold nanorods are 25–45 nm long. Gold nanorods are prepared from chloroauric acid with a gold-seed and a stabilizer, i.e., cetyltrimethylammonium bromide (CTAB). It was found that gold particles with less than 5–6 nm size show enhanced kidney clearance. For drug delivery use, spherical gold nanoparticles are used. The drug is conjugated to the nanoparticles [136, 137]. Further gold cores are nontoxic and biocompatible. Gold nanoparticles can be incorporated into a larger drug carrier such as liposomes for increased diagnostic, therapeutic, and imaging applications. Multifunctional gold particles have also been developed.

To prepare functional nanoparticles, gold nanoparticles are PEGylated to increase circulation times and then various ligands can be attached via thiol-PEG monolayer [141]. Covalent grafting of thiolated-polyethylene glycol (mPEG-SH) was done on gold nanoparticle surface. Thiolated-polyethylene glycol solution was added to citrate-capped gold nanoparticles solution with stirring. Stirring of solution was done for approximately 1 h allowing exchange of citrate-ligands with mPEG-SH. Centrifugation at 15,000 rpm was done for approximately 45 min to remove excessive mPEG-SH. PEG covalently attaches to gold nanoparticles because thiol groups have strong affinity for gold. Colloidal solution obtained was stable for many months [142].

Gold particles have enhanced absorption and scattering of light in near infrared range (NIR), 650–950 nm upon exposure to a near NIR laser source. This property of gold particle is finding use in the diagnosis and treatment of cancer. On exposure to laser source, the scattered light produces hyperthermia in cancer cells due to surface plasmon resonance effect [143]. In a study, a mouse was implanted subcutaneously with colon cancer cells. After IV administration of gold nanoshell-PEG conjugates, the accumulation of gold nanoshells within cancer cells resulted. On exposure to an 808 nm NIR laser, cancer was ablated due to heat [144]. The combined use of NIR laser thermal therapy and MRI imaging via gold nanoshells was effective in the treatment of breast cancer [145]. Since breast cancer is near the skin, the cancer can be easily treated by the limited use of NIR laser. It was concluded that gold particles provide an opportunity/commitment in the treatment of breast cancer [137].

Gold particles as a contrast agent in X-ray CT scans have been extensively studied. Gold nanoparticles provide superior images. They have longer circulation times than conventional iodine based contrast agents enabling prolonged imaging of specific cells [141]. In a study, PEG-coated AuNPs were injected to mice for X-ray CT scan. The scan could be recorded even after 24 h [146]. Gold nanoparticles coated with a Gadolinium chelator Gd-DTDTPA provided images for both CT scan and MRI [147]. van Schooneveld et al. [148] prepared gold/silica nanoparticles for CT scan, MRI, and fluorescent imaging.

6.3.7 Mesoporous Silica Nanoparticles

Mesoporous silica nanoparticles (MSNPs) have emerged as drug delivery devices around two decades ago. They have unique properties: large surface areas, large pore volumes, high loading capacity, biocompatibility, and tunability regarding size and pore diameter. First MSNP was Material MCM-41 (Mobil Composition of Matter no. 41) which was synthesized in Japan and later in the USA at Mobil Corporation. It had 2D hexagonal pore structure, ~60–100 nm size, pore diameter ~1.5–3.5 nm, surface area ~1000 m² g⁻¹ and pore volume ~1 cm³ g⁻¹. The well-studied MSNPs include MCM-41 and SBA-15. The latter were produced at University of California, Santa Barbara and had pore size ~5–30 nm. MSNPs have surface silanol groups, i.e., free silanol group ($\equiv\text{Si}-\text{OH}$) and geminal silanol group ($=\text{S}(\text{OH})_2$) which have affinity for head groups of phospholipids. Thus affinity for adsorbing on cell surfaces that finally leads to endocytosis (transport of substances into cells). The strong silicon-oxygen bonds provide stability to silica nanoparticles and these are mechanically stable as compared to liposomes.

Surface modification of MSNPs is done by three methods: co-condensation, grafting, and imprint-coating method [149–152]. They are PEGylated to avoid uptake by the RES system. A research group has reported PEGylation of MSNPs. 25 mg MSNPs was dispersed in 30 ml of pH 4 HCl solution of ethyl-alcohol and water (volume ratio 1:2) with stirring. The solution was termed as solution-A.

Dissolution of 100 mg PEGxk-silane was done in 30 ml of ethyl-alcohol. The solution was termed as solution-B. A specific volume of solution-B was added dropwise in solution-A. The mixture of both solutions was vigorously stirred for 24-h and then dried at 100 °C for 12-h in vacuum. The experimentation gave covalently-PEGylated MSNPs [153]. Different ligands can be conjugated to the surface of MSNPs for site-specific targeting of drugs against overexpressed receptors such as Epidermal Growth Factor Receptors (EGFRs) in cancer cells. They are made of constituents such as tetraethyl orthosilicate (ETOS), a silica source and C16TAB (a surfactant) which are not costly. They are used for the controlled release of antibiotics, anti-inflammatory agents, etc. [149–152].

Stimuli-sensitive MSNPs have been developed for the delivery of drugs at specific sites. The stimuli include external (temperature, electric field, magnetic field, light) and internal (pH, redox potential, enzymes). The stimuli make drug carrier leaky, thereby drug is released at the desired site [154]. In a study, it was found that the combined use of photodynamic therapy using a photosensitizer (porphyrin) and lectin-targeted MSNPs containing anticancer drug (camptothecin) was very effective in the treatment of cancer [155]. Further the combined use of doxorubicin and siRNA employing MSNPs against the target (the P-glycoprotein drug transporter), showed more than 50% efficiency in treating multidrug resistant breast cancer than free drug alone in a xenograft model in mice [156].

6.4 Challenges and Limitations

The drug carriers are not without challenges and limitations. For example, liposomal preparations are advised to be stored at refrigeration temperature, not in a freezer to avoid disruption of lipid bilayers [157]. Solid lipid nanoparticles have low entrapment efficiency [2]. Quantum dots can be incorporated in nano drug carriers such as liposomes but long-term toxicity issue needs to be addressed [132]. A lot of research has been carried out on nanocarriers but more research is required for optimization of nanocarriers using cutting edge surface engineering techniques to tailor a nanocarrier for treatment of fatal diseases, i.e., cancer. In this regard, multifunctional nanocarriers obtained by surface modification of a nanocarrier with a targeting ligand, pro-drug, and imaging agent is the latest approach. Research on multifunctional nanocarriers should be focused for more efficient treatment of diseases. Each nanocarrier should be considered unique and must be individually evaluated. Systematic studies on various aspects of nanocarrier's surface modification need to be carried out before proceeding to clinical trials [158].

PEGylation has significantly improved circulation time of liposomes. PEGylation has reduced the liposomal uptake by RES but it does not completely inhibit liposomal uptake [159]. Liposomal uptake is also possible through pathways which are independent of opsonization suggesting more research on surface modification of liposomes for further increment in circulation time [160, 161]. Modification of liposomes by various synthetic components may lead to antibody

production against various components of liposomes, if recognized as a foreign particle by the body [162–164]. Some liposomal formulations trigger innate immune-response leading to hypersensitivity syndrome called complement activation-related pseudoallergy (CARPA) [165]. Development of new surface modification techniques can resolve the issues and have the potential to decrease immunogenic-reactions to liposome based treatment preventing their loss of efficacy, altered pharmacokinetics, and toxicity.

Impressive-progress has been made in preparation and targeting of nanoparticles for treatment of diseases but only few nanoparticle-based drugs are commercially available. This is due to following main reasons. Nanoparticles have low drug loading capacity, i.e., less than 5% drug with respect to nanocarrier weight. Burst release of drug after administration is another challenge, needs to be addressed. Burst release is rapid drug-fraction release of adsorbed or anchored drug from nanocarrier's surface leading to lower therapeutic response [166].

Hydrogels typically have higher water content resulting in rapid drug release from gel-matrix especially hydrophilic drugs. The drug release profile of hydrogels is usually shorter than some other nanocarriers. Sensitive molecules, e.g., antibody, proteins, and nucleic acid are deactivated by interaction with hydrogel. Similarly, hydrogel mediated delivery of hydrophobic drugs also requires improvement. Complexation or pre-encapsulation of biomolecules may be helpful in addressing these challenges [167].

Gold nanoparticle is a promising nanocarrier. However, challenges such as toxicity, biodistribution, and pharmacokinetics needs to be addressed [158]. Biodistribution of gold- nanoparticles is often affected by the route of administration. Therefore, nanomaterials should be properly characterized and in vivo studies can be carried out in animal models with apt statistical analysis [168]. Gold nanoparticles have low inherent toxicity [169]. More research on interaction of gold nanoparticles with tumor microenvironment should be done to reduce their toxicity by adapting suitable surface engineering techniques. Detection of toxicity of gold nanoparticle, i.e., core, capping agents and ligands is another research area, needs to be explored. Certain ligands attached to gold nanoparticles cause toxicity, e.g., cationic ligands leads to moderate toxicity [170].

Surface engineering has played a vital role in formulation and optimization of nanocarriers for better targeting, cellular-internalization (endocytosis), and therapeutic response. Surface engineering aided in addressing the challenges and limitations associated with nanocarriers. It can provide multifunctional properties to these nanocarriers, i.e., imaging, prognosis, diagnosis, and additionally theranostics. Research on surface modification and engineering based on the incorporation of desired bioactive compound to get anticipated results will lead to achieve a targeted drug and gene delivery with enhanced drug loading capacity and better cellular uptake for prolonged duration of action.

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