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Core–shell-type lipid–polymer hybrid nanoparticles as a drug delivery platform

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Abstract

The focus of nanoparticle design over the years has evolved toward more complex nanoscopic core–shell architecture using a single delivery system to combine multiple functionalities within nanoparticles. Core–shell-type lipid–polymer hybrid nanoparticles (CSLPHNs), which combine the mechanical advantages of biodegradable polymeric nanoparticles and biomimetic advantages of liposomes, have emerged as a robust and promising delivery platform. In CSLPHNs, a biodegradable polymeric core is surrounded by a shell composed of layer(s) of phospholipids. The hybrid architecture can provide advantages such as controllable particle size, surface functionality, high drug loading, entrapment of multiple therapeutic agents, tunable drug release profile, and good serum stability. This review focuses on current research trends on CSLPHNs including classification, advantages, methods of preparation, physicochemical characteristics, surface modifications, and immunocompatibility. Additionally, the review deals with applications for cancer chemotherapy, vaccines, and gene therapeutics.

From the Clinical Editor: This comprehensive review covers the current applications of core–shell-type lipid–polymer hybrid nanoparticles, which combine the mechanical advantages of biodegradable polymeric nanoparticles and biomimetic advantages of liposomes to enable an efficient drug delivery system.

Key words: Lipid-polymer hybrid nanoparticles; Core–shell; Drug delivery; Lipoparticles; Cancer

Nanoparticles (NPs) have attracted much attention because of their ability to deliver drugs to the therapeutic targets at relevant times and doses. Of all the common nanoparticulate systems, liposomes and biodegradable polymeric NPs (PNPs) have emerged as the two dominant classes of drug nanocarriers, as evidenced by increasing numbers of clinical trials, research reports, and approved drug products.[1–3] Both classes have advantages and limitations in terms of their physicochemical and biological properties. Historically, lipids have been used for several decades in various drug delivery systems including liposomes,[1] solid lipid NPs,[4] nanostructured lipid carriers,[5] and lipid–drug conjugates.[6] Most liposomes are biocompatible, biodegradable, nontoxic or mildly toxic, flexible, and nonimmunogenic for systemic and nonsystemic administration if their component lipids are from natural sources.[7] However, liposomal drug products have several limitations from the viewpoint of physical and chemical stability, batch-to-batch reproducibility, sterilization, drug entrapment, and manufacturing scale-up.[3,7–9] Generally, PNPs are advantageous in terms of smaller particle size, tissue penetrating ability, a greater variety of preparation methods, availability of various polymers, improved stability in biological fluids, versatile drug loading, and release profiles.[2,10] The limitations of PNPs include use of toxic organic solvents in the production process,[11] poor drug encapsulation for hydrophilic drugs, drug leakage before reaching target tissues, polymer cytotoxicity, polymer degradation, and scale-up issues.[10]

Novel, integrated systems known as lipid–polymer hybrid nanoparticles (LPHNs) have been introduced in an effort to mitigate some limitations associated with liposomes and PNPs.[12] Briefly, the biomimetic characteristics of lipids and architectural advantage of polymer core are combined to yield a theoretically superior delivery system. LPHNs are solid, submicron particles composed of at least two components: the polymer and the lipid.

Conflict of interest and disclosure: The authors report no financial interest that might pose a potential, perceived, or real conflict of interest.

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Table 1
Various classes of lipid–polymer hybrid nanoparticles (LPHNs).

Type	Description	Synonyms	Reference
Polymer core–lipid shell	Colloidal supramolecular assemblies consisting of polymer particles coated with lipid layer (s)	Lipoparticles Lipid–polymer particle assemblies Lipid-coated NPs Nanocell Polymer-supported lipid shells	Troutier et al,[20] Hetzer et al[62] Troutier et al,[19] Thevenot et al,[17,18] Bathfield et al[63] Messerschmidt et al[59] Sengupta et al[55] Bershteyn et al[78]
Core–shell-type hollow lipid–polymer–lipid NPs	Hollow inner core surrounded by concentric lipid layer, followed by polymeric layer, again followed by lipid layer along with lipid–PEG.		Shi et al[68]
Erythrocyte membrane-camouflaged polymeric NPs	Sub-100-nm polymeric particles are coated with RBC membrane derived vesicles to mimic complex surface chemistry of erythrocyte membrane	Biomimetic NPs	Hu et al[76]
Monolithic LPHNs	Lipid molecules are dispersed in a polymeric matrix	Mixed lipid–polymer particles	Gao et al[14]
Polymer-caged liposomes	These systems are composed of polymers, anchored or grafted at the surfaces of the liposomes to provide stability		Lee et al[8,9]

Various bioactive molecules such as drugs, genes, proteins, and targeting ligands can be entrapped, adsorbed, or covalently attached in the hybrid system. The common choices of biodegradable polymers include polylactic-*co*-glycolic acid (PLGA), polycaprolactone (PCL), dextran, or albumin because of their biocompatibility, biodegradability, nontoxicity, and previous use in approved products.[13,14] Lipids used are often zwitterionic, cationic, anionic, and neutral phospholipids such as lecithin, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), or 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE).[15–21] Various classes of LPHNs are summarized in Table 1 and are classified by the arrangement of lipid and polymer in the hybrid system.

Because of their perceived advantages over other existing hybrid systems, significant effort has been directed toward understanding CSLPHNs.[22–31] The primary objective of this review article is to discuss CSLPHNs, which are composed of polymeric core and lipid shell. Discussion of other types of LPHNs is limited as it is not within the scope of this communication.

Core–shell-type LPHNs

CSLPHNs continue to gain recognition in drug, gene, protein, and vaccine delivery.[32–35] Based on the CSLPHN concept, a new nanoparticulate drug delivery system, known as "Supra molecular bio-vector™" (SMBV™), was introduced in the early 1990s by Biovector Therapeutics.[36] SMBV is an artificial analog of virus composed of a modified polysaccharide hydrogel core covered with phospholipids acting as a shell. Because of its size (~60 nm) and architecture mimicking the structure of viruses,[37] SMBV has been investigated for various purposes such as delivery of anticancer agents,[38] nasal vaccines,[37] and antisense oligonucleotides.[39] Originally, core–shell-type hybrid microparticles and NPs were synthesized with a lipid shell and a core that was made from inorganic materials such as silica,[40] magnetic iron oxide,[41] or organic materials such as polysaccharides,[42] polystyrene,[43] polyelectrolyte capsule,[44] or polymer microgels.[45] Comprehensive reviews by Troutier and Ladaviere[46] and Richter et al[47] are available on lipid membrane systems supported by various organic and inorganic colloidal solid cores and are not highlighted in this review. Instead, our main focus is on polymeric cores (preferably biodegradable) that can be used in drug delivery systems.

CSLPHNs systems can be described as a polymeric core coated with single or multiple layers of lipids that constitute the shell. Based on the concept of core–shell architecture, lipoparticles or lipid/polymer particle assemblies were first synthesized for various biotechnological and biomedical applications such as immunological kits and biosensors for amplifying biomolecular recognition.[17,19] The special features of lipoparticles are imparted by their method of preparation and use of the types of lipid materials. They are generally prepared by mixing liposomes and PNPs to form lipid–polymer complexes in which a lipid bilayer or lipid multilayers cover the surface of the polymeric core. The space between polymeric core and lipid layer is usually occupied by water or aqueous buffer (Figure 1, *A*). Cationic or zwitterionic phospholipids have been used to construct the shell of the lipoparticles to promote electrostatic interactions with oppositely charged polymers.

In a recent report, Zhang et al[12] designed a novel CSLPHN system composed of three functional building blocks, each having distinct attributes that influence the whole hybrid delivery system (Figure 1, *B*). The first building block is a polymeric core composed of a biodegradable hydrophobic polymer (e.g., PLGA) and acts as the carrier for poorly water-soluble drugs. This core imparts controlled drug release from the system. The second component is the shell or the outer corona of the hybrid particles composed of hydrophilic substrates, most commonly

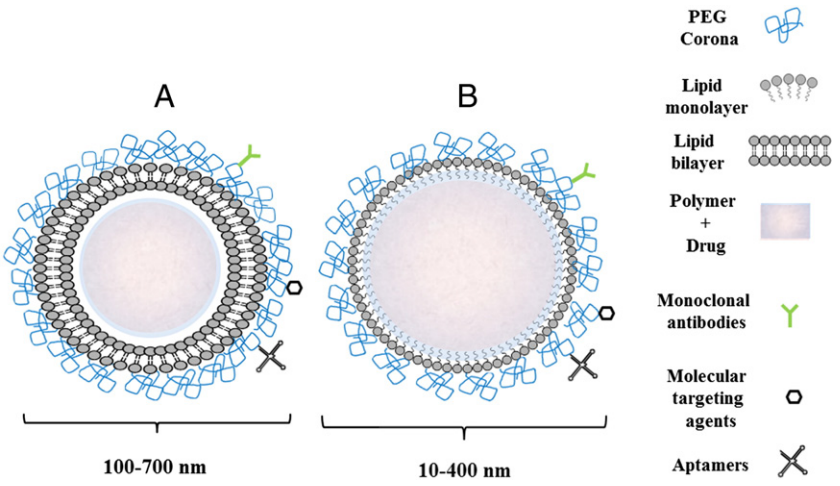

Figure 1. Schematic representation of CSLPHN with its structural components. **(A)** Lipoparticles with a lipid bilayer. **(B)** CSLPHNs with a lipid monolayer.

lipid–PEG conjugates. This layer allows the particles to evade uptake by the immune system and imparts long-circulating characteristics. The shell can also be manipulated to facilitate the attachment of targeting ligands. Finally, the third component is composed of a lipid monolayer at the interface of core and shell. This layer helps to reduce drug diffusion from the core and water penetration into the core, thereby increasing drug encapsulation and altering drug release rates.

Advantages of CSLPHNs

Some of the perceived advantages are summarized below:

- The solid polymeric core acts as a cytoskeleton that provides mechanical stability, controlled morphology, biodegradability, narrow size distribution, and high available specific surface area.[48–50]
- The lipid shell enveloping the core is biocompatible and exhibits behavior similar to that of cell membranes. The shell has the ability to interact with a wide variety of molecules, either within the membrane or on the surface.[51]
- Improved encapsulation of hydrophobic drugs with therapeutically effective drug entrapment efficiency and drug loading has been reported for a number of drugs compared to liposomes or PNPs.[26,29,32]
- Amphiphilic character of lipids facilitates the adsorption of hydrophilic compounds on the bilayer surface and insertion of hydrophobic molecules into the hydrophobic lamellar region.[51–54] This feature allows CSLPHNs to entrap and deliver multiple hydrophilic and hydrophobic therapeutic agents simultaneously.[29,55]
- Optimization of the core and shell can result in tunable and sustained drug release profiles.[56]
- CSLPHNs exhibit storage and serum stability over prolonged periods.[34,56]
- Besides passive targeting of CSLPHNs based on particle size, they can be conjugated with appropriate targeting ligands such as aptamers,[56] folic acid,[27,57] transferrin,[58] anticarcinoembryonic antigen half-antibody,[24] or single-chain tumor necrosis factor[59] to deliver NPs at the target tissues for treating cancers.
- Particles smaller than 100 nm (similar to virus-like architecture) are promising for intracellular drug targeting and vaccine adjuvants.[60]

Methods of preparation

Methods used to prepare CSLPHNs broadly fall into two categories; the two-step method and the single-step method.

Two-step method

The polymeric core and lipid shell are prepared separately using two independent processes; then the two components are combined by direct hydration, sonication, or extrusion to obtain the desired lipid shell–polymer core structure (Figure 2). Several investigators have prepared lipid–polymer particle assemblies or lipoparticles to obtain solid supported lipid bilayers that act as a model for artificial cell membrane and also for drug delivery applications.[17,19,34,55,57,61–63] In the two-step process, cationic lipid vesicles and anionic PNPs are drawn together by electrostatic interactions.[20]

The fusion of the PNPs and lipid vesicles can be accomplished using different mixing protocols.[46] The dry lipid film can be hydrated with the PNP dispersion or the PNPs can be introduced into preformed lipid vesicles. Such a process is usually followed by low-energy mixing processes such as vortexing the resulting mixture. This mixture is heated at a temperature above phase transition temperature (T_m) of the lipid to facilitate reorganization of the lipid onto the particle surface.[19] The nonadsorbed lipids, micelles, and free PNPs are separated by centrifugation to obtain a final CSLPHN dispersion.

Several factors affect final particle size of lipoparticles: methods applied to produce lipid vesicles (direct hydration, sonication, or extrusion), mixing protocol of lipid vesicles/PNPs, type of polymers/lipids, pH and ionic strength of buffers used, surface charge of lipid vesicles, vesicle-to-particle ratio, and temperature of incubation.[19,46] In general, adding water or an

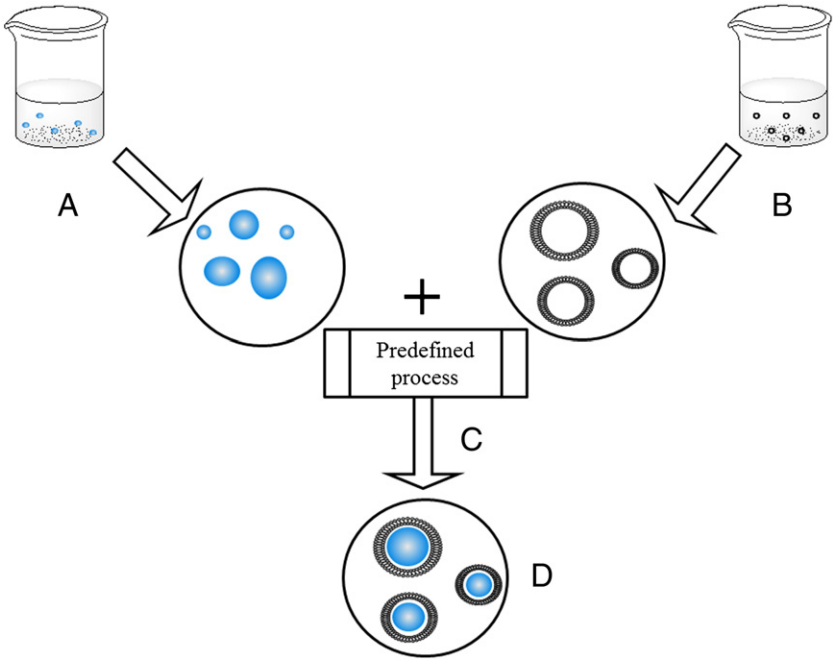

Figure 2. Schematic representation of the steps involved in lipoparticle synthesis by the two-step method. **(A)** Polymeric nanoparticle cores (PNPs) are prepared separately. **(B)** Lipid shells (liposomes) are prepared separately. **(C)** Both polymeric cores and lipid shells are mixed and incubated. **(D)** Finally, lipoparticles are obtained.

aqueous buffered solution to dry lipid film forms large, multilamellar vesicles. However, using additional steps such as sonication or extrusion leads to formation of small, unilamellar vesicles with smaller particle sizes and a lower polydispersity index. Troutier et al[19] reported that particles of approximately 100 nm were obtained using membrane extrusion compared to 250 nm using hydration or 500 nm using sonication. Simultaneous loading of two drugs, doxorubicin and combretastatin, into nanocells by using the two-step approach has been reported.[55] In this study, doxorubicin–PLGA-conjugated PNPs were prepared using an emulsion/solvent evaporation technique. Then, combretastatin-loaded lipid vesicles were prepared using phosphatidylcholine, cholesterol, and PEG-DSPE. Finally, hybrid dual-drug-loaded nanocells were obtained by extruding the mixture of PNPs and lipid vesicles. The size of the particles ranged from 180 to 200 nm. The authors reported that combretastatin was released from the CSLPHNs at a faster rate compared to doxorubicin. The differential drug release was attributed to localization differences inside nanocells. The authors postulated that combretastatin was entrapped at/in the shell, whereas doxorubicin was located in the core.

In the two-step process, particle size and drug loading of the core can be precisely controlled to produce final lipid–polymer hybrid nanoparticles of appropriate size and drug loading and release characteristics.[19,55] In addition, the theoretical amount of the lipid required to uniformly coat the core with a uniform bilayer of phospholipids can be calculated based on the properties of the core and phospholipids.[64,65] However, the two-step method may reduce drug encapsulation efficiency for water-soluble drugs in the incubation step, because drug molecules may leak from the core before a lipid coat is formed on the core surfaces.[66] Some limitations of this method are the technical complexity and less efficient processes of preparing both polymeric core and liposome vesicles separately.

Single-step method

To circumvent the problems of time-consuming preparation steps, a relatively simple approach that combines the dual steps of the two-step method into a single step has been evaluated. Here a nanoprecipitation process is synchronized with a simultaneous self-assembly process (Figure 3). One of the critical factors influencing successful preparation of CSLPHNs using this method is the amount of lipid needed for uniform lipid coating of polymeric core particles. Variations of the single-step method have been reported in the literature, including modified solvent extraction/evaporation and nanoprecipitation methods.

Modified solvent extraction/evaporation method

This method is a modification of the emulsion/solvent evaporation method first reported by Gurny et al.[67] The method has been used to prepare CSLPHNs loaded with docetaxel[16,27] and paclitaxel.[28] Briefly, the polymer and drug are dissolved in a water-immiscible organic solvent such as dichloromethane, chloroform, or ethyl acetate. A predetermined amount of lipid is then dispersed in water by bath sonication, mechanical stirring, or sometimes heat. The organic solution is mixed into the aqueous phase, and the resulting dispersion is sonicated using a probe sonicator and ice bath. The organic dispersed phase is broken into tiny nanodroplets, which are solidified into nanospheres coated with a lipid layer. The organic solvent is usually

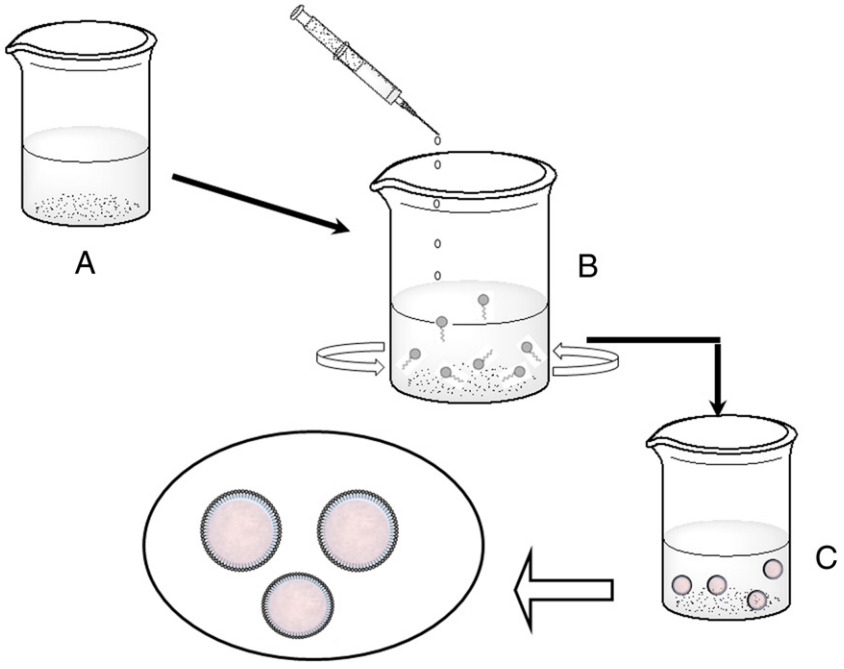

Figure 3. Schematic representation of the single-step method involving nanoprecipitation and self-assembly processes. **(A)** Drug, polymer dissolved in organic solvent forming organic phase. **(B)** The organic phase is added dropwise into the aqueous phase containing phospholipids. **(C)** The resulting dispersion is sonicated or homogenized to obtain CSLPHNs.

removed by evaporation in a rotary evaporator under reduced pressure or stirred overnight. The particle suspension is purified by centrifugation followed by controlled washing. The washed particles are freeze-dried to obtain a dry powder.

Liu et al[27] used this method to prepare folic acid-conjugated docetaxel-loaded CSLPHNs having a particle size of approximately 200–300 nm and drug encapsulation efficiency of 60%–66%. They observed a decrease in mean particle size of CSLPHNs with increasing concentrations of lipid. This phenomenon was attributed to the presence of 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC) lipid that acts as an emulsifier, thereby lowering the surface tension of the lipid monolayer and resulting in lower surface free energy and smaller CSLPHNs.

A typical approach to entrap hydrophilic small and macromolecules in microparticles/NPs is to use a multiple emulsion/solvent evaporation method. A similar approach used to prepare hollow core–shell-type lipid–polymer–lipid hybrid NPs. This method uses a modified double-emulsion/solvent evaporation for encapsulation and delivery of nucleic acids.[68] The hollow aqueous core acts as the reservoir for hydrophilic small interfering RNA (siRNA). Briefly, in the first step, a primary water-in-oil (w/o)-type emulsion was formed by dispersing with sonication the aqueous siRNA into an organic solvent containing a polymer and a cationic phospholipid. The phospholipid layer helps stabilize the aqueous droplets and increase the loading of siRNA by polycomplexation. A secondary oil-in-water (o/w)-type emulsion is prepared by adding the primary emulsion into aqueous dispersion of another phospholipid (lecithin) and DSPE-PEG. Finally, the organic solvent is evaporated to prepare multilayered CSLPHNs. Not only macromolecules but also water-soluble hydrophilic small-molecular-weight drugs such as antibiotics have been encapsulated within CSLPHNs via the double-emulsion solvent evaporation method.[66]

Modified nanoprecipitation method

In this method, polymer(s) and hydrophobic drug(s) are dissolved in a water-miscible organic solvent (e.g., acetonitrile or acetone). The organic solution is then added, drop by drop, to the aqueous dispersion containing lipid and/or lipid–PEG conjugate. The mixture is vortexed and subsequently homogenized or ultrasonicated to reduce the particle size to nanometer range. Valencia et al[69] reported a similar method based on rapid mixing of lipid and polymer solutions by using a continuous flow microfluidic device that used hydrodynamic flow in combination with passive mixing structures to prepare CSLPHNs in a single step. Their study indicated that, to ensure proper dispersion of lipid and lipid–PEG conjugate, it is necessary to heat the aqueous dispersion (generally ~65 °C) before adding the organic solution. To uniformly coat the polymeric core with a lipid shell and to evaporate the organic solvent, the dispersion was stirred for several hours with a magnetic or mechanical stirrer. CSLPHNs formed were purified by ultracentrifugation, centrifugal ultrafiltration, or dialysis.

The critical factors to be optimized for particle size, polydispersity, and surface charge include the type of the lipid, lipid/polymer ratio, phase/volume ratio of organic to aqueous phase, and viscosity of the polymer.[12,23,69] Docetaxel CSLPHNs were prepared by this method to produce particles of mean size of 66 nm and encapsulation efficiency of approximately 60%.[56]

Recently, a fast and simple method of using sonication to produce CSLPHNs was reported by Fang et al.[23] They prepared CSLPHNs of uniform and controllable size (~65 nm) and low polydispersity index (~0.08) by using bath sonication for 5 min

Table 2
Examples of CSLPHN applications.

Encapsulant	Polymer	Lipid	Particle size	EE/DL	Application	Reference
Doxorubicin and combretastatin	PLGA	PC/Chol/DSPE-PEG	180–200 nm	NR	Melanoma, Lewis lung carcinoma	Sengupta et al[55]
Doxorubicin and GG918	HPESO	Tristearin	150–270 nm	70%–90%	MDR breast cancer	Wong et al[29]
Doxorubicin	HPESO	Stearic acid	290 nm	76%	MDR breast cancer	Wong et al[30]
Doxorubicin	PLGA	DPPC	195 nm	DL 0.52%	MDR breast cancer	Li et al[61]
Paclitaxel	PLGA	Lecithin	83–95 nm	NR	Pancreatic cancer	Hu et al[24]
Verapamil HCl	Dextran	Decanoic acid	342.5 nm	90%–99%	NR	Li et al[26]
Paclitaxel	PLGA	DLPC	200–300 nm	43%–56%	Cancer	Liu et al[28]
Paclitaxel	PLGA	OQLCS	184–194 nm	84%–88%	Cancer	Zhao et al[57]
Docetaxel, indium 111 and yttrium 90	PLGA	DMPE-DTPA/lecithin	65 nm	60%	Prostate cancer	Wang et al[56]
AChE	PMOXA–PDMS–PMOXA	EPC/DPPC	75 nm	NR	Protein delivery	Ruysschaert et al[21]
Docetaxel	PLGA	Soy lecithin	60–70 nm	NR	Cancer	Chan et al[16]
Docetaxel	PLGA	Lecithin/DSPE-PEG	70–80 nm	~60%	Cancer	Zhang et al[12]
Plasmid DNA	PEI	Triolein/EPC/DSPE-PEG	128 nm	NR	Gene delivery	Li et al[25]
Plasmid DNA	PLGA	DOTAP/DC-Chol	100–400 nm	NR	Gene delivery	Zhong et al[138]
Plasmid DNA	PLA	DPPC/DPTAP	325–340 nm	NR	Gene delivery	Troutier et al[33]
mRNA	PBAE	DOPC/DOTAP	230–300 nm	NR	mRNA based vaccine delivery	Su et al[146]
siRNA	PLGA	EPC/Lecithin/DSPE-PEG	225 nm	78%-82%	Tumor suppression	Shi et al[68]
7α-APTADD	PLGA	Egg PC/DOPE/TPGS	170.3 nm	36.3%	Breast cancer	Zheng et al[58]
Fluoroquinolone antibiotics	PLGA	Phosphatidyl choline	260–420 nm	4%–25%	Lung biofilm, infection therapy	Cheow et al[66]
5-Fluorouracil	PGA/Dextran	Cetyl alcohol/Tripalmitin	600–1100 nm	DL 4.4%–18%	Lung cancer	Hitzman et al[52–54]
FITC-BSA	Protamine sulfate	Cholesterol/DSPC/DHA	130–200 nm	19%–59.6%	Protein delivery	Chang et al[34]

Abbreviations: EE, entrapment efficiency; DL, drug loading; NR, not reported; HPESO, hydrolyzed polymer of epoxidized soybean oil; MDR, multi-drug resistant; PLGA, poly(lactic-*co*-glycolic acid); DLPC, dilinoleoylphosphatidylcholine; DMPE-DTPA, 1,2-ditetradecanoyl-*sn*-glycero-3-phosphoethanolamine-*N*-diethylenetriaminepentaacetic acid; DSPE-PEG, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[amino(polyethylene glycol); PMOXA-PDMS-PMOXA, poly(2-methyloxazoline)-block-poly(dimethylsiloxan)-block-poly(2-methyloxazoline); DPPC, dipalmitoylphosphatidylcholine; PEI, polyethyleneimine; EPC, 1,2-dimyristoleoyl-*sn*-glycero-3-ethylphosphocholine; PGA, poly(glutamic acid); DPTAP, 1,2-dipalmitoyl-3-trimethylammonium-propane; PLA, poly(lactic acid); OQLCS, octadecyl-quaternized lysine-modified chitosan; DHA, *cis*-4,7,10,13,16,19-docosahexanoic acid; PBAE, poly-(β-amino ester).

compared to a few hours for other fabrication approaches. The size and polydispersity of the particles were effectively controlled by optimizing the ratios of lipid–PEG/polymer and lipid/lipid–PEG/polymer.

Drug loading and entrapment efficiency

Many small-molecular-weight chemotherapeutic drugs, proteins, and nucleotides have been encapsulated/entrapped in CSLPHNs (Table 2). One reason for poor drug loading (DL) and entrapment efficiency (EE) in CSLPHNs is the presence of excess lipids that can form vesicles by entrapment or adsorption of drug via hydrophobic interactions and/or hydrogen bonding.[28] Additionally during purification, these vesicles are washed away, leading to drug loss. Therefore, the amount of the lipid required to uniformly coat the core nanoparticles has to be optimized using empirical and/or experimental techniques.

Various techniques exist for drug loading into CSLPHNs. The drug can be loaded into both the polymeric core and the lipid shell, thereby increasing the total drug payload. Moreover, two different drugs can be loaded into the core and the shell.[29] The most commonly used strategy is to incorporate the drug during core production or lipid film formation. Another option is to adsorb or absorb the drug with the cores and lipid vesicles separately before combining to form CSLPHNs. However, the DL is generally expected to be better in the incorporation approach than the adsorption approach.[70] The adsorption method has been used to load DNA into lipoparticles composed of PLA core/DPPC–DPTAP lipid shell.[33] The macromolecules or proteins show greatest loading efficiency near their isoelectric point when they have minimum solubility and maximum adsorption.[71] For small molecules, using ionic interactions between the drug and polymer can be an effective way to increase drug loading.[26]

Examples of the factors that may influence DL and EE are aqueous solubility of the drug, affinity and miscibility of the drug in both polymer and lipid phases,[26] amount of lipid,[28] drug–lipid charge interactions,[66] aqueous phase pH,[72] and methods of preparation. Often, in-depth physicochemical characterization during preformulation studies is required to optimize LC and EE.[73] For instance, Li et al[73] analyzed the combined solubility parameters and partition coefficients for screening the best lipid and polymer for the highest LC and the maximum binding capacity to the cationic drug, verapamil. They have reported drug EE greater than 90% and DL between 5% and 36.1%.[26] The amount of the lipid is also a decisive factor for EE of lipophilic drugs in CSLPHNs. Liu et al[28] reported the decrease in EE from 42% to 15% when the lipid component was lowered from 0.1% to

0.01% for paclitaxel particles. Drug–lipid charge interactions may be important for encapsulation of drugs. Cheow and Hadinoto[66] reported successful encapsulation of zwitterionic levofloxacin and ofloxacin when PLGA polymer and phosphatidyl choline (PC) lipid were used, whereas formation and loading of cationic ciprofloxacin into the CSLPHNs were unsuccessful. When PC was replaced with nonionic polyvinyl alcohol, ciprofloxacin-loaded CSLPHNs were successfully produced. The results suggested the possibility of unfavorable ionic interactions between the anionic PC and cationic ciprofloxacin for the failed formulation. The method of preparation also affects DL and EE. The method used during core PNP preparation, such as solvent displacement, leads to poor DL and EE for hydrophilic compounds.[74] Another problem of the two-step method is that encapsulated drugs leak out before the lipid coat is formed.[66]

Surface modification

In addition to the incorporation of drug, the outer surface of the CSLPHNs can be functionalized to make long, circulating particles with the capability of active targeting. PEGs have become a standard for creating long-circulating NPs, thereby reducing plasma protein adsorption, macrophage uptake, and particle aggregation, while increasing circulation time.[75] For long-circulating CSLPHNs, the outer surface is coated with hydrophilic polymeric chains of PEGs anchored in the bilayer with DSPE. The functional coating of PEG stabilizes the particles in storage because of the steric hindrance by its long polymer chains.[23] Another surface modification relates to the acidic environment of tumors. A pH-sensitive PEG coating shed its coating under the acidic condition, fused with cell membrane, and entered into tumor cells.[22] A red blood cell approach to particle surface functionalization was made by coating biodegradable PNPs with natural erythrocyte membranes. The membrane included both membrane lipids and associated proteins.[76] The erythrocyte membrane that covered the polymeric core mimicked the natural endogenous erythrocyte, thus escaping from recognition by the reticuloendothelial system and producing a prolonged circulation time. Erythrocytes have different surface antigens (blood groups), and patients should be cross-matched before injecting these erythrocyte-membrane-camouflaged NPs.

CSLPHNs surfaces can also be modified with folic acid, monoclonal antibodies, or therapeutic cytokines for targeting tumors located in various parts of the body. In general, antibodies or other targeting ligands are attached to the surface of liposomes and NPs by using various covalent and noncovalent coupling techniques, as reviewed by Nobs et al.[77] For example, anticarcinoembryonic antigen (CEA) half-antibody was conjugated to the LPHN surface by a maleimide–thiol coupling reaction.[24] CSLPHNs containing attached folic acid on the surface can be prepared by using presynthesized DSPE-PEG_{5k}–folic acid.[27] Alternatively, CSLPHNs can display on the outer surface a cell death ligand such as tumor necrosis factor-α (TNF-α) that mimics the bioactivity of membrane-bound TNF-α. In one study, the dual attachment of TNF-α in both the core and the shell showed strong and specific binding to TNF receptor-expressing cells.[59]

Table 3
Summary of the instrumental techniques used for physicochemical and in vitro biological characterization of CSLPHNs.

Parameter	Method of characterization
Particle size distribution	Photon correlation spectroscopy (PCS)[24,32,56,66,110]
Surface charge	Zeta potential by PCS[32,56,110]
Morphology	Transmission electron microscopy (TEM),[16,19,32,56] scanning electron microscopy,[24,66,139] atomic force microscopy (AFM),[145] confocal laser scanning microscopy (CSLM),[19,78] fluorescence microscopy[19,56]
Lipid shell thickness	Small angle x-ray scattering (SAXS),[17] TEM[17]
Interface chemical composition	X-ray photoelectron spectroscopy (XPS)[19,28]
Lipid shell fluidity	Fluorescence recovery after photobleaching (FRAP),[78] fluorescent probes[36]
Lipid shell transition	Nuclear magnetic resonance (NMR),[17,18,26] Fourier transform infrared spectroscopy (FTIR),[26] differential scanning calorimetry (DSC),[26] powder x-ray diffraction (PXRD)[26]
Drug loading and entrapment	High-performance liquid chromatography (HPLC),[28] dialysis,[16,56] centrifugation,[66] membrane filtration[32]
Drug release	Dialysis followed by HPLC[16,56]/UV-visible spectrophotometry,[26,66] sample and separate method[28,32]
In vitro cellular uptake	Fluorescence[24,27,56]
Cell viability and cytotoxicity	MTT cell viability assay,[16,28] MTS cell proliferation assay,[56] trypan blue staining,[32] clonogenic assay,[32] ATPLite1-step luminescence ATP detection assay[24]

Abbreviations: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; ATP, adenosine triphosphate.

Physicochemical characteristics

Several physicochemical and biological techniques for characterizing CSLPHNs are summarized in Table 3. Additionally, the following section includes discussions on the mechanism of hybrid particle formation, structure and stability of CSLPHNs.

Interaction and mechanism of hybrid particle formation

The interactions between lipids and polymer particles to form hybrid particles have not been well defined. Generally, different mechanisms of lipid–polymer hybrid particle formation can be distinguished based on the method of preparation. In the single-step method, polymer particle formation involves the precipitation of polymer from an organic solution and the diffusion of the organic solvent in an aqueous medium.[23] Then, the lipid molecules self-assemble spontaneously by hydrophobic interaction on the polymeric particle surface to form a monolayer. In cases when the lipid–PEG component is incorporated, the lipid moiety of the lipid–PEG conjugate is inserted into the lipid

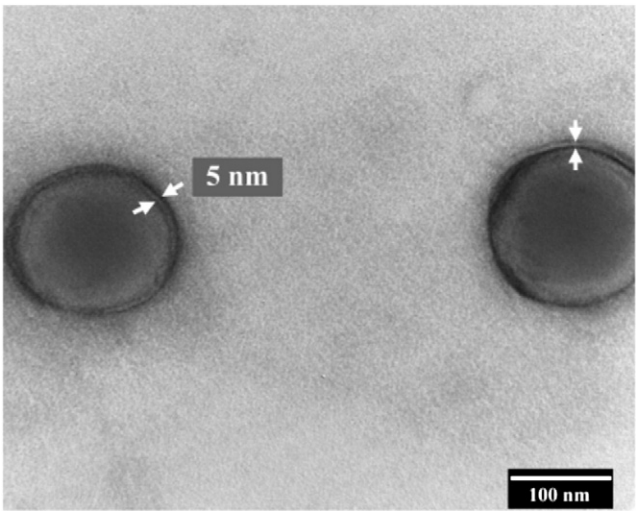

Figure 4. Lipoparticles synthesized in pure water and observed by TEM after staining with sodium silicotungstate (1% wt/vol in water). White arrows indicate the lipid bilayer thickness measured with AnalySIS software (average determined with 30 measurements on 10 particles). Reprinted with permission from Thevenot et al.[17] Copyright [2007] American Chemical Society.

monolayer, and the polar PEG moiety faces outward into the external media to form the stabilizing shell for the hybrid particles.

The possible mechanism of hybrid particle formation in the two-step method can be understood from a study by Carmona-Ribeiro and de Moraes Lessa.[43] Their study involved phospholipid adsorption by polystyrene particles. According to the authors, the process occurs in two steps. First, the phospholipid forms a bilayer in aqueous solution and attaches to the polystyrene particle surface by adsorption to form homodispersed and stable phospholipid vesicle-covered particles. Second, after bilayer attachment, hydrophobic attractions between the polystyrene surface and hydrocarbon chain of the phospholipid bilayer collapse the bilayer structure and leave a monolayer covering the polymer particle. In the process, the lipid and polymer contact is favored by electrostatic interactions, hydrophobic attractions, or van der Waals forces. In addition, the input of external energy such as heating, sonication, or agitation helps to rearrange lipids onto the polymer particles. Surface charges also play a major role in forming the lipid layer onto polymer particles. Stable particles are formed by electrostatic interactions between a negatively charged polymer and a cationic lipid. Moreover, affinity of the phospholipid for the polymer particle depends on the hydrophilicity of the polystyrene surface. Surface hydration of the polystyrene particles can shield the attractive forces and decrease affinity for the lipid monolayer coverage.

Structure

The morphology, two-dimensional fluidity, lipid shell permeability, and distribution of lipids in polymeric particles have been assessed using confocal laser scanning microscopy and cryo-transmission electron microscopy (Cryo-TEM).[78] Often, samples in TEM are stained with uranyl acetate, osmium tetraoxide, or phosphotungstic acid for better imaging contrast to differentiate the core–shell structure (Figure 4). Using negative staining of the low electron-dense lipid layer, Zhang and co-workers[12] deciphered the structure of the PLGA–lecithin–DSPE-PEG LPHNs by TEM. Information about the structure of the hybrid particles is obtained by using conventional fluorescence microscopy and confocal laser scanning microscopy (CSLM). For example, the coexistence of a polymer core and lipid layer has been confirmed after overlay of the fluorescent images of nitro-2-1,1-benzoxadiazol-4-yl phosphatidyl choline (NBD-PC) at 365 nm for the polymeric core and at 534 nm for the lipid layer (Figure 5).[19] As indicated previously, lipid composition and its concentration play a significant role in the formation of various nanostructures of hybrid particles. Thus, the presence of excess lipid during preparation leads to the formation of multilamellar lipid coatings on the particle or may form free liposomal vesicles. Bershteyn et al[78] reported two distinct structures when an excess concentration of lipid (DOPC) and lipid–PEG conjugate (DOPC–PEG) were used to prepare lipid/PLGA hybrid NPs. In the first case, when excess DOPC was used, it formed an onion-like structure with multilamellar stacks of lipid packed together around the polymer core. When 10 mol% of DOPC was replaced by DOPC–PEG, lipid "flowers" were formed with "petals" extruding from the polymer core (Figure 6).

Stability

Evaluation and optimization of physical (colloidal), as well as chemical stability are required for any nanocarrier system. The phospholipids that constitute the shell of the CSLPHNs may act as surfactants to stabilize the hybrid nanoparticles.[79–81] Often, the phospholipids alone are not enough to stabilize the system. For instance, the electrostatic repulsion between colloidal particles failed to stabilize a hybrid system prepared from poly(lactic acid) core and lipid mixtures composed of DPPC/DPTAP when incubated in 10 mM aqueous salt solution.[82]

Four major factors that affect the colloidal stability of lipoparticles have been identified; pH and ionic strength of the aqueous medium, temperature, curvature of radius of lipoparticles, and vesicle-to-particle ratio.[17] These factors are discussed below.

Lipoparticles usually become unstable with an increase in ionic strength of the continuous phase. For lipoparticles composed of a poly-lactic acid (PLA) core and DPPC/DPTAP lipid shell, a significant increase in particle aggregation was seen when the ionic strength of the aqueous phase increased from 1 to150 mM of NaCl solution. This phenomenon can be explained as ion screening of electrostatic charges on the particle surface.[83] The adsorption of lipid onto polymer particle is affected by incubation temperature. When incubation occurs at temperature (T) below glass transition temperature (T_g), the entire vesicle adheres onto particles without rupturing. However, when T is greater than T_g, lipid reorganization onto the polymer particle is accelerated. Sicchierolli and Carmona-Ribeiro[84] studied the adsorption of DPPC lipid on the surface of polystyrene microspheres at two different temperatures (25 °C and 65 °C) for 1 h. High adsorption of DPPC at room temperature suggested entire vesicle adhesion on latex particles. However, at a temperature above T_g for the lipid, monolayer coverage on polystyrene particles was observed because of a change in the physical state of the lipid into a liquid-crystalline state.

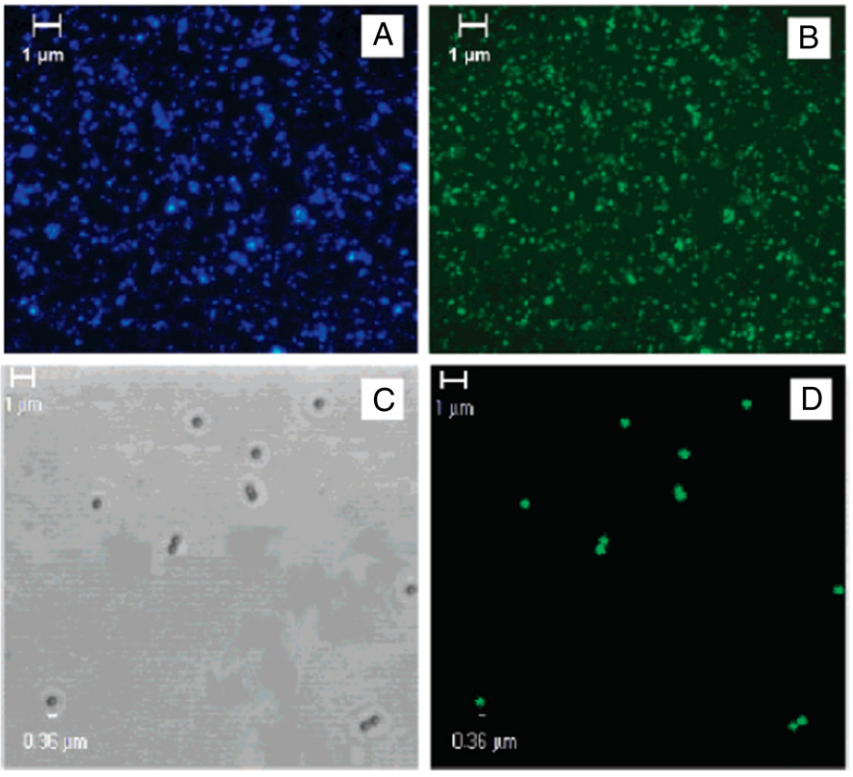

Figure 5. Lipoparticles observed by fluorescent microscopy at two emission wavelengths (identical location): at 365 nm **(A)**, to detect the core particles, and at 534 nm **(B)**, to examine the fluorescent lipids (scale bar = 1 μm). Lipoparticles observed simultaneously by either transmitted light **(C)** or fluorescence microscopy **(D)** (excitation wavelength 458 nm) (scan zoom × 5, scale bar = 1 μm). Reprinted with permission from Troutier et al.[19] Copyright [2005] American Chemical Society.

Spontaneous or intrinsic curvature of the lipid monolayer assemblies arises from the geometric packing of the lipid molecules by intermolecular interactions.[85] Generally, small vesicles having a higher curvature radius tend to coat the smaller polymer particles.[86] As shown in Figure 7, for spherically shaped monolayers, intrinsic curvature (R) of the lipid monolayer membrane can be derived from the following equation:

$$1/R = [2(V/Al-1)/l]/[1\sqrt{(4V/Al-1)/3}]$$

where V is the volume of the entire lipid molecule, l is the length, and A is the area of the lipid head group at the lipid-water interface.

The proportion of lipid vesicles with regard to polymeric particles is an important parameter affecting overall size and stability of lipoparticles. This parameter can be expressed by the following equation:

$$\text{Vesicle to particle ratio} = \text{Total surface area of lipid vesicles (Av)} / \text{total surface area of polymer particles (Ap)}$$

Ap can be determined from the particle number and mean diameter. Based on the study by Troutier et al,[20] it can be hypothesized that the stability of the lipoparticles depends on the value of Av/Ap. For instance, a high Av/Ap value suggests electrostatic stabilization of the lipoparticles while a low Av/Ap value suggests that aggregation will occur. The aggregation behavior at low Av/Ap values can be attributed to the formation of bridges between lipid and polymer and incomplete coating that exposes the anionic zone of the polymer.

One approach to improve the colloidal stability of CSLPHNs is by steric repulsions between particles after incorporating a lipid–PEG conjugate into the formulation.[87,88] Thevenot et al[82] reported that lipoparticle stabilization was improved drastically from 1 mM to at least 150 mM sodium chloride solution for a period of 1 year at 4 °C when 10 mol% lipid–PEG conjugate was added to the formulation. In the process of stabilization by lipid–PEG conjugate, two important aspects were identified: PEG degree of polymerization (n) and molar percentage of lipid–PEG conjugate, which affected the final stability of lipoparticles. The stability of lipoparticles toward ionic strength revealed that the longer the PEG degree of polymerization (i.e., chain length), the greater the stability in polar salt solution. The decreasing order of lipoparticle colloidal stability was reported as a function of PEG degree of polymerization: $PEG_{113} > PEG_{45} > PEG_{16}$. The molar percentage of lipid–PEG conjugate also affected the amount of lipid adsorbed onto particles, thereby affecting the surface coverage by PEG. The amount of lipid–PEG adsorbed decreased when n increased. Because of the steric hindrance by long PEG chains, lipid–PEG_{45} conjugate adsorption was 3 mol% compared to the initial 10 mol%.

Another approach to improve the colloidal stability of CSLPHNs is to incorporate suitable amounts of additional surfactants along with the phospholipids.[66] For example, adding

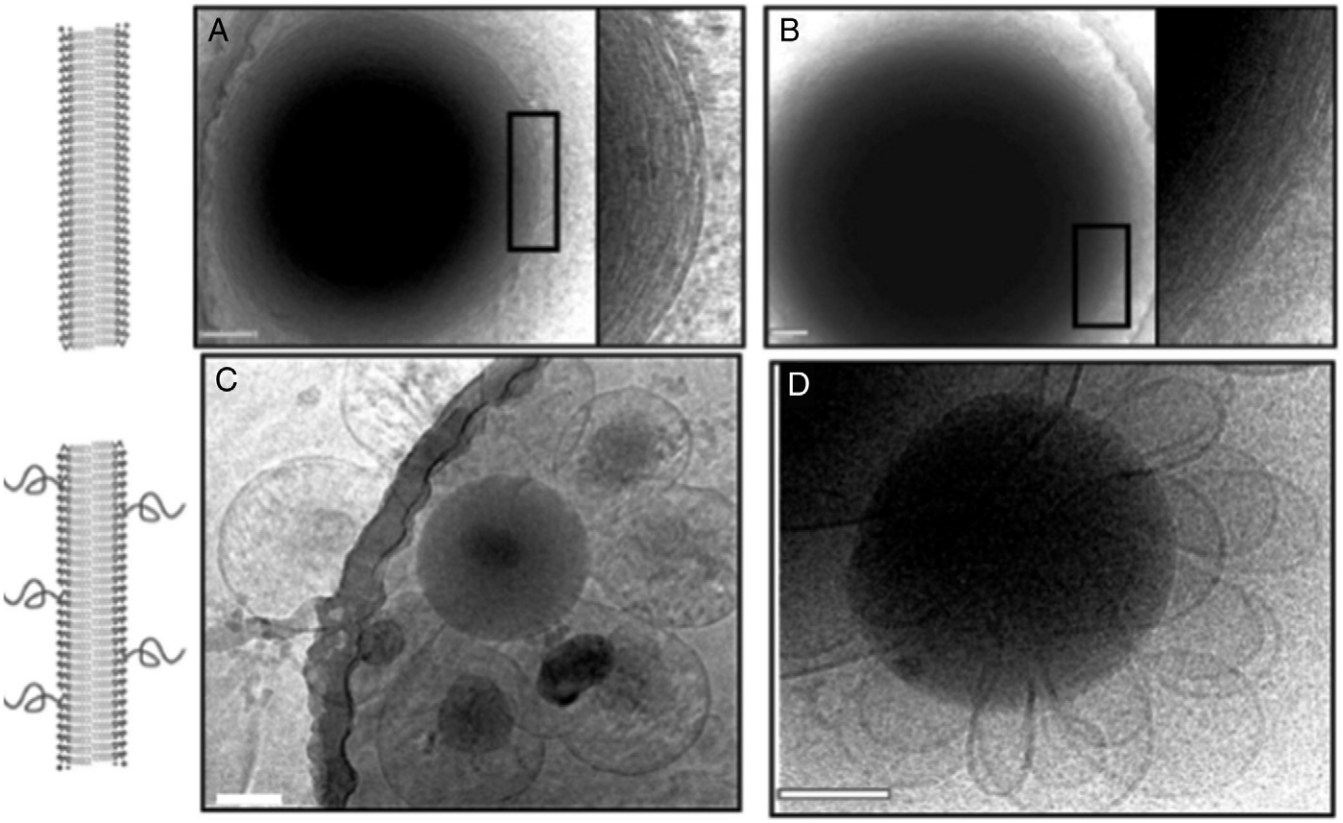

Figure 6. Cryo-TEM micrographs of lipid-enveloped particles made with ~25:9 wt/wt. PLGA/lipid. **(A, B)** PLGA/DOPC particles exhibit "onion" morphologies, with multilamellar stacks of lipid packed together in conformal rings around the particle core. **(C, D)** When 10 mol% PEG-conjugated lipid is included with DOPC as the lipid component, lipid "flowers" form, with "petals" extruding from the polymer core. Scale bars: 100 nm.[78] Reproduced with permission from The Royal Society of Chemistry.

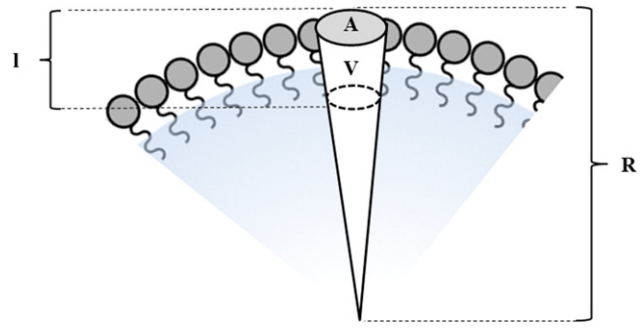

Figure 7. Schematic of the geometry of lipid membrane curvature.

10% D-α-tocopherol polyethylene glycol 1000 succinate, TPGS (an amphiphilic biocompatible, biodegradable surfactant) along with PC confers stability of CSLPHNs in phosphate-buffered saline.[66] Reasonably, the projection of the long and bulky PEG chain of the TPGS enhances stability as compared to small choline head group of PC.[89] Finally, lyophilization may be used to further enhance the colloidal stability of CSLPHNs in storage.[90]

Unlike the physical stability issue that is a common concern for CSLPHN dispersions, the chemical stability is drug specific, depending on the presence of susceptible functional groups and the aqueous solubility of the compound. For example, drug molecules containing esters and amides are susceptible to hydrolytic degradation, while oxidative degradation is common for amine compounds.[91] For poorly water-soluble drug molecules, the possibility of chemical reactions in CSLPHNs is not as substantial as that in solution-based formulations. Considering the inactive ingredients of CSLPHNs, the phospholipids may degrade by hydrolysis and oxidation reactions during storage in aqueous dispersions.[92] The common strategy to enhance the chemical stability of CSLPHNs is to transform the nanoparticle dispersion into dry, solid dosage form by using lyophilization with suitable cryoprotectants.[90,93]

Immunocompatibility

Drug delivery systems including CSLPHNs should be biocompatible, hemocompatible, and immunocompatible to avoid undesirable interactions with the immune system.[94] The recognition of therapeutic nanoparticles as foreign entities may result in multilevel immunological responses (e.g., cytokine release, interferon response, and lymphocyte activation) and lead to severe toxicity and/or lack of therapeutic benefit.[95]

Since a CSHLPN system is composed of polymeric core nanoparticles and lipid shell, the immunocompatibility

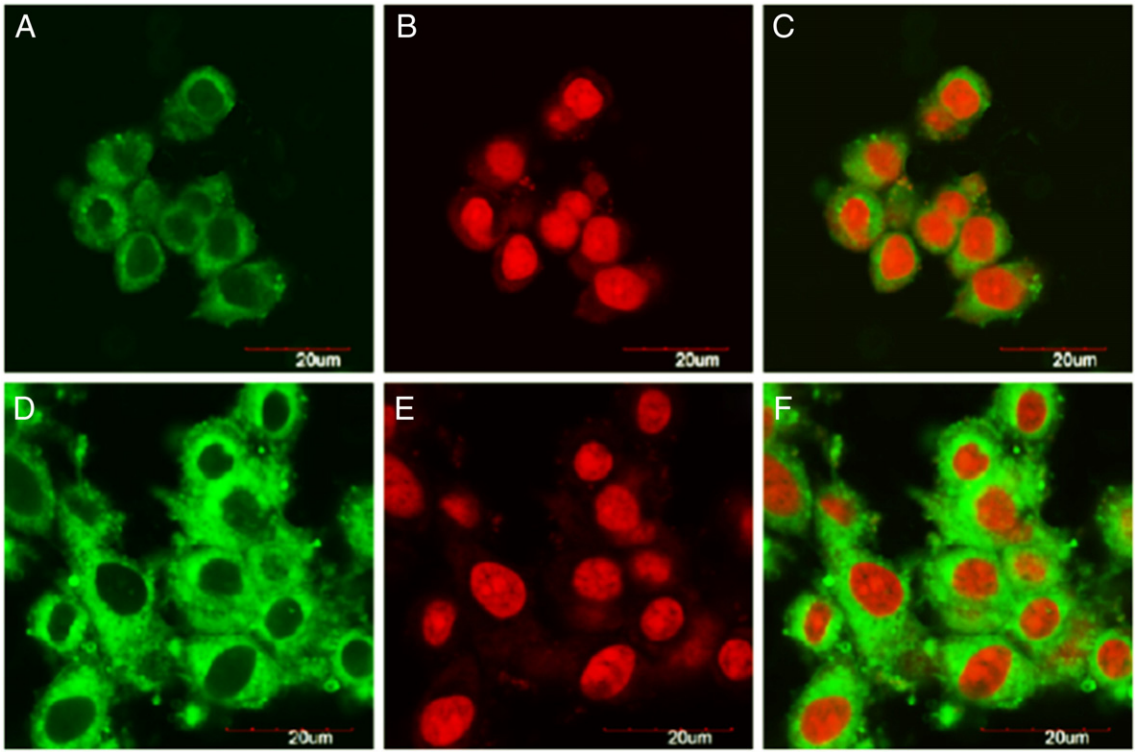

Figure 8. The confocal laser scanning microscopy (CLSM) images of MCF-7 cancer cells after 0.5-h (the upper row) and 2-h (lower row) incubation with the coumarin-6 loaded. DLPC shell and PLGA core NPs at 250 μg/ml NP concentration at 37 °C. Left column **(A** and **D)** shows the green fluorescence in the cells stained in FTIC channel. Middle column shows the red fluorescence from the cell nuclei stained in the propidium iodide (PI) channel. Right column shows the combination of the corresponding left and middle pictures in the same row and obtained from the merged channels of the FITC and PI. All the scale bars represent 20 μm. Reprinted from Liu et al.[28] Copyright (2010), with permission from Elsevier.

properties of the individual components should be considered. Ample evidence exists to show the immunogenic properties of polymeric nanoparticles composed of synthetic polyesters and polyanhydrides.[96,97] Although, the phospholipid bilayers are made up of natural phospholipids found in the body, therapeutic liposomes containing paclitaxel or docetaxel activate the complement system resulting in adverse immune phenomenon C activation-related pseudoallergy.[98,99] Complement activation can be enhanced by the physicochemical properties of liposomes including size,[100,101] charge,[102] aggregation,[103] polyamino coating,[104] presence of endotoxin contaminants,[105] drugs like doxorubicin,[103] and PEGylation.[106] Liposomes are vulnerable to immune recognition since the vesicles mimic the size and shape of some pathogenic microbes, ectosomes, nanobacteria, and viruses. Additionally, lack of self-discriminating molecules (e.g., C control proteins) on the phospholipid bilayers makes them susceptible to immune attack.[94,107–109] Currently, there is a scarcity of immunocompatibility studies on the CSLPHNs, and detailed investigations are warranted. The pioneering work on the immunological characteristics of CSLPHNs including complement system activation, plasma/serum protein binding, and coagulation cascade activation, was reported by Salvador-Morales et al.[110] Among the three surface functional groups of CSHLPNs tested, the methoxy group induced the lowest level of complement activation compared to the amine and carboxyl groups. These researchers showed that the surface chemistry of the CSLPHNs also changed human plasma and serum protein adsorption profiles. The findings of the complement activation and coagulation assay of their study provided evidence for good biocompatibility of CSLPHNs.

Based on the immunocompatibility issues of polymeric core nanoparticles and liposomes, it is necessary to evaluate the immunocompatibility properties of the CSLPHNs. Several in vitro and in vivo techniques such as complement activation assay, platelet count and function test, plasma coagulation, and protein binding studies are available.[94,111] Assessing the complement activation proteins (e.g., SC5b-9, Bb, C4d) by using enzyme-linked immunosorbent assay is one of the most useful in vitro predictors of immunological reactions.[112]

The immunocompatibility of a complex drug delivery system such as CSLPHNs is often challenging to predict based on their physicochemical properties because of the composition of formulations that differs both in nature and percentage of lipids and polymer. Moreover, the immunological response depends not only on the biomaterials but also on the host innate immune reactivity.

Applications in drug delivery

Various drug delivery applications of the CSLPHNs are summarized in Table 2. Among their versatile applications, some major areas with significant clinical implications will be discussed here.

Vaccine adjuvants

NPs are promising adjuvant delivery systems for enhancing and directing the adaptive immune response of vaccine antigens.[113] Biodegradable polymeric microparticles and NPs composed of PLGA have been investigated as potential vaccine delivery systems because of their ability to control the release of antigens and to codelivering immunostimulatory molecules along with antigens in the same particle.[114] However, low antigen EE and denaturation of the antigen during nanoencapsulation have limited their development.[115,116] Antigens adsorbed or covalently coupled onto the surface of presynthesized CSLPHNs could be viable strategies for vaccine delivery.[35,117] Synthetic pathogens, which are surface-modified biodegradable CSLPHNs, can be used to imitate structural features of pathogens for designing vaccine adjuvants.[60] In this context, polymeric core nanoparticles (e.g., PLGA) are advantageous since they activate inflammasome in antigen-presenting cells and enhance innate/adaptive immune responses.[118] In addition, lipid bilayers displaying protein antigens and molecular "danger signals" (such as pathogen-associated molecular patterns) create pathogen-mimicking antigens and related motifs to boost the immune response.[35] The surface display of antigen onto lipid-based NPs has been shown to induce robust antibody responses by mimicking the structure and surface chemistry of microbial pathogens.[119] For example, high IgG titers ($>10^6$) were observed with sustained levels over 100 days after immunization with nanograms of ovalbumin antigen conjugated onto the surface of CSHLPNs along with monophosphoryl lipid A or α-galactosylceramide as molecular danger signals.[35] Moreover, the strategy allows the conjugation reaction to proceed under mild aqueous conditions, thus avoiding harsh processing during encapsulation.[101] Additionally, the immune response can be altered by the presence of heterogeneous surface functional groups. It has been reported that the presence of the amine terminal group of DSPE-PEG on the PLGA–lecithin CSLPHNs induced the highest complement activation and could be considered as vaccine adjuvant.[110]

Cancer targeting

Recent advancements in nanotechnology have fuelled NP development of different sizes, shapes, core physicochemical properties, and surface modifications to potentially treat cancers. CSLPHNs are being developed for tumor-selective delivery of anticancer agents to increase the cell-kill effect while protecting he healthy tissue from exposure to cytotoxic agents, thereby reducing systemic toxic effects.[67] The following section discusses selected studies dealing with in vitro evaluation and in vivo evaluation. Most of the literature on CSLPHNs has focused on in vitro cell culture models as the means to proof of concept.

Breast cancer is the most common form of cancer and affects more than 200,000 females in 2010 in the United States. Multidrug resistance (MDR) is a common cause of failure of chemotherapy in breast cancer patients.[120] MDR is caused by overexpression of membrane drug efflux transporter P-glycoprotein (P-gp), which reduces intracellular uptake of anticancer drugs.[121] Excellent reviews are available on the cause and strategies for overcoming MDR.[122,123] For example, a CSLPHN system containing doxorubicin was developed and evaluated for cytotoxicity against MDR breast cancer cells by Wong et al.[29–32] The particle size and EE of the CSLPHN were reported to be 50–200 nm and 65%–80%, respectively. Cell-kill and cellular uptake were significantly enhanced in CSLPHN forms compared to the solution formulation. Two possibilities for the mechanism of cytotoxicity of doxorubicin-loaded CSLPHNs were proposed: i) free drug was released from CSLPHNs and acted on the cells, and ii) drug-loaded CSLPHNs entered and released the drug inside cells, thereby evading the P-gp efflux pump. In a subsequent publication, the authors proposed that the second mechanism was more likely to happen. Drugs in CSLPHNs entered the cells by a combination of diffusion and phagocytosis. Because of the physical association of the drug with the anionic polymer, the drug was not easily removed by the P-gp efflux pump. Therefore, chronic suppression of MDR cell proliferation was observed because of the continued buildup of drug inside cells (Figure 8).[28]

Another potential strategy to overcome MDR of breast cancer cells is to simultaneously use a combination of chemotherapeutic drug and P-gp inhibitor/chemosensitizer such as verapamil in a single nanoparticle cargo.[123] A similar strategy using a CSLPHN capable of codelivering doxorubicin and elacrider (chemosensitizer) was developed and evaluated by Wong et al.[29] The particle size was found to be 187–272 nm. EE was 71%–76% for doxorubicin and 80%–88% for elacrider. In this study, the dual agents coencapsulated in CSLPHNs showed greatest uptake and anticancer activity in human MDR breast cancer cell line MDA435/LCC6/MDR.

In another study, a CSLPHN system having a PLGA core and phosphatidyl choline shell was designed for loading 7α-APTADD, an investigational aromatase inhibitor for treating estrogen-responsive breast cancer.[58] Transferrin, a natural 80-kDa glycoprotein, was conjugated to CSLPHN to target SKBR-3 breast cancer cells with overexpressed transferrin receptors. EE and mean diameter were measured to be 37% and 170 nm, respectively. Aromatase inhibition activity of the targeted CSLPHN was significantly higher in SKBR-3 cells compared to nontargeted CSLPHNs.

Prostate cancer is the second leading cause of cancer mortality in men over the age of 40 in the United States.[124] Prostate-specific membrane antigen (PSMA) is a type II membrane integral glycoprotein overexpressed in prostate cancer cells and has been identified as a biochemical marker.[125] Several PNPs and liposomal-targeted delivery systems were developed for prostate cancer.[126–128] A new CSLPHN system composed of a PLGA core and lecithin/DMPE-DTPA lipid shell was developed for prostate cancer by codelivering the chemotherapeutic drug docetaxel (Dtxl) and the therapeutic radionuclide yttrium 90 (^{90}Y).[56] The CSLPHNs were termed as chemorad NPs. They were prepared by the single-step nanoprecipitation method to produce a mean particle size of 65 nm. Oligonucleotide aptamer A10, which has high affinity and selectivity to PSMA-positive prostate cancer cells, was attached to the outer surface of the CSLPHNs via coupling reaction with DSPE-PEG to produce targeted particles (Apt-Dtxl-^{90}Y-NPs). An increase in uptake of chemorad NPs was observed in the LNCaP prostate cancer cell lines. Chemorad NPs were able to kill 80% of the

LNCaP cells (PSMA-positive) compared to the PC3 cell line (PSMA-negative) and untargeted control groups. The experimental findings of this study suggested the potential of chemorad NPs to improve chemoradiotherapy in prostate cancer patients.

Pancreatic cancer, especially adenocarcinoma of the exocrine pancreas, is the fourth leading cause of cancer death in the United States.[129] However, the current chemotherapeutic regimen has shown little or only modest improvement in patient survival because of poor vascularization and inadequate perfusion of the tumor.[130] Liposomal paclitaxel and gemcitabine,[131] cisplatin and gemcitabine,[132] curcumin-encapsulated PLGA NPs,[133] and EGFR-targeted gemcitabine-loaded PLGA NPs[134] showed promising efficacy in refractory pancreatic cancer in animal studies and clinical trials. Anticarcinoembryonic antigen (CEA) half-antibody was conjugated to paclitaxel-loaded CSLPHNs, which were investigated for targeting ability against BxPC-3 (CEA-positive) and XPA-3 (CEA-negative) pancreatic cancer cells.[24] Antibody-conjugated CSLPHNs with particle size of 95 nm were prepared by nanoprecipitation via self-assembly of PLGA, lecithin, and DSPE-PEG. Monoclonal antibody was attached to CSLPHNs through a maleimide–thiol coupling reaction. Targeting specificity, as well as enhanced cellular cytotoxicity, of paclitaxel-loaded CSLPHNs was observed in CEA-positive cells compared to their nontargeted counterparts, a result that can be explained by the occurrence of the receptor-mediated endocytosis process, which facilitated particle internalization into cells. Thus, the delivery platform showed the therapeutic potential of CSLPHNs in targeting pancreatic cancer.

Few available studies reported in the literature are based on in vivo evaluation of drug-loaded CSLPHNs in animal cancer models.[31,53–55,57] To the best of our knowledge, the pioneering research involving in vivo evaluation of dual-drug-loaded CSLPHNs, known as nanocells, was reported in 2005 by Sengupta et al.[55] The delivery system was composed of chemotherapeutic agent doxorubicin conjugated to PLGA to form a polymeric core (nucleus, similar to a cell) and the antiangiogenic agent combretastatin entrapped within the lipid shell. Tumors were induced by implanting GFP-positive BL6/F10 melanoma cells or Lewis lung carcinoma cells in male c57/BL6 mice. Compared to CSLPHNs with other combinations, intravenous administration of different combinations of CSLPHNs containing doxorubicin and combretastatin showed that CSLPHNs containing dual agents exhibited distinctly greater reduction in tumor volume with increasing survival (%) in Kaplan–Meier survival graphs. The study proved that the dual-agent loaded-CSLPHN treatment induced inhibition of tumor growth in a dose-dependent manner with more susceptibility toward melanoma than lung carcinoma. In addition, white blood cell count assay indicated that the delivery system resulted in the least systemic toxicity compared with other combinations.

Another study dealt with the in vivo evaluation of the doxorubicin-loaded CSLPHNs in the solid tumor model induced by injecting EMT6 mouse mammary cancer cells intramuscularly into the hind legs of BALB/c mice.[31] The cationic anticancer agent doxorubicin was complexed with anionic polymer HPESO to form a core, which was then covered by the lipid mixture of stearic acid and tristearin. The mean time for the tumor to reach the cutoff size was significantly prolonged by 7 days. The tumor growth delay value was 100% in mice after receiving 0.2 mg of doxorubicin in the form of CSLPHNs compared to blank CSLPHNs injected into the tumor. The normal tissue toxicity of the particles was minimal after a single dose of intratumoral injection, suggesting the usefulness of the delivery system for local treatment of breast cancer.

Delivery of nucleic acids

Delivery of nucleic acids represents a challenge and great opportunities to treat chronic diseases, genetic disorders, and cancers.[135] Cationic liposomes and biodegradable PNPs have been investigated as gene delivery carriers.[136] Polymer-based nonviral carriers have received significant attention because of the death of a patient in a clinical trial of viral-based gene therapy.[137] Among various nonviral-based approaches, polymer and lipid-based nonviral carriers have several particular advantages: low immunogenicity, low toxicity, absence of viral recombination, low production cost, and the possibility of repeated administration.[138]

Cytotoxicity, stability in serum, duration of gene expression, and particle size of the nonviral-based carriers still remain major limitations of lipid and polymer-based systems. Recently, CSLPHNs have emerged as an alternative, biodegradable, stable, and long-lived nanoparticle vector delivery system. Plasmid DNA encoding luciferase reporter gene was entrapped in CSLPHNs composed of PLGA and cationic lipids DOTAP/DC-cholesterol.[139] The CSLPHNs (100–400 nm) were able to transfect the luciferase gene in adherent 293 human prostate cancer cells 500–600 times more efficiently than did unbound DNA after 48 h. Another CSLPHN was reported by Li et al[25] for efficient nonviral gene delivery with higher transfection efficiency and lower toxicity compared to commercial Lipofectamine 2000. In another study, CSLPHNs with a mean particle size of 128 nm were prepared by the emulsion evaporation technique by using different combinations of triolein, polyethylenimine (PEI), egg yolk phosphatidylcholine (EPC), and PEG-DSPE. Plasmid DNA was complexed with NPs by adsorption. A green fluorescent protein intensity study revealed that the transfection efficiencies of CSLPHN/DNA complexes were 37% and 34% for HEK293 and MDA-MB-231 cells, respectively. Transfection efficiency was significantly higher than that of commercial Lipofectamine 2000. Additionally, the proton-sponge effect destabilized the endosomal membrane and enhanced transfection. PEG helped as a protective layer and reduced the degradation of plasmid DNA by lysosomal enzymes after entering the lysosome.

For siRNA delivery, cationic nanoscale complexes such as lipoplexes or polyplexes were used successfully to deliver siRNA.[140] However, some of these systems have disadvantages such as toxicity, induction of inflammatory responses, and instability in serum. Shi and coworkers[68] designed a relatively neutral surface charged hybrid nanostructure capable of protecting siRNA and lipoplexes from physiological environments. This delivery system was termed “differentially charged hollow core/shell lipid–polymer–lipid hybrid nanoparticles,” which were composed of four functional building blocks: a positively charged inner hollow core made up of cationic lipid, a hydrophobic PLGA layer, and a neutral lipid layer having outer PEG chains. A combination of a modified double-emulsion/solvent evaporation

method and a self-assembly method yielded an average particle size around 225 nm and a neutral surface charge. The hybrid system was capable of releasing siRNA in a sustained manner, enhanced in vivo gene silencing, and inhibited luciferase gene expression in murine xenograft tumors. This strategy has opened another potential avenue for successful gene delivery for treating multidrug resistant cancers.

Summary, future prospects and challenges

CSLPHNs are the alternative platform for drug delivery. This particle design uses an integrative approach by combining two classes of nanocarriers, namely polymeric nanoparticles and liposomes. These particles have several beneficial features for treating various diseases, particularly cancers. Often treatment of a single type of cancer requires administering multiple drugs, and, in this aspect, CSLPHNs are promising because they have the potential to deliver multiple drugs simultaneously from a single platform. Specifically, incorporating two drugs into the core and lipid layer can offer a viable approach to treating MDR and life-threatening diseases. Apart from small-molecular-weight drugs, delivery of diagnostic agents such as quantum dots, macromolecules such as proteins, and genes offers other exciting strategies with CSLPHNs. Because of their structural similarity to the viral architecture, CSLPHNs offer potential as vaccine adjuvants. Furthermore, recent advancements in the CSLPHNs delivery system such as coating PNPs with natural erythrocyte membrane, entrapping quantum dots inside these hybrid particles, and concurrent administration of chemotherapy/radiotherapy have shown potential for theranostic applications in treating malignancies and other diseases.

The design and development of CSHLPNs as drug delivery platforms have been concentrated in the architecture and in vitro efficacy. The complexities of these systems afford new challenges in translating the in vitro efficacies into tangible therapeutic options. More focused research is warranted, especially in key areas of development including stability, scale-up, optimization of targeting ligand density, in vivo fate, toxicity, and pharmacokinetic profiles.

Stability of new drug products is an essential prerequisite. Therefore, the long-term physical and chemical stability of these hybrid nanoparticles in various environmental stress conditions needs to be systematically evaluated to have a shelf-life assigned to the marketed product. The critical parameters that should be evaluated include, but are not limited to, particle size distribution, drug entrapment, retention of entrapped drug in the system, physical robustness of the system, and effect of stressed environments on any of the aforementioned parameters.[141] As with any colloidal system, stability can be a challenge in the liquid state. Thus, if instability is observed in aqueous states, other strategies could be evaluated including lyophilization or other stabilization techniques to address instability issues.[93]

Active targeting has been considered to be a significant paradigm shift for therapeutic efficacy of nanoparticulate drug delivery systems.[142] Although these systems show potential in early in vitro or proof-of-concept studies, a number of factors that can impact their efficacy need to be addressed. One such factor is the optimization of the targeting ligand on the hybrid NP surface. The process of surface modification for the NPs with the targeting ligands should be reproducible and yield consistent outcomes. The selection of the targeting ligands should additionally be evaluated because some of these targeting agents possess pharmacological activity.[143] Understanding the targeting ligand is crucial to address therapeutic outcomes and also to address confounding outcomes due to polypharmacological inconsistencies.

The pharmacokinetic and pharmacodynamic (PK/PD) effects of these systems should be critically evaluated. Traditional PK evaluations depend on the availability of the free drug in the biological system to postulate its PD or metabolic fate. When drugs encapsulated in CSLPHNs are administered, the PK/PD profile may be altered when compared to only the free drug due to altered release of the drug from these systems. Therefore, the appreciation of this phenomenon is essential in understanding the final therapeutic outcomes of these systems. A recent review by Li and Huang[144] specifically addressed this aspect of the PK/PD fate of NPs and should be a valuable resource for researchers.

Safety and toxicological issues should be considered for a drug delivery system such as CSLPHNs. It can be envisioned that the hybrid drug delivery platform should be non-toxic or minimally toxic based on the formulation ingredients which are regarded as safe for human use. However, the chemical toxicity of the ingredients may be completely different from the toxicity of the integrated nanoparticles due their size and ability to cross various biological barriers. Currently, there is little understanding of the possible adverse reactivity of CSLPHNs with living cells, organs and organisms. Specific emphasis should be given to the toxicity of "empty" non-drug-loaded particles, especially for slowly or non-degradable nanoparticles.

A primary requirement for any product entering the pharmaceutical market is the availability of large-scale production methods that need to be cost-effective and meet regulatory requirements. Current bench-scale processes used for developing CSLPHNs systems are labor intensive and are not amenable to direct scale-up. Moreover, most of these delivery systems are intended for parenteral administration and thus directly impact their aseptic production. Although, significant advances in aseptic processing have been used for manufacturing CSLPHNs systems, they often come with a high price-tag and can be cost-prohibitive.

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