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REVIEW ARTICLE

Highlights on Cell-Penetrating Peptides and Polymer-Lipid Hybrid Nanoparticle: Overview and Therapeutic Applications for Targeted Anticancer Therapy

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Abstract
Polymer-lipid hybrid nanoparticles (PLHNs) have been widely used as a vehicle for carrying anticancer owing to its unique framework of polymer and lipid combining and giving the maximum advantages over the lipid and polymer nanoparticle drug delivery system. Surface modification of PLHNs aids in improved targeting and active delivery of the encapsulated drug. Therefore, surface modification of the PLHNs with the cell-penetrating peptide is explored by many researchers and is explained in this review. Cell-penetrating peptides (CPPs) are made up of few amino acid sequence and act by disrupting the cell membrane and transferring the cargos into the cell. Ideally, we can say that CPPs are peptide chains which are cell specific and are biocompatible, noninvasive type of delivery vehicle which can transport siRNA, protein, peptides, macromolecules, pDNA, etc. into the cell effectively. Therefore, this review focuses on the structure, type, and method of preparation of PLHNs also about the uptake mechanism of CPPs and concludes with the therapeutic application of PLHNs surface modified with the CPPs and their theranostics.

Keywords anticancer · cell-penetrating peptides · nanotechnology · polymer-lipid hybrid nanoparticles · targeted delivery · theranostics

Introduction

Cancer nanotechnology is used as an optimistic tool for many anticancer drugs. Innovation in the design and framework of drug delivery to carry the chemical entities to the different sites in the body and their characterization to carry the drug allows for the treatment of a wide range of clinical problems. Genes (DNA/RNA), synthetic substances, recently discovered pharmaceuticals, or altered versions of drug molecules from the past are examples of such chemicals. Optimizing nanotechnology as a carrier for drug delivery to the human system is crucial for the therapeutics of many different diseases [1]. In this regard, a wide variety of submicron materials has been synthesized to act on cancer cells. Lipid-based and polymer-based nanoparticles have been largely used in the development of delivery vehicles for anticancer drugs, contrast agents, and therapeutics as well as in theranostics [2, 3].

Polymer-based nanoparticles have been widely studied in the cancer field. Various natural, semi-synthetic, and synthetic polymers have been used for designing polymer-based nanoparticles. Two of the most popular biodegradable and biocompatible polymers are poly(lactide-co-glycolide) (PLGA) and chitosan, both of which have received FDA approval for oral and parenteral administration [4]. Although polymer-based nanoparticles have several benefits, including sustained and controlled release, higher drug loading capacity, and stability, they also have some disadvantages, including a lack of polymer solubility, the use of organic reagents, and drug interactions with the polymer's monomer units [5, 6].

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Decades of research have been done on liposomes, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC) as a part of lipid-based nanoparticles used as a drug delivery vehicle. Liposomes showed advantages like low toxicity, biocompatibility (as phosopholipid either from natural or synthetic source is used in the structure), controlled drug release, targeted drug delivery, and large-scale manufacture. They can encapsulate and distribute both hydrophilic and lipophilic substances. However, the main drawbacks include drug leakage from the carrier as well as oxidation and hydrolysis of liposomal phospholipids, which results in structural deterioration and burst drug release [7]. Another disadvantage of liposomes is their quick clearance by reticuloendothelial system (RES), although this can be avoided by using lipid PEG (polyethylene glycol), which will cover the particles and lengthen the duration they are in circulation [8, 9]. Consequently, the ability to enhance vesicle performance by using both natural and synthetic polymers was quickly tapped into by liposome preparation technology.

Evolution of Polymer-Lipid Hybrid Nanoparticles

The combination of liposomes with polymeric nanoparticles such as polymeric micelles, polymersomes, polyplexes, and polymer protein/drug conjugates is a contemporary trend in the pharmaceutical industry which are found to have wide applications in targeted delivery of anticancer drugs. All of these systems strive to deliver therapeutic agents with maximal effectiveness while causing the least amount of harm to the body's healthy cells. They make it possible for drug molecules to effectively and safely enter the target region while preventing any possibility of systemic side effects. Moreover, these particles act as a barrier against the immune system and RES, extending the duration that anticancer medications are in circulation. These particles can be given the desired features, such as those needed for imaging, stimuli-triggered release, and prolonged circulation time [10, 11].

Liposome hybrid nanocarriers known as polymer-caged or polymer-incorporated liposomes or polymer-modified liposomes are made of polymers that have been grafted or attached to their surfaces (Fig. 1a). The creation of liposomes with enhanced shape, particle size, zeta potential, surface characteristics, and membrane flexibility is another advantage of using polymers; these features are crucial to how they behave *in vitro* and *in vivo* [12, 13]. Polyethylene glycol (PEG) was conjugated to the lipid anchor in the first novel modification of liposomes, which significantly lengthened circulation time, stabilized stress levels, and provided protection from opsonins. These extra characteristics caused hepatic RES to drain more slowly, considerably extending circulation duration [14]. Table I shows different clinical trial data that suggest the importance of the PEG modification on to the liposomes.

Polymer-modified liposomes (Fig. 1b) and cell-based bio-mimetic (Fig. 1c) offer potent carriers and permit considerably more effective drug administration, and their application has witnessed a tremendous development in recent years as the next generation of lipid-polymer hybrid nanocarriers. Synthetic nanocarriers will experience clearance by the reticuloendothelial system (RES)/

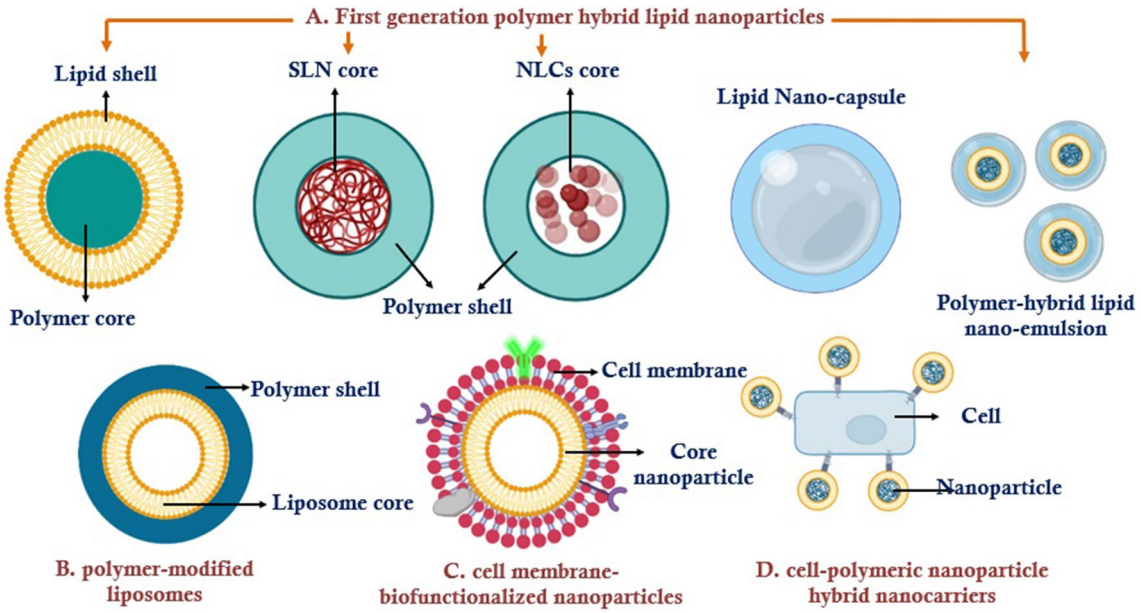

Fig. 1 Schematic representation of evolution of polymer-lipid hybrid nanoparticles. **a** First-generation polymer hybrid lipid nanoparticles; **b** polymer-modified liposomes; **c** cell membrane-biofunctionalized nanoparticles; **d** cell-polymeric nanoparticle hybrid nanocarriers

Table I Clinical Trial Data of PEG Modified Liposomes

SL. No	Study title	Condition	Phase
1	Clinical Application of Polyethylene Glycol Liposome Doxorubicin (PLD) in Primary Lymphoma	Lymphoma, non-Hodgkin; Hodgkin disease	Phase 4
2	Pegylated Liposomal Doxorubicine and Prolonged Temozolomide in Addition to Radiotherapy in Newly Diagnosed Glioblastoma	Glioblastoma	Phase 1 Phase 2
3	A Phase 3, Randomized, Double-Blind Trial of Pegylated Liposomal Doxorubicin (PLD) Plus AMG 386 or Placebo in Women With Recurrent Partially Platinum Sensitive or Resistant Epithelial Ovarian, Primary Peritoneal, or Fallopian Tube Cancer	Fallopian tube cancer Ovarian cancer Primary peritoneal cancer	Phase 3

mononuclear phagocyte system (MPS) once they have entered the bloodstream. Bio-mimetic nanoparticles based on cell membranes have demonstrated greater potential in establishing lengthy blood circulation, precise recognition for targeting, and immune evasion because natural cell membranes feature particular antigen and structure essential for biological processes [15].

Recent years have seen the emergence of cell nanoparticle hybrid vectors as a means of addressing significant difficulties in nanocarriers linked to the incorporation of nanoparticles into living cells (Fig. 1d). Hybrid cell nanoparticle vectors were predicted to control drug release, particularly for substances affected by efflux transporters and passive diffusion. They were also created to lessen the toxicity of the integrated medicine to the carrier cells, protecting the health and tumor-homing capacity of normal cells [16].

Although there are many reasons to use polymers in liposome preparations, they can all be categorized into the group to overcome some drawbacks of first-generation liposomes.

- Increasing the residence time, stealth abilities, and physicochemical stability in biological fluids.
- Modifying the drug release profile, minimizing payload leakiness, and increasing encapsulation efficiency.
- Possessing mucoadhesive and mucopenetrating characteristics.
- Possessing qualities that respond to stimulus.
- Offering a flexible targeting platform.

To address the drawbacks of the aforementioned two vehicles, a new generation of nano dimensional carrier systems called polymer-lipid-hybrid nanoparticles (PLHNs) has been developed. It is a hybrid made up of both lipids and polymers. In this manner, the inherent properties of polymers and lipids can be combined in a way that simultaneously avoids the disadvantages outlined above.

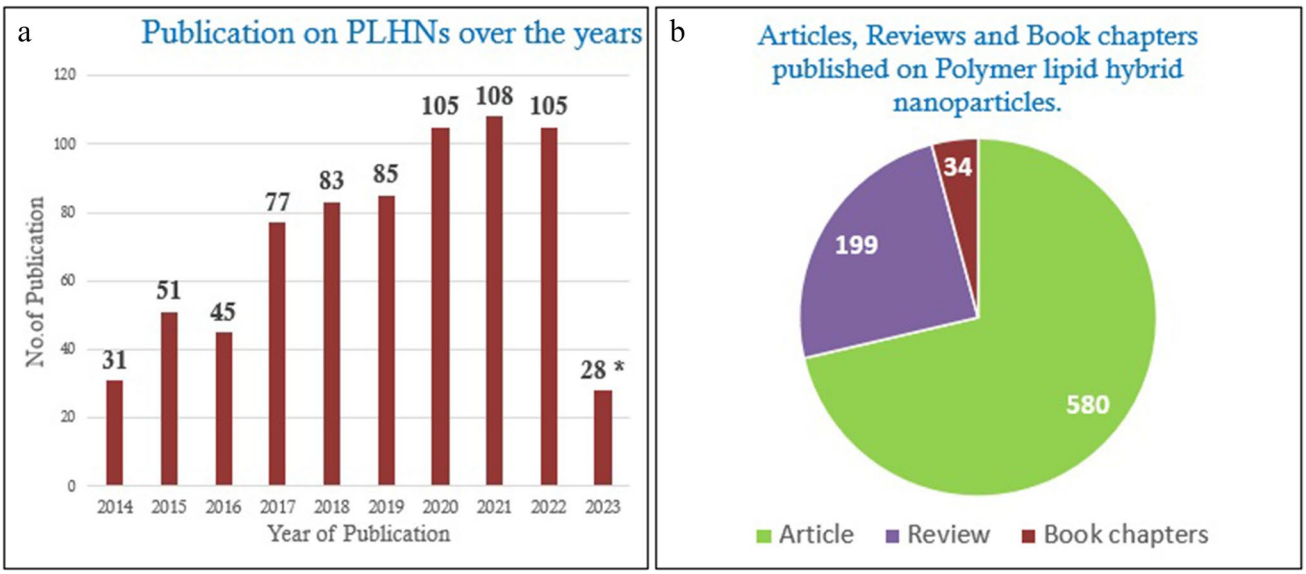

Fig. 2 **a** Publication on polymer-lipid hybrid nanoparticles over the years. *More publications under process; **b** statistics of published polymer-lipid hybrid nanoparticles articles, reviews, and books

PLHNs have increased interest in recent years, and as mentioned in Fig. 2, prove to be one of the nanocarrier for the improved delivery and sustained release.

To improve the targeting capacity and delivery of the PLHNs, several surface modification strategies were used. One such strategy is using cell-penetrating peptides on the surface of PLHNs which helps in transport and targets certain receptors that are over expressed in respective cancer pathology. Cell-penetrating peptides are also termed or referred to as protein-transduction domains (PTDs). They contain less than 30 amino acid residues with a net positive charge. CPPs are peptides that can transport active molecules into the cell through biologically active conjugates and act as a promising strategy for cancer diagnosis and treatment [17].

Peptides present on the cell membrane act by disrupting it and then they pass through the membrane, or reside at the membrane interface and fuse with the cell membrane. These classes of peptides are known as membrane active peptides (MAPs) [18]. MAPs particularly have greater advantages like higher specificity and increased affinity for therapeutic agents and biomolecules, ease in synthesis, flexibility for any modification or changes, compatibility with other therapeutic agents, low immunogenicity, and favorable physicochemical parameters which makes them compatible [17]. When we talk about membrane active peptides, we can say that majorly they are divided into 2 classes: the first one is antimicrobial peptides which destroy the cells and the second class is cell-penetrating peptides (CPPs) which transport cargo (ability to load the therapeutic agents) across lipid bilayers [18].

Thirty years ago was the first time the term "cell-penetrating peptide" (CPP) was used and it originated when a small cationic sequence was identified in HIV-1 protein that is Tat protein, which was called as TAT peptide. CPPs were first discovered in *Drosophila melanogaster* (Antennapedia, *Antp*) which was an organism model and in human immunodeficiency virus (HIV Tat) retrovirus [19]. This TAT peptide had the ability to carry active molecules or the protein into the cell and nucleus [18, 20]. The first discovered CPP was cationic TAT (YGRKKRPQRRR) [17] and second was penetratin (RQIKIWFQNRRMKWKK). Penetratin has the ability to carry large cargoes, including oligonucleotides, proteins, or other peptides, through intracellular components of the cell [21].

Therefore, CPP contains broad range of peptides with various mechanisms, efficiency, specificity, and intracellular targets in trans-membrane transport [22, 23]. This broad range of CPPs makes its application in drug delivery and its transport characteristics difficult. Surface-modifying PLHNs with the CPPs to enhance transport characteristics are very useful which can be developed by selecting certain CPPs for transportation purpose in order to deliver PLNHs with drug inside the cell for intracellular target of action [20].

In this review, we have focused on structure of PLHNs, classification of PLHNs and CPPs, method of preparation of PLHNs and CPPs, oral absorption of PLHNs, therapeutic application of PLHNs, intracellular delivery mechanism of CPPs and finally therapeutic applications of PLHNs surface modified with CPPs.

Structure of PLHNs

By overcoming the drawbacks of lipids and polymers, PLHNs offer tremendous potential for the development of nanotechnology [24]. The three different building units that make up PLHNs are (i) the inner polymeric core, (ii) the lipid monolayer, which surrounds the polymeric core, and (iii) the outer lipid conjugated PEG layer (Fig. 3) which helps to stabilize and ensure longer retention time in the human system [25]. The most common types of polymer used in the synthesis of PLHNs are PCL, PLGA, PLA, PbAE, and chitosan [26, 27]. These materials are also highly biocompatible and biodegradable [28]. The lipid and polymer can be bound by different interactions like hydrophobic, van der Waals force, electrostatic interactions, or other non-covalent connections where the core of PLHNs is made of polymer with lipid coated in the surface [29]. Based on the individual medicinal drugs, the outer lipid layer can be modified to promote effective uptake. To create a tailored drug delivery system, the outer lipidic membrane can also be changed by affixing particular antibodies, ligands, aptamers, and bioactive materials like DNA, RNA, or proteins that can be bound covalently or non-covalently [30].

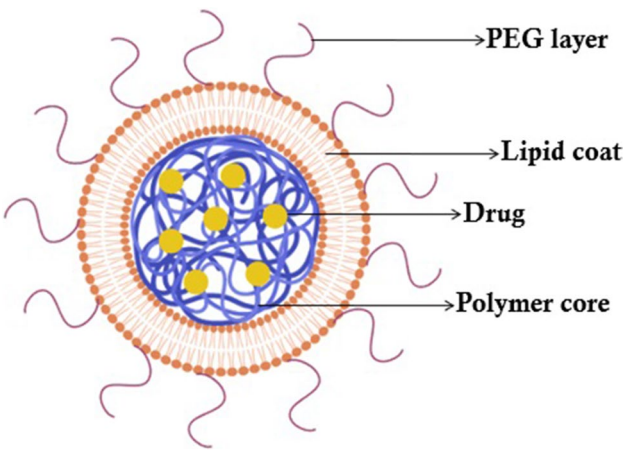

Fig. 3 Structure of polymer-lipid hybrid nanoparticle

Classification of PLHNs

The structural organization of the polymer and lipid in PLHNs determines how they are classified.

Polymer Core Lipid Shell PLHNs

As the name suggests, polymer core lipid shell consists of a polymer core surrounded by the lipid layer (Fig. 4a). This system can encapsulate both hydrophobic and hydrophilic drugs because of the amphiphilicity of the polymeric core and lipid surface [7]. The polymeric core protects the drug from systemic metabolism and the lipid surface provides excellent biocompatibility as it mimics the biological membrane [31].

Core–Shell-Type Hollow Lipid PLHNs

This type of PLHNs consists of concentric multi-layers of A-B-A type (Fig. 4b) made up of the outermost lipid layer covering the polymer layer which in turn covers the hollow lipidic layer [32]. The outer layer of lipids can be PEGylated to improve the systemic circulation time of the system. The multilayer provides improved stability and helps the drug by encapsulating it from the external environment [33, 34]. The gap between the polymer and the lipid layers is usually filled with aqueous buffer and cationic or zwitterion phospholipids along with oppositely charged polymers favor electrostatic interactions [24].

Monolithic PLHNs

Another term for monolithic PLHNs (Fig. 4c) is mixed lipid polymers in which the lipid is dispersed throughout the polymeric core forming the colloidal carrier for therapeutic agents [35, 36]. Here, the polymeric layer can be made using co-polymers and lipid layer should bio-mimic the internal membrane; therefore, phospholipids can be used [37].

Polymer-Caged Liposome

Liposomes serve as a carrier for delivering anticancer drugs, DNA, siRNA, proteins, and peptides but have drawbacks like drug leakage, and stability problems; therefore in this type, the liposomes are coated with the polymer (Fig. 4d) to give enhanced drug release and improved stability [38]. The polymers that are coated can be selected based on the desired nature of drug release for example stimuli responsive polymers [39].

Erythrocyte Membrane–Coated PLHNs

The polymer-lipid hybrid nanoparticle is coated with the erythrocyte membrane (Fig. 4e) which can be done by the extrusion method. This coating forms distinct nanoparticles which can bio-mimic the surface chemistry of the RBC, therefore, making them hemocompatible [40]. These system escape from macrophages because of the erythrocyte layer and therefore escape uptake by reticuloendothelial system [41].

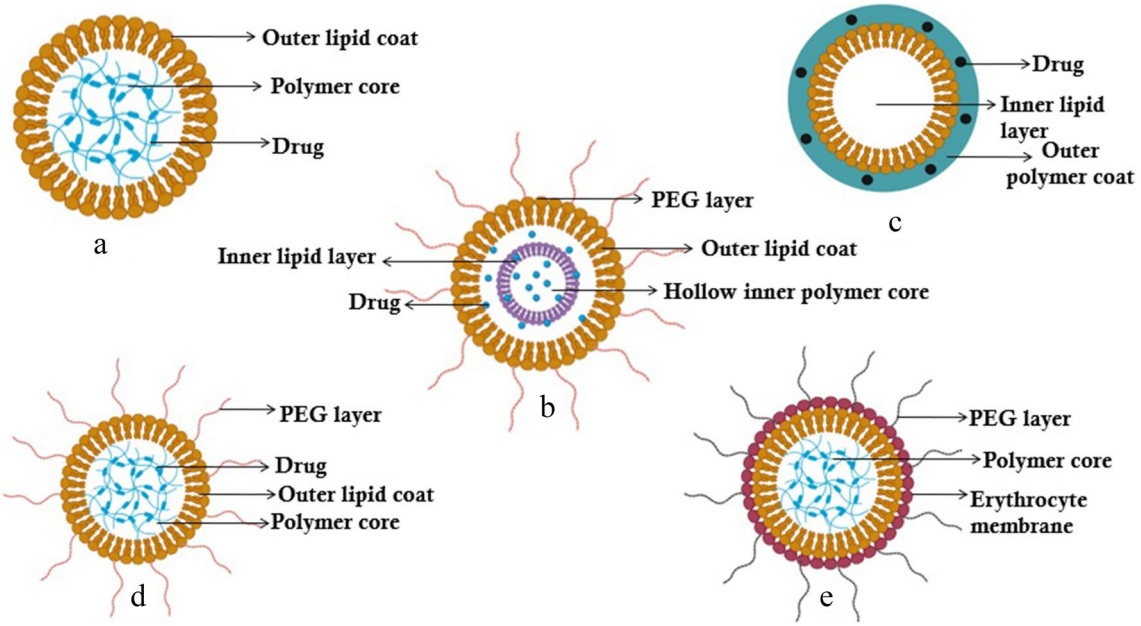

Fig. 4 Structure of **a** polymer core lipid shell PLHNs; **b** core–shell-type hollow lipid PLHNs; **c** monolithic PLHNs; **d** polymer-caged liposome; **e** erythrocyte membrane–coated PLHNs

Method of Preparation of PLHNs

There exist different methods of preparation of PLHNs, which broadly falls under two processes:

Two-Step Method

For preparing PLHNPs, two-step method is regularly used in which the polymeric and lipid-based nanoparticles are prepared separately (Fig. 5a) and then integrated by electrostatic interactions [12, 24]. This method yields single, double, or multilayered shells. Polymer-based nanoparticles can be prepared by dissolving the water immiscible polymer in the organic solvent by nanoprecipitation, emulsification solvent evaporation (ESE), or high-pressure homogenization [42–44]. This forms a polymeric core that can encapsulate hydrophobic drugs. Lipid-based nanoparticles can be prepared by various methods like micro-emulsification, ultrasonication, high-pressure homogenization, melt emulsification, solvent emulsification, and solvent injection methods Two-step method is further divided into (i) conventional and (ii) non-conventional process.

Conventional Method

This type of technique is used in small-scale preparation where the polymeric and lipid nanoparticles were prepared separately as in a two-step method. Desired quantity of lipids was dissolved in an organic solvent which forms a thin layer inside the round bottom flask. Then, the polymeric nanoparticles suspension was added to this formed layer of the lipid. Another way is to hydrate the lipid film with the aqueous solvent which forms a vesicle and to this performed vesicle, polymeric nanoparticles were added [24]. Then, the formed hybrid is subjected to vortexing or ultrasonication and centrifugation to separate lipids and PLNs [45].

Non-Conventional Method

Large-scale production of PLHNs can be done using this method. Here, methods like spray drying and soft lithography particle molding are used to mix the separately prepared polymeric- and lipid-based nanoparticles [24]. Wang *et al.* and co-workers used spray drying technique to prepare PLHs using lactic-co-glycolic acid as the polymer, lecithin as lipid to formulate dry powder inhaler of levofloxacin where spray drying was used to form small-scale micro aggregates of the nanoparticles.

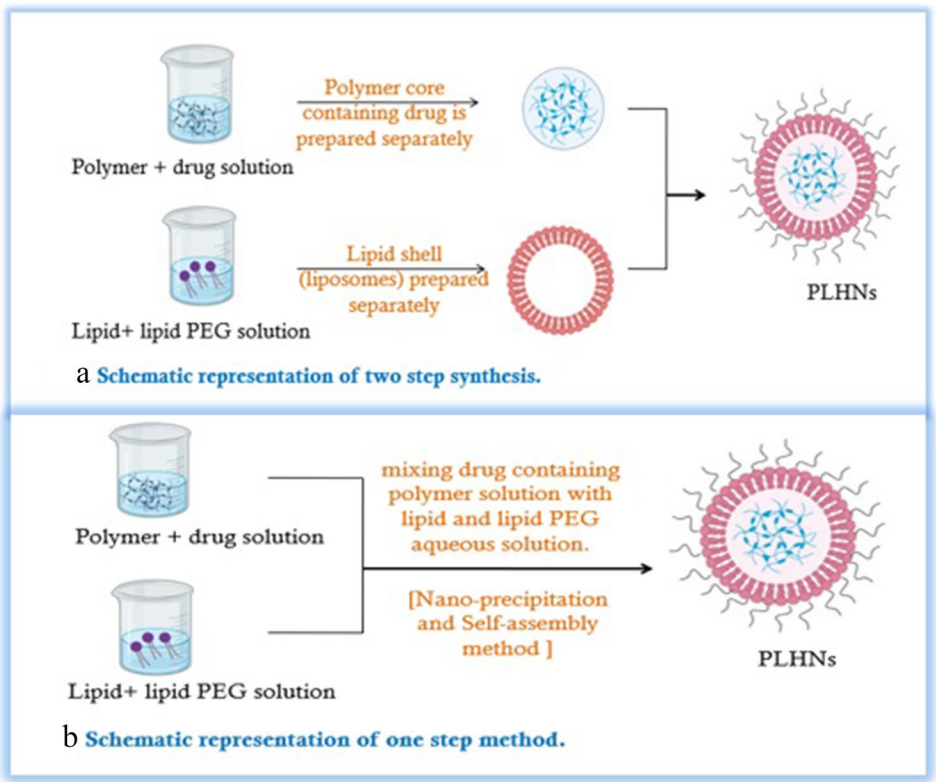

Fig. 5 **a** Schematic representation of two-step synthesis; **b** schematic representation of one step method

One-Step Method

In order to prepare the PLHNs more efficiently by decreasing the time and energy spent, one-step method is used where the lipid and polymer is mixed together (Fig. 5b) which eventually self-assemble to form PLHNs. Precipitation of the organic polymeric solution takes place when aqueous solution of lipid is added to it which then self-assembles on the polymeric core through hydrophobic interactions to give PLHNs. This can be prepared by various methods like emulsification solvent evaporation and nanoprecipitation method as described below [24].

Emulsification Solvent Evaporation Method

This process is categorized into single and double ESE. It was introduced by Gurny *et al*. [24]. The method was selected based on the water miscibility of the drug. In the case of water immiscible drugs, a single ESE process was used as it forms an O/W emulsion in which the aqueous phase dissolves lipids. In contrast, the organic solvent dissolves the polymer and hydrophobic drug. The aqueous lipid phase was added to this organic solvent mixture. Water-insoluble lipids can also dissolve in organic solvents. Lipid molecules self-assemble onto the hydrophobic polymer core and encapsulate the drug. For the final step, the organic solvent evaporates to produce PLHNs after homogenization and sonication [31]. A double ESE process was used when the encapsulated drug (antibiotics and anticancer drugs) was found in the hydrophilic matrix. This method yields core–shell-type hollow lipid PLHNs. By dropwise addition of the aqueous phase with the drug to the oil phase containing polymer and lipid, the primary W/O emulsion type was obtained. The final obtained emulsion was added into the aqueous phase carrying another lipid may be phospholipid, which gives a secondary W/O/W emulsion [34].

Nanoprecipitation

PLHNs were prepared by dissolving the drugs and polymers first in water or any organic solvent and then adding lipids or lipid PEGs to the aqueous medium for dispersion. The organic solvent was added to the aqueous phase and heated to 65–70 C to precipitate the polymer. As a result of hydrophobic interactions between the polymer core and the lipid or lipid PEG, a uniform distribution was achieved. To reduce particle size, the solution was continuously agitated, sonicated, or homogenized. Centrifugation removes excess polymer and organic solvent. Therefore, this process works by precipitation of the polymer via solvent diffusion and the lipid self-assembles onto the polymer core [24, 31].

Oral Absorption of PLHNs

Understanding the intestinal uptake of lipids is crucial to understanding PLHN absorption since they contain lipids on their outer surface. Due to its finger-like projections called villi, the small intestine absorbs many substances because of its increased surface area. Consequently, cholesterol esters, phospholipids, triacylglycerols (TAGs), and other lipids are absorbed through the intestinal epithelium. A variety of cell types exist in the small intestine, including enterocytes that contain micro-villi, goblet cells that secrete mucus, paneth cells that secrete antimicrobial enzymes and lysozymes, and endocrine cells. The epithelial cells also have M cells associated with the payer's patch which are involved in GLAT-gut-associated lymphoid tissue components [46].

Nanoparticles are generally transported through the intestinal epithelium by 4 major process known as paracellular (transport of molecules between the cells), transcellular (transport of molecules through the cells), and transcytosis (receptor specific active transport of the molecules) by which they enter the systemic circulation through blood or lymph [47].

The enterocytes absorb lipids from the liver's bile. As lipids reach the stomach, gastric lipase (pH-4.0) fragments them into diacylglycerols (DAGs) and fatty acids. Upon reaching the intestine, these lipids are hydrolyzed by pancreatic lipase to form monoacylglycerols (MAG) and fatty acids. Cholesterase breaks down cholesterol esters into free cholesterol and fatty acids, and pancreatic phospholipase breaks down phospholipids into lysophospholipids and fatty acids. The enterocyte cells of the small intestine absorb these byproducts of the corresponding lipids. They are re-esterified by proteins like monoacylglycerol acyltransferases (MGATs), diacylglycerol acyltransferases (DGATs), and acyl-CoA: cholesterol acyltransferases (ACATs) to TAGs, cholesteryl esters, and phospholipids, which are then packed into pre-chylomicron with apolipoprotein B-48 (apoB-48) or stored as intracellular lipid droplets. Pre-chylomicron transport vesicles (PCTVs) transport pre-chylomicrons from the endoplasmic reticulum to the Golgi apparatus for maturation via microsomal triglyceride transfer protein (MTP) (Fig. 6). In the MTP, lipidation of TAGs, cholesteryl esters, and phospholipids along Apo-B-48 occurs within the pre-chylomicron. By exocytosis, the mature chylomicrons are transported to the lamina propria, where they are absorbed into the circulation by the lymphatic system [48–50].

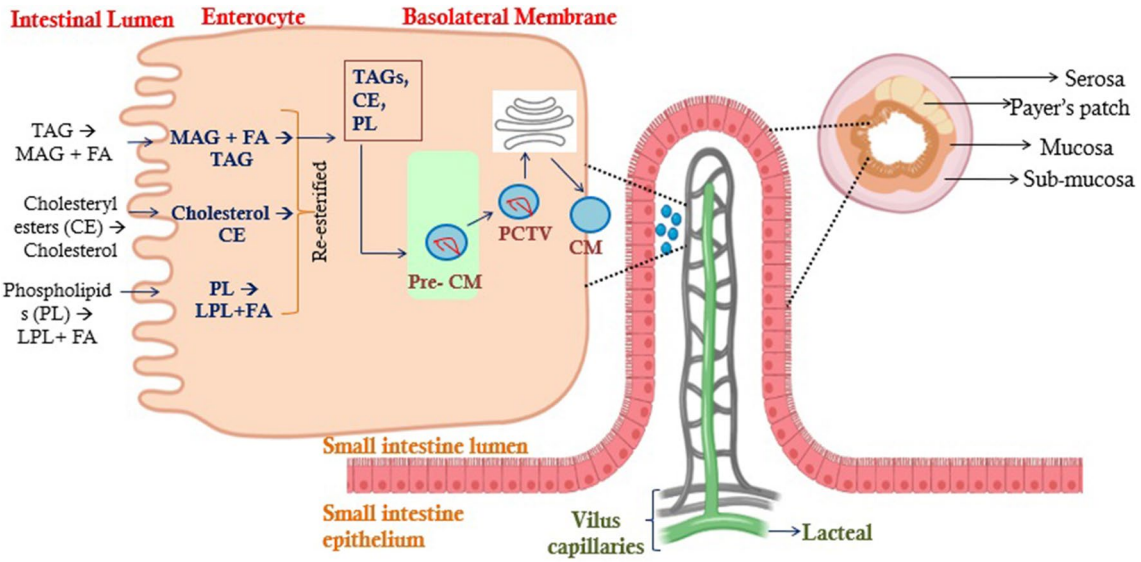

Fig. 6 Absorption of lipids through enterocytes

Application of PLHNs in Cancer Therapy

Cancer Therapy

Many studies used PLHNs in various types of cancer to increase the ability to target, penetrate, and increase the cytotoxic effect of the chemotherapeutic agents. PLHNs have been used in different types of cancers like breast cancer, lung cancer, osteosarcoma, hepatocellular carcinoma, liver cancer, nasopharyngeal carcinoma, cervical cancer, melanoma, ovarian cancer, prostate cancer, and glioblastoma (Table II).

Dual Drug Delivery

PLHNs help encapsulate and deliver both hydrophilic and lipophilic drugs and therefore have a greater advantage in co-delivering chemotherapeutic agents, increasing the formulation's efficiency. Alhajamee *et al*. used chitosan and lecithin as polymers and lipids, respectively, to prepare lipid-polymer hybrid nanoparticles (LPHNP) that encapsulate curcumin (Cu) and tamoxifen (Tmx) to improve anticancer efficacy and cytotoxicity. Results revealed that ovarian cancer cells were more severely inhibited by Tmx-Cu-LPHNPs than healthy cells. Based on flow cytometry, AO/PI staining, and upregulation of P53 and caspase-9, Tmx-Cu-LPHNPs induce pro-apoptotic effects. A scratch assay and a decrease in metalloproteinase (MMP-2, 9) gene production both indicate that cancer cells suppress metastasis [57].

To distribute erlotinib (ERL) and bevacizumab (BEV) and also to target, inhibit NSCLC, Pang *et al*. produced lipid-polymer hybrid nanoparticles (LPHNPs) with HA embellishments and pH sensitivity. The HA-ERL/BEV-LPH NPs group had higher tumor inhibition rate and the highest tumor tissue accumulation concentration with minimal systemic toxicity compared to the 0.9% NaCl control after 21 days [58].

To increase the anticancer efficacy in osteosarcoma, Yang *et al*. developed a new doxorubicin (DOX)– and edelfosine (EDL)–loaded lipid-polymer hybrid nanoparticle (DE-FPLN). The targeted nanoparticles showed improved cellular uptake and sub-cellular distribution when compared to non-targeted nanoparticles. The MTT assay and caspase-3/7 activity assay showed that DE-FPLN formulations were more effective at killing cancer cells than other anticancer agents. It appears that the co-administration of medications in a folic acid–targeted nanoparticle causes apoptosis and cell death, as well as a significant reduction in tumor formation, without any noticeable adverse effects [59].

Tumour-Targeted Drug Delivery

Tumor-targeted drug delivery is very important to bring out the effective chemotherapy and increasing the therapeutic efficacy. Sakpakdeejareon reported the use of transferrin to achieve tumor-targeted delivery, whose receptors melanoma highly expressed, which was coupled with lipid-polymer hybrid nanoparticles entrapping plumbagin. Following intravenous injection, results showed that 40% of B16-F10 tumors vanished and 10% of tumors regressed [60]. Chen *et al*. presented a study in which they developed lipid-polymer hybrid nanoparticles (LPNs) encapsulating carboplatin (CBP) and paclitaxel (PTX) together to display synergistic effects in cervical

Table II Application of PLHNs in Cancer Therapy

Drug	Cancer	Preparation method	Polymers and Lipids	Size, zeta, PDI, and EE	Ref
Emodin (EMO)	Breast cancer	Nanoprecipitation method	Polymer: PLGA. Lipid: soybean phospholipid	Size: 122.7 ± 1.03 nm, zeta: − 28.5 ± 1.55 mV, PDI: 0.118, EE:	[51]
5-fluorouracil	MCF-7 (breast cancer) and HeLa cells (human cervical) cell lines	Single nanoprecipitation method with minor modifications	Polymer: PCL. Lipid: Lipoid E 80 S	Size: less than 270 nm, PDI: less than 0.186, zeta: within 25.6 ± 2.16, EE: 93.57 ± 0.656	[52]
Methotrexate	Glioblastoma	Solvent injection and homogenisation method	Polymer: PLGA and glyceryl tripalmitate	Size: 173.51–233.37 nm, zeta: 25.78–36.31 mV, EE: 70.56–86.34%	[53]
Sorafenib (SFN)	Breast cancer	Bulk nanoprecipitation and microfluidic (MF) co-flow nanoprecipitation techniques	Polymer: PLGA. Lipid: Lecithin and DSPE-PEG 2000	Size: 187.9 ± 4.5 to 302.0 ± 2.0, PDI: 0.100 ± 0.1 to 0.246 ± 0.1, Zeta: − 37.7 ± 0.9 to − 30.0 ± 0.7	[54]
Gemcitabine hydrochloride	Breast cancer	Modified double emulsion solvent evaporation method	Lipid: Soya phosphatidylcholine (SPC) and DSPE-PEG2000, Polymer: PLGA	Size: approx. 200 nm, PDI: approx. 0.2, Zeta: approx. − 18 mV, EE: 45%	[55]
Anastrozole (ANS)	Breast cancer	Direct emulsification solvent evaporation method	Lipid: stearic acid. Polymer: polycaprolactone (PCL)	Size: 193.6 2.9 to 218.2 1.9 nm, Zeta: 0.50 0.52 to 6.01 4.74, PDI: 0.1, EE: 80%	[56]

cancer. The results showed high tumor distribution and antitumor efficacy, which were confirmed by both *in vitro* and *in vivo* studies [61].

Multidrug Resistance

One of the main reasons for cancer patients' chemotherapeutic treatments failing is multidrug resistance (MDR). It is also very critical to address MDR in order to improve the efficacy of cancer treatment. PLHNs have several advantages, including increased mechanical stability and shape control. Huang *et al*. developed polymer-lipid hybrid nanoparticles (PLN) for the delivery of psoralen (PSO) to treat breast cancer. PSO-PLN exhibited a strong reversal effect on MCF-7/ADR cells according to *in vitro* experiments and was modestly localized in liver. As a result, PSO-PLN improves PSO's ability to release into the body continuously, which not only helps to reverse MDR but also improves the efficacy of treatment [62]. Yuan *et al*. also developed polymer-lipid hybrid nanoparticles (LPN) containing psoralen (P) to improve the efficacy of doxorubicin in multidrug-resistant HepG2 cells. In multidrug-resistant HepG2/ADR cells, psoralen LPNs (P-LPNs) increased the cytotoxicity of doxorubicin (DOX) 17 times more than free DOX. Additionally, pro-apoptotic activity, elevated ROS, and depolarization were all observed in P-LPNs. According to mechanistic investigations, P-LPNs boosted the cytotoxicity of DOX by enhancing caspase-3 cleavage and cytochrome C release, which led to apoptosis in HepG2/ADR cells [63]. By encapsulating doxorubicin (DOX) in the PLGA core and a NO photodonor (NOPD) in the phospholipid shell, Fraix *et al*. reported the delivery of nitric oxide (NO) via customized lipid-polymer hybrid nanoparticles (NPs) under the control of visible light as a method of overcoming DOX resistance in melanoma. The release of NO by multi-cargo NPs is sufficient to obstruct the efflux transporters principally responsible for DOX cellular extrusion, according to research on M14 DOX-resistant melanoma cells. DOX's anticancer activity was subsequently boosted by increased cellular retention [64]. To overcome paclitaxel (PTX) MDR in lung cancer, Pramual *et al*. used a nanoparticle-mediated photodynamic treatment (NPs/PDT) method. They combined PTX with a photosensitizer known as pTHPP in PLGA-lipid hybrid nanoparticles. Various MDR models were used to assess the photocytotoxic effects of the nanoparticles, and the results through flow cytometry showed that combination hybrid system killed the cancer cells through apoptosis. The PLGA-lipid hybrid nanoparticles' ability to carry photosensitizers or chemotherapeutic drugs suggests that they have the potential to treat both drug-selected and metastasis-associated MDR lung cancer cells [65].

Gene Delivery

PLHNs have been used to target specific enzyme by favoring gene delivery. Yang *et al*. and his colleagues created lipid-polymer hybrid nanoparticles (LPHNs) for gene targeting and efficient delivery, as well as CRISPR/Cas9 for targeting the enzyme that causes resistance to the glioblastoma drug temozolomide (TMZ). In this study, they used the nanoprecipitation method (polymer: PLGA; lipid: lecithin) to produce LPHNs containing clustered regularly interspaced short palindromic repeats (CRISPR)–associated protein 9, and the peptide chain CRGD in order to overcome resistance to TMZ treatment caused by the O6-methylguanine-DNA methyltransferase (MGMT) enzyme. A focused ultrasound technique (FUS)–microbubble (MB) was used to help facilitate gene delivery into glioblastomas *in vivo* without affecting the BBB. With focused ultrasonic irradiation (FUS), this formulation enhanced the sensitivity of temozolomide towards GBM cells, could locally and safely improve blood–brain barrier permeability *in vivo*, and made it easier for nanoparticles to collect at the tumor site in mice with orthotropic tumors. With a high level of biosafety, the developed LPHNs also improved the therapeutic effects of temozolomide in treating glioblastoma, suppressed tumor growth, and extended the longevity of tumor-bearing animals [66].

Theranostics

Potential use of PLHNs in cancer theranostics have not been explored that well. Gu *et al*. reported the preparation of Cisplatin encapsulated in polymer-lipid hybrid nanoparticles (PLHN), where they used the near-infrared fluorescent dye indocyanine green to treat MCF-7 cells. LPHN treated with folate showed excellent near-infrared radiation (NIR) penetrability and improved stability. According to cell uptake tests, folate modification targets MCF-7 cells that overexpress the folate receptor more effectively than A549 cells that express the receptor insufficiently. With the use of an 808 nm NIR laser, LPHN significantly increased apoptosis and necrosis [67]. Huang and his team reported the use of GSH-sensitive Pt (IV) prodrug–loaded transitional phase lipid-polymer hybrid nanoparticles for theranostic delivery against ovarian cancer. Under ultrasonic energy, the ready LPHN demonstrated robust echogenic signals. A delivery approach that uses ultrasonic stimulation and GSH sensitization improved therapeutic results while lowering toxicity. Mechanistic investigations showed that GSH consumption and an increase in the amounts of reactive oxygen species cause cell death [68].

Types of CPPs

Cationic CPPs

Cationic CPPs are peptides containing small amino acid series that are largely composed of arginine, lysine, and histidine amino acids. As a result of these amino acid residues, CPPs that bear a positive charge interact with the anionic or acidic contents on the surface of the plasma membrane. The first cationic TAT (YGRKKRPQRRR) was identified in the HIV-1 Tat protein, which constitutes the TAT peptide. It contains 1 tyrosine, 1 glycine, 5 arginines, 2 lysines, 1 proline, and 1 glutamine sequence (a total of 11 amino acid series). As we can see above, there are 5 amino acid residues in TAT which gave rise to the investigation of its derivatives of various lengths like oligoarginine [20, 69].

Both oligoarginine and oligolysine are positively charged peptides. But interestingly, both of these oligo-peptides showed a difference in cellular uptake and also in intracellular localization [21, 70]. And the above statement was confirmed by using a method called as sub-cellular fractionation method or the other way is by the localization of these oligopeptides in the cytoplasm which was confirmed by their internalization at 4 C *versus* 37 C. The sequence of cationic CPPs can be oriented in clustered form or mixed form. In clustered form, arginine or lysine amino acids are adjacent to each other. An example of this is the TAT peptide, CPPs derived from the SV40 protein, and synthetically prepared oligoarginine CPPs [70].

Amphipathic CPPs

In the above section, we have seen that cationic CPPs contain arginine and lysine, which can be oriented in a cluster or mixed sequence, while amphipathic CPPs have arginine amino acid and lysine amino acid residues as well as hydrophobic residues such as valine, leucine, isoleucine, and alanine.

Because of the increased levels of hydrophobic residues in the amphipathic CPPs, their binding characteristics to the hydrophobic lipid bilayer in the cell membrane are very distinct from those of the cationic CPPs. As a result, strong hydrophobic interactions were observed in amphipathic CPPs compared to cationic CPPs. When it comes to the insertion of CPPs into the hydrophobic lipid bilayer, it was also seen that insertion was higher in amphipathic CPPs than in cationic CPPs [71]. Penetratin is one of the secondary amphipathic CPPs which has very unique properties as it contains both charge and the amphipathic nature [72].

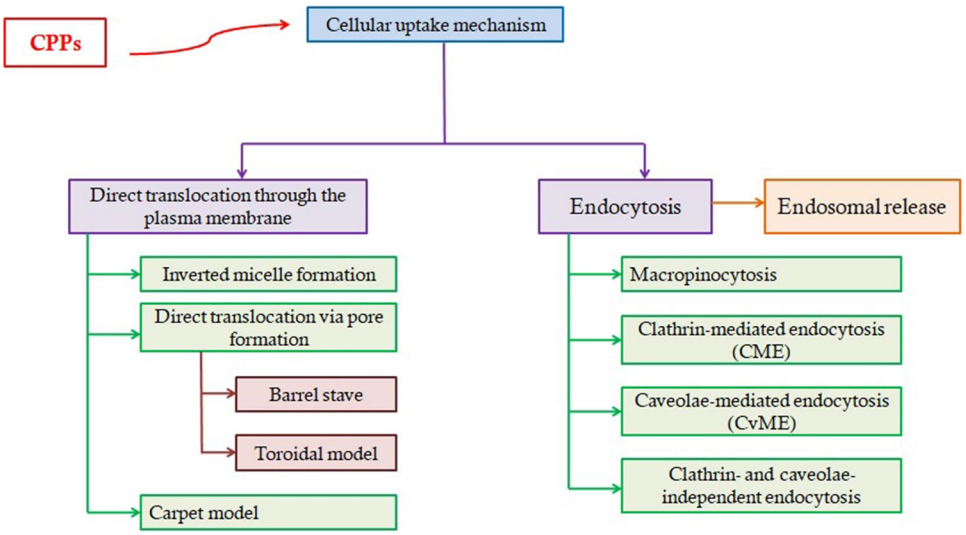

Fig. 7 Schematic representation of different cellular internalization mechanism of CPPs

Hydrophobic CPPs

Hydrophobic CPPs are the series that accommodate only apolar residues, which are either attached to the peptide chain [73], or prenylated peptides [74], and/or peptucins [75]. A few examples discovered are integrin β3 (-val-threo-val-leu-ala-leu-gly-ala-leu-ala-gly-val-gly-val-gly-) and Kaposi fibroblast growth factor (-ala-ala-val-ala-leu-leu-pro-ala-val-leu-leu-ala-leu-leu-ala-pro-) [76]. Hydrophobic CPPs also serve a great importance as they are also involved in enhancing the uptake as well as the delivery of the active components [77].

Cellular Uptake and Release Mechanism of CPPs

The assessment of the internalization behavior of the CPPS is essential, as it affects the safety and efficacy profile. The cellular uptake of CPPs depends on several factors, like peptide structure, molecule length, charge distribution, cargo molecules and their behavior, and the plasma membrane structure and its environment. These above characteristics cause the CPPs to interact with many molecules on the cell surface and therefore greatly impact their route of entry into the cell [78]. Therefore penetration of CPPs is discussed below (Fig. 7).

Direct Translocation Through the Plasma Membrane

This process has interesting characteristics as they are energy independent process, occur in single step, and occur at low temperature (Fig. 8). The translocation/entry of the CPPS to the lipid bilayer cell membrane is explained using 2 models, i.e., inverted micelle model and the carpet model [79, 80].

Inverted Micelle Formation

An example of an inverted micelle model, where it was involved in the early stages of the uptake into the cell, is the penetratin peptide series. Penetratin was one of the first described CPPs which was derived from homeoprotein

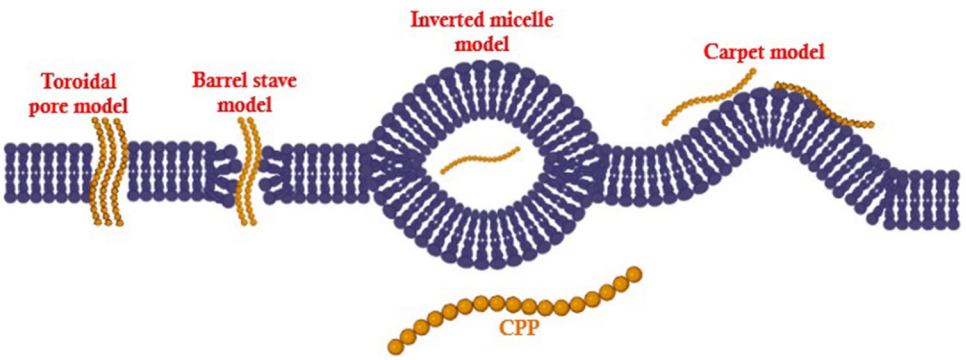

Fig. 8 Mechanisms of peptide uptake across the phospholipid bilayer membrane by direct translocation

Antennapedia. It transferred the active molecule across the phospholipid bilayer and studied in detail [81].

The internalization process begins with the electrostatic attraction between the peptide chain and the lipophilic cellular membrane. As a result of this interaction, the lipid bilayer membrane is disturbed, trapping the CPP peptide chain within the hexagonal-shaped inverted micelles. These micelles have an internal hydrophilic compartment where the CPP peptide is trapped until it is transferred to the cytosol. Once the inversion process takes place, these inverted micelles undergo destabilization, so they release the peptide chain into the cell. Nuclear magnetic resonance (NMR) data studies support this model [82].

Direct Translocation Via Pore Formation

Two models are used to explain direct translocation by the formation of pores on the membrane. The first is the barrel-stave model and the second is the toroidal model.

Barrel-Stave Model Barrel-stave is a model which shows that CPPs can diffuse across the phospholipid bilayer and is the characteristic feature of amphipathic α-helical peptides. Pore formation includes the following processes: Firstly, the peptide chain interacts with the phospholipid membrane and attaches to it. This results in an increased concentration of peptides on the surface of the cellular membrane. As the concentration of peptides increases, the resting lipid bilayer membrane gets distorted. They form a pore with the outward hydrophobic group, which interacts with the lipid bilayer membrane, and the inwardly facing hydrophilic group, which forms the surface of the pore [83, 84].

Toroidal Model In the case of the "toroidal" model, the peptide chain interacts with the phospho group and accumulates between the phospho and carbon lipid bilayers of the cell membrane. This incorporation of the peptide sequence between the phospholipid bilayer results in thinning of the cell membrane. Also, as the pH increases, the guanidinium group of the CPP peptide chain interacts with the fatty acids. Because of this, the CPPs enter the cell through a transient toroidal pore formation. But as the pH decreases, the CPPs attached to the fatty acids are released into the cell, and eventually, the pore closes [85].

Carpet Model

As the name suggests, in the carpet model, a series of peptides adhere to the cellular surface and form a structure similar to the carpet (Fig. 9). Due to this kind of arrangement, the hydrophobic component of the peptide interacts with the hydrophobic area of the phospholipid cell membrane and causes disruption of the cellular membrane, leading to micellization [86, 87].

Endocytosis

The endocytosis pathway is a very complex process or mechanism. Active substances or therapeutic agents are transported into the cell in two distinct ways, namely, through endocytosis and endosomal escape [88].

Endosomal Uptake

Generally, endocytosis is divided into two categories, namely (a) phagocytosis and (b) pinocytosis. Phagocytosis generally occurs in specialized cells (macrophages, monocytes, and

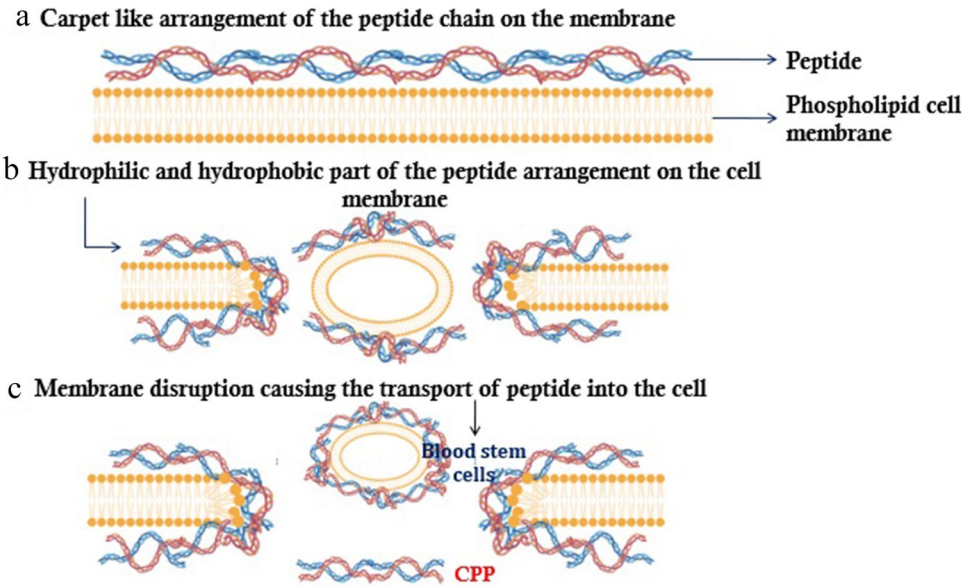

Fig. 9 Model representing carpet arrangement of the peptide on the cell membrane

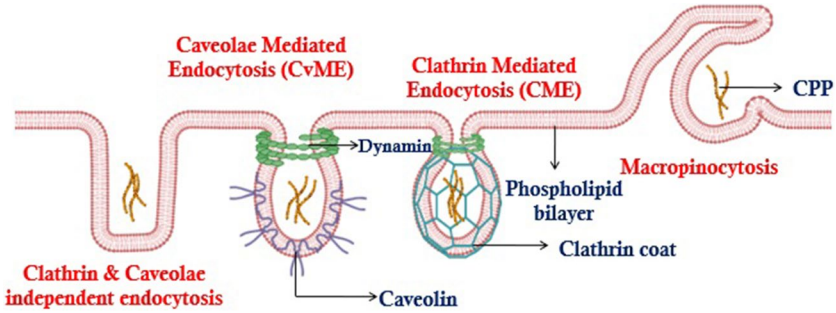

Fig. 10 Mechanisms of peptide uptake across the phospholipid bilayer membrane by endocytosis

neutrophils) where they take up large particles. Pinocytosis is involved in the transport of fluids and solutes. The uptake of the solute occurs most efficiently when the solute is diluted. This is because the dilute solutes are attracted by receptors with high affinity and are concentrated in the endocytic transport vesicle. Different mechanisms (Fig. 10) of the pinocytosis pathway are (i) macropinocytosis; (ii) clathrin-mediated endocytosis (CME); (iii) caveolae-mediated endocytosis (CvME); and (iv) clathrin- and caveolae-independent endocytosis [89].

Macropinocytosis Macropinocytosis is a form of lipid raft-dependent endocytosis that occurs more rapidly and is receptor independent. A large amount of fluid content is taken up, and this type of uptake is termed fluid phase endocytosis (FPE). Due to the simulation of growth factors or other signals, membrane ruffling occurs. Similar to phagocytosis, there is a cascade of steps that include signals and regulators belonging to the kinase group (such as Src, PI3) and GTPases (Rho family, Ras family, Rab proteins), and induce the macropinocytosis process. These regulators trigger actin-mediated membrane protrusions. The so-formed protrusions collapse and eventually fuse with the cellular membrane. This fusion produces large endocytotic vesicles called macropinosomes. These vesicles named macropinosomes mature [89–93].

Clathrin-Mediated Endosomal Uptake CME is a form of endocytosis that is typically engaged in the uptake of LDL (low-density lipoprotein). The transportation of the active component from outside to inside the cell is via the endocytic pathway. All the processes make use of clathrin and the GTPase dynamin, as this process is receptor-dependent [94]. The initiation of the CME process starts with the gathering of major proteins required for coat formation, i.e., clathrin triskelia, which forms clathrin-coated pits. This clathrin triskelion was composed of three heavy chains of clathrin along with closely bound light chains that are made up of clathrin

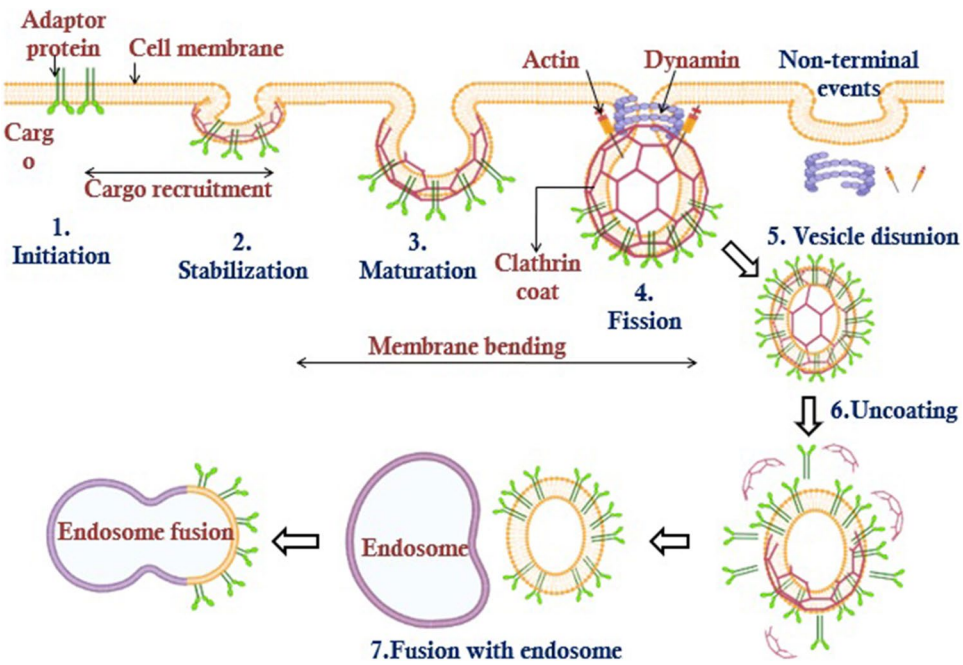

Fig. 11 Stages and process involved in clathrin-mediated endocytosis

and also an adaptor protein-2 (AP2). This AP-2 is a heterotetramer, which consists of alpha, beta-2, gamma-2, and μ2 subunits [95].

An endocytotic process begins when a ligand binds to a specific cell surface with a strong binding interaction. Because of this strong interaction, clathrin starts assembling on the cellular surface in a polyhedral lattice. As a result of the invagination of the clathrin-coated surface, they tend to move towards the cytoplasm by forming pits that acquire a spherical shape along the membrane [96]. Therefore CME contains 5 stages (Fig. 11): (1) initiation of CME events, (2) cargo loading, (3) membrane bending/folding, (4) vesicle disunion, (5) disassembly of the outer coat.

Initiation of CME Events Initiating endocytotic events is the first step in this pathway. It is very critical for regulating the series of biochemical events because it paves the way for the formation of the endocytic vesicle. This is because they determine where the vesicle will be formed on the cellular membrane. The endocytic proteins start clustering on the plasma membrane, and because of this clustering, they form a vesicle on the cellular membrane. That is the major components of the plasma membrane, phosphatidylinositol 4,5-biphosphate (PI(4,5)P2), and the adaptor proteins like AP-2 interact with each other and favor the formation of vesicles [97].

The allosteric regulation of AP2 and the change in conformation play a very prominent role in controlling the initial steps of CME. When the cystolic AP2, which is a protein, comes into contact with the cellular membrane, it interacts with the plasma membrane, which is rich in PI4,5P2, and because of this interaction, nucleation of clathrin assembly occurs. The cargo, as well as the PI4,5P2, also bonds to the EAPs (endocytic accessory proteins), which triggers the stabilization of the open conformation and prevents the early apportion of the clathrin-coated pits. This sustained interaction of the cargo molecules, PI4,5P2, and the EAPs contributes to the growing stages of the clathrin-coated vesicles, and once the growth is complete, maturation of the so-formed vesicle takes place [95].

Cargo Loading Cargo loading is the second step, right after the initiation of endocytic events. The principle driving this step is the assembly of the clathrin coat on the specific site on the plasma membrane as well as the assembly of the cargo molecules. This type of arrangement triggers the clustering of the cargo molecules onto the specific site on the membrane and contributes to the formation of the curvature on the plasma membrane resulting in a clathrin-coated vesicle. After the vesicle forms, particular cargo is incorporated into the vesicle, and the concentration of cargo molecules is very critical because if that concentration is not reached, it can lead to abortion of the clathrin-coated vesicles or scission of the vesicles [98].

Membrane Bending The following step of CME is membrane bending. The endocytosis process requires the bending of the membrane, which is contributed by several modules like the coat, the actin filaments, and the scission proteins. Clathrin first interacts with an adapter protein residing on the plasma membrane. This interaction leads to the assembly of clathrin onto the coat in an icosahedral manner. This results in the polymerization of clathrin and causes membrane curvature, or bending. Actin also plays an important role in membrane bending as the membranes undergo polymerization and attach to the surface and base of the growing membrane vesicle. The association of clathrin and actin filaments gives a driving force for membrane bending, and actin filaments undergo depolymerization in seconds once the process is complete [89, 96, 97].

Vesicle Disunion As the word implies, vesicle disunion is the dissociation of the formed vesicle from the parent cellular membrane. The GTPase dynamin is primarily responsible for catalyzing the above-mentioned process. A GTPase dynamin arranges them around the neck of the forming vesicle in a helical fashion and dissociates the vesicle from the membrane. Dynamin is generally involved in membrane vesicle scission based on the following process. The first dynamin assembles in a helical manner around the vesicle in the form of a tightly bound oligomer. This results in the constriction of the so-formed vesicle neck. GTP hydrolysis results in membrane fission. Upon the addition of GTP, the dynamin further constricts the formed vesicle, which then undergoes a spontaneous transition into the half-fission state, and finally, to the fission state, releasing the free clathrin-coated vesicle [97, 99].

Disassembly of the Coat Once the vesicle is dissociated from the membrane, the next step is the shedding or disassembly of the coat. This will enable the newly formed vesicle to fuse with the early endosome. The initially attached endocytic proteins released in this process can also be used for future events or saved for the next event. The ATPase also influences the disassembling activity, which results in the depolymerization of clathrin and dynamin [95, 98].

Caveolae-Mediated Endocytosis Pathway Palade was the first one to describe caveolae in 1953. He described it as a flask or an omega-shaped substance that appears as a small cave-like structure attached to the cellular membrane.

It was found that it played a very crucial role in endocytosis, lipid digestion, and cell signaling [100]. Caveolae consist of a structure called caveolin, which is a dimeric protein. This caveolin can bind to cholesterol and insert it into the inner part of the cellular membrane in a loop-like manner, which further self-associates and forms a striated coat on the membrane, which undergoes growth [89]. There are three members of the Cav family, namely Cav-1, Cav-2, and Cav-3. Cav-1 and Cav-2 are mostly found in fibroblasts, adipocytes, endothelial cells, and pneumocytes, whereas Cav-3 communicates with skeletal muscle fibers and cardiac myocytes independently of Cav-1 and Cav-2 [101, 102].

Caveolae-dependent endocytosis is largely dependent on the GTPase dynamin. Cav-1 ligands on a cellular membrane interact with the dynamin domain and disrupt the cytoskeleton arrangement of actin filaments. Due to this process, dynamin-II replaces the plasma membrane where vesicle internalization occurs. When this happens, dynamin undergoes oligomerization as well as GTP-dependent conformational changes and results in inserting itself around the neck collar (Fig. 12), leading to the formation of new vesicles called caveosomes, which help in the transportation of cargo molecules. Afterward, the vesicles undergo vesicle disunion from the originally attached plasma membrane[100, 103].

Clathrin- and Caveolae-Independent Endocytosis Basically, this pathway involves in the uptake of lipids and fluids [104]. Caveolae, as previously stated, are dimeric proteins that have a proclivity to interact with the cholesterol-rich domain and are cave-like structures on the plasma membrane with a diameter of 40–50 nm. As a result of their highly lipidic nature, these cave-like structures are very effective in the endocytosis process in any vesicle, sorting the membrane proteins and favoring their internalization [105, 106].

The molecular-level process underlying this type of endocytosis can be dynamin dependent or clathrin independent. They make use of the scission GTPase dynamin, or they may function independently of dynamin without clathrin. The dynamics of the actin cytoskeleton determine the stability and scission processes [107]. Another process is by clathrin- and dynamin-independent endocytosis pathway, which makes use of endophilin-A2 and endophilin-A1. These endophilin directly facilitates endocytosis process by binding to the selected G protein–coupled receptors [108].

Clathrin-dynamin independent is also an endocytic pathway that is involved in the internalization process of glycosyl-phosphatidylinositol anchored proteins (GPI-APs) and fluid phase in multiple cell lines and tissues [109]. In the electron microscope picture, uncoated, tubular intermediates [110] described as clathrin-independent carriers (CLICs), were evident, subsequently fusing to form GPI-AP-enriched early endosomal compartments (GEECs) [111].

Endosomal Release

The endosomal release is considered the rate-limiting step because, once the cargo molecules have been uptaken, they must reach the cytosol to exhibit any therapeutic response or inhibit or induce certain actions. There are several mechanisms by which cargo molecules reach the cytosol, mainly membrane disruption and ionic pair formation.

By interacting with the negatively charged phospholipid content of the cellular membrane, the positively charged CPPs can induce membrane disruption [112]. Because of this interaction, pores are formed or can cause leakage in the plasma membrane, through which the CPPs are released. TAT is the most prominent example of this, as it causes leakage when it interacts with the plasma membrane's phospholipids [113]. Another mechanism was the formation of an ion pair when the CPPs interacted with the negatively charged, high-lipid plasma membrane, resulting in the partitioning of the CPPs across the membrane lining and the release of the CPPs; a clear example of this is how the oligo-arginines reached the cytosol [114].

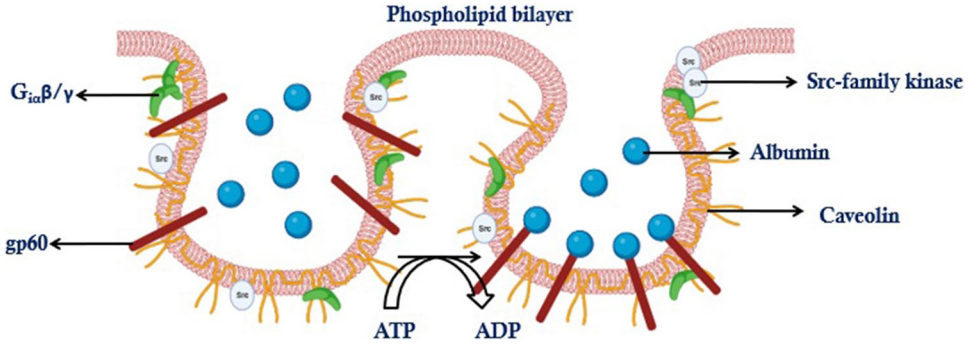

Fig. 12 Caveolae-mediated pathway where caveolin dimer inserts themselves in vesicles, in loop manner and favors phagocytosis

Synthesis of CPPs

Emil Fischer carried out the first effective coupling of two amino acids in 1903 using acyl chlorides, but at the time, no adequate amino-protecting group was available for the synthesis of longer peptides. Bergmann and Zervas' and other inventors' discovery of the benzyloxycarbonyl-protecting group enabled the production of the neurohypophysial nonapeptide hormone oxytocin, for which du Vigneaud won the Nobel Prize in 1955. The invention of solid-phase peptide synthesis (SPPS) by Bruce Merrifield, a trailblazer in the field, eased the time-consuming and difficult stages of purification involved in solution phase synthesis [115, 116].

The basic idea behind solid-phase peptide synthesis is resin preparation, in which the amino acid is joined to the resin by carboxyl, hydroxyl (or chloro), and amino groups to create ester- or amide-linked peptides, which give us a C-terminal acid or amide peptide, respectively. The first peptide loads into the resin with a straight, linear path from the C-terminal to the N-terminal. Here, where urethane derivatives are frequently utilized, many cycles of deprotection and amino acid coupling reactions will take place; the amino group is protected by temporary protecting groups (T), while the side chain of the amino acid group is protected by permanent protecting groups (Pn). Under benign circumstances, it is simple to remove the temporary protective group (T), preventing the peptide from becoming epimerized. Filtration and washing cycles are used to get rid of excess reactants. So, the required sequence of the peptide is achieved by repeatedly performing deprotection and coupling reactions. Finally, the peptide is released from the resin along with the Pn side chain. The desired sequence of peptides necessary for distribution via the phospholipid bilayer can be generated using this accepted methodology for solid-phase peptide synthesis [115, 116].

Polymer-Lipid Hybrid Nanoparticles Decorated with Cell-Penetrating Peptides for Therapeutic Applications—Cancer Therapy

The hybrid system can take advantage of polymeric and lipid nanoparticles and can also encapsulate both hydrophilic and hydrophobic drugs. The targeting of this hybrid system can be achieved by conjugating or decorating the surface with the CPPs, which help to bind the specific receptors. On the other hand, it can also be used to overcome the MDR associated with certain types of cancer.

One such example is using a CPP called RGD peptide to decorate the surface of the PLHNs containing the hydrophilic or lipophilic chemotherapeutic agent to target the integrin receptors overexpressed in certain types of cancer. Integrin is a cell surface protein that is important for cell adhesion which is composed of α and β subunits non-covalently bond to each other [117]. During angiogenesis, integrin is involved in regulating cell growth, survival, and migration. These integrins include the heterodimers α1β1, α2β1, α4β1,α5β1, α6β1, α6β4, α9β1, αvβ3, and αvβ5 [118]. One of the models of endothelial apoptosis is the caspase activation model, in which RGD peptides directly activate caspases that trigger apoptosis, and the RGD peptide also has a binding site in an integrin that leads to apoptosis [117].

Shi et. al. developed a hybrid system using a W/O/W emulsion solvent extraction evaporation process with PLGA as a polymer and soybean lecithin as a lipid. The surface was then coated with RGD CPPs in order to target integrin αvβ3 which is overexpressed in 90% of glioblastoma multiforme. Due to receptor-mediated endocytosis, the RGD surface decorated polymer-lipid hybrid system (RGD-L-P) exhibited a higher absorption efficiency (in C6 cells) than PLGA, lipid nanoparticles, and the hybrid system without the RGD surface coating. Better tumor spheroid penetration confirmed the RGD-L-P's ability to target C6 cells that express integrin 3. RGD-L-P loaded with docetaxel showed greater cytotoxicity in C6 cells due to rapid internalization facilitated by the RGD peptide sequence. Biodistribution studies using DiR were conducted using near-infrared fluorescence probes, which showed that DiR-loaded RGD-L-P showed better brain-localized fluorescence signals, suggesting better localization and specific binding. Altogether, these findings suggest that RGD-PLHNs are a potential drug delivery strategy for the targeted therapy of GBM [119].

In order to administer isoliquiritigenin (ISL), a dietary supplement with natural anti-breast cancer properties but poor transport and limited bioavailability, Gao *et al*. also created a lipid-polymer hybrid nanoparticle. A method known as modified single-step nanoprecipitation was used to synthesize the hybrid system containing PLGA as a polymer and lecithin as a lipid. The surface of the hybrid system is conjugated with an iRGD peptide so as to preferentially bind αvβ3 and αvβ5 integrin receptors and Nrp1 receptors (permeability-regulating receptors). Results showed higher cellular accumulation, cell toxicity, and apoptosis against breast cancer cells. Higher cellular accumulation was attributed to the surface conjugation of the iRGD peptide onto the hybrid system, which recognizes integrin receptors overexpressed in breast cancer cells, which resulted in rapid internalization and longer circulation due to the stealth nature of the hybrid system, resulting in enhanced inhibition of tumor growth [120].

Wang *et al*. reported the used of RGD peptide with the paclitaxel (PTX) in the form of redox-sensitive lipid-polymer

nanoparticles (LPNs) along with its prodrug form and also used cispaltin (CDDP) in order to deliver PTX in the form of dual-loaded drug delivery system. LPN was prepared using PLGA as a polymer and soybean lecithin as a lipid system by the emulsification-sonication method. Lung cancer cells and tumor-bearing animal models were used to assess the anticancer efficacy of PTX-loaded LPNs *in vitro* and *in vivo*. The IC50 values of the PTX-loaded LPN when compared others showed much stronger anticancer activity. The most effective anticancer effectiveness was also demonstrated by RGD-modified PTX or CDDP LPNs, which reduced mouse tumor size with less systemic toxicity [121].

Applications

Receptor Targeting

Integrin Receptor Targeting Integrin receptors, which help in cell adhesion and mediate contacts between cells and the surrounding extracellular glycoproteins composed of α and β subunits, have a significant impact on the behavior of cancerous cells. There are at least 25 distinct integrins that can be created from the 18α and 8β subunits. Integrins function as crucial surface adhesion and cell signaling receptors, which have an impact on growth factor signaling, cell survival, proliferation, and migration [118]. They may also have a significant impact on tumor behavior and metastasis**.** Therefore, targeting the over expressed integrins in many type of cancers helps in effective delivery of the chemotherapeutic agent. One such approach to target integrin receptor is using CPPs, called RGD peptide (arginine-glycine-aspartic acid) which recognizes integrin receptors mainly integrin αvβ3, αvβ1, and αvβ5 which are over expressed in many types of cancer and therefore brings about effective targeting. Yang *et al*. used modified emulsification technique to develop and optimize lipid-polymer hybrid NPs for targeted delivery. The PLHNs core was made up of PLGA whose 90% of the surface was coated with lipid monolayer using egg lecithin (EPC) and DSPE-PEG. In addition, hybrid NPs loaded with 10-hydroxycamptothecin (HCPT) and the arginine-glycine-aspartic acid (RGD) peptide were created to target integrin 3-positive breast cancer cells. It abundantly expressed integrin 3 and as a result, head-to-tail cyclic RGD-containing peptides, or c(RGDyk), have a high affinity for their overexpressed integrin 3 receptors, resulting in tumor angiogenesis and metastasis. Initial DSPE-PEG-OH synthesis was followed by succinic anhydride treatment and RGDyk peptide conjugation. This DSPE-PEGc(RGDyk) was conjugated to the surface of the prepared polymer-lipid hybrid structure. The cellular uptake and cytotoxicity study revealed enhanced permeability to tumor cells via endocytosis and an increased ability to kill cancerous cells due to the conjugation of the cell-penetrating peptide, c(RGDyk). The HCPT, which was encapsulated into hybrid NPs, showed higher drug loading compared to PLGA NPs alone [122].

Using an emulsification solvent volatilization method, Zhao *et al*. reported the utilization of Arg-Gly-Asp (RGD) peptide–modified lipid shell and polymer-core hybrid nanoparticles (lpNPs) loaded with curcumin (Cur). PLGA-mPEG was used as the polymer core with lecithin/cholesterol surface–coated lipids. In order to improve targeting, lpNPs were surface decorated with Chol–PEG–RGD. Cytotoxicity analysis results showed that the curcumin-encapsulated RGD adorned lpNPs maintained strong antitumor activities. A subcutaneous B16 melanoma tumor model was significantly more sensitive to curcumin-loaded RGD-lpNPs for arresting tumor growth. The results of immunofluorescence and immunohistochemistry analysis showed a higher number of apoptotic cells, a reduced number of microvessels, and a reduced number of proliferative cells in comparison to those using curcumin-free RGD-lpNPs. Curcumin-loaded RGD-lpNPs are a strong formulation of curcumin that targets tumors because of their enhanced antitumor effect in melanoma [123]. Zang *et al*. reported the used of PLHNs to administer doxorubicin (DOX) and sorafenib (SOR) simultaneously, shell-core lipid-polymer hybrid nanoparticles (NPs) coated with the iRGD (CPPs) which can be used to achieve tumor-targeting delivery. Human liver cancer cells that expressed integrin showed synergistic cytotoxicity, pro-apoptotic potential, and enhanced internalization rates of both DOX and SOR after adding iRGD to the hybrid system. DOX + SOR with iRGD NPs had a longer circulation and bioavailability, according to *in vivo* pharmacokinetic research. More impressively, DOX + SOR with iRGD PLHNs markedly improved antitumor effectiveness in mice models with hepatocellular carcinoma xenografts [124].

CD13 Receptor Targeting Many different forms of solid tumors exhibit high CD13 expression. A multipurpose protein receptor called CD13, tumors use it to grow, invade, and spread. One of the CPPs named kNGR (Asn-Gly-Arg) peptide can target by recognizing and binding to the overexpressed specific isoform of CD13 receptors in solid cancers [125]. Gupta *et al*., in his project used kNGR peptide which was attached to lipid-polymer-based nanoparticles to deliver paclitaxel (PTX), an anticancer agent. Spectral analysis was used to construct and analyze the kNGR-PEG-DSPE compound. The dual-targeted PLGA-lecithin-PEG core–shell nanoparticles (PLNs-kNGR-NPs) were made using a modified nanoprecipitation process. The findings showed that PLNs kNGR-NPs are significantly more cytotoxic than other NPs due to increased intracellular absorption and apoptosis. It highlights the necessity of stratified

nanoparticle design to achieve a synergistic effect in the HT-1080 tumor–induced paradigm, with a tumor volume reduction percentage rate of 59.7% compared to other formulations developed in Balb/c mice. The hybrid lipid-polymer nanoparticles based on PLNs-kNGR-NPs showed the greatest therapeutic efficacy against solid tumors over-expressing CD13 receptors [126].

Human Epidermal Growth Factor Receptor-2 (Her2/neu) Targeting Her2/neu, which is overexpressed in 30% of breast cancers, is a great therapeutic target for the development of breast cancer medications. For the treatment of breast cancer, Yang *et al*. reported using a hybrid system that was conjugated with the dual ligands anti-HER2/neu peptide (AHNP) and modified HIV-1 TAT (mTAT). Modified emulsification technique was used to prepare polymer-lipid hybrid (NPs) system encapsulating docetaxel with PLGA as polymer and egg lecithin as lipid. An analysis of ligand density showed that altering the surface-ligand densities might change how well the NPs were absorbed by cells. Several endocytic pathways helped for the internalization of NPs according to cell uptake kinetics and mechanism. The dual ligand PLHNs with DTX loading displayed a slower sustained drug release and a smaller burst release. The dual-ligand PLHNs modified with DTX were found to be more potent than other NP formulations for treating SK-BR-3 cancer cells due to the synergistic effect [127].

Dual Drug Delivery

To increase the effectiveness of the therapy, PLHNs and CPPs work together to deliver biomolecules like DNA and RNA together with chemotherapeutic drugs. Dong *et al*. reported the use of hybrid system (LPNs) for the co-delivery of plasmid DNA (pDNA) and a chemotherapeutic drug, docetaxel (DTX). Solvent evaporation method was employed which used (PEI-PEG) [poly (ethylene imine) polyethylene glycol] and Compritol1 888 ATO as polymer and lipid respectively. The surface of the hybrid system was decorated with CPPs named TAT to facilitate cellular uptake of gene vectors. The results of the *in vitro* (PC3 cancer cells) and *in vivo* (murine prostate cancer model) studies demonstrated increased anticancer effect both *in vitro* and *in vivo*, as well as efficient gene delivery of TAT-modified DTX and pDNA LPNs. Therefore, it was stated that PLHNs and CPPs could be a promising co-delivery nanosystem [128]. Qiao et al. studied multi-targeted polymer-lipid hybrid delivery system in order to target brain glioma by using a therapeutic gene delivery strategy to deliver and target the blood–brain barrier and improve therapeutic efficacy and synergistic effect. In order to achieve BBB penetration, receptor-mediated endocytosis was favored using Angiopep2 peptide (Ang) and for rapid endosomal uptake and release, CPP name TAT was used. Tumor-targeting ability under the magnetic field was further improved with the use of iron oxide magnetic nanoparticle (Fe3O4 MNPs). PCL which forms core and DOTAP covers the surface was used as polymer and lipid respectively to prepare the hybrid system. The results demonstrated that the Ang-TAT decorated iron oxide LPHNs considerably increased both the transfection efficiency of C6 cells using pEGFP-C1 as the reporter gene and the penetration efficiency of the BBB model produced by hCMEC/D3 cells [129].

Gene Delivery

As an alternative to polyethyleneimine (PEI) derivatives or peptide-protein conjugates, PLHNs are used for delivering mRNA-based gene therapy. An improved mRNA delivery method, DMP-039, was described by Gao *et al*., as using cRGD-R9 as a CPP and a cationic nano-sized DMP backbone. The DMP gene vector's structure is built from DOTAP lipid and mPEG-PCL polymer, which self-assemble. The cRGD-R9 peptide was added to the DMP backbone to create a peptide-functionalized hybrid delivery system, which increased its capacity to disperse mRNA. The produced DMP-039 hybrid nanoparticles demonstrated high mRNA distribution efficiency during trans-membrane transit via a variety of routes. By loading the suicide gene Bim's encoded mRNA, a locally injected mBim/DMP-039 combination effectively inhibited the growth of two colon cancer models. Additionally, intravenous treatment of the mBim/DMP-039 complex effectively and safely slowed the growth of the C26 lung metastatic tumor [130].

Multidrug Resistant

A prominent barrier to effective treatment is multidrug resistance (MDR), which causes chemotherapy failure in the majority of patients with cancer. Zang *et al*. reported the use of iRGD peptide decorated on a lipid-polymer hybrid nanosystem (LPN), which was synthesized via two-step synthesis consisting of PLGA as the polymer and egg lecithin (EPC), cholesterol (CHOL) as the lipid layer. LPNs were produced with a specific combination proportion of paclitaxel (PTX) and tetrandrine (TET) to improve cellular internalization and control drug release in ovarian cancer. The major MDR mechanism in PTX therapy is the upregulation of P-glycoprotein (P-gp) receptors, which promote the efflux of the chemotherapeutic agent, reducing its anticancer efficacy. TET was used as a P-gp inhibitor to promote the increased concentration of PTX inside the cancer cells. iRGD CPP was used to target the over-expressed αvβ 3/5integrin receptors to improve drug delivery *in vivo*. As a result of the suppression of P-gp (by TET) and conjugation of iRGD CPP, there was an increase in the concentration of PTX within cancer cells, as confirmed by cellular uptake studies (Table III), and they also effectively increased apoptosis, produced ROS, and arrested the cell cycle [132].

Table III Therapeutic Applications of Polymer-Lipid Hybrid Nanoparticles Surface Modified with Cell-Penetrating Peptides

Sl. No	CPP sequence	Drug	Cancer	Targeting/use of CPPs	Polymer and lipid	Preparation method	Outcome	Ref
1	RGD peptide	10-hydroxycamptothecin	Breast cancers	Integrin αvβ3	PLGA and Egg lecithin	Modified single emulsification solvent evaporation method	Increased endocytosis and enhanced anticancer activity	[131]
2	RGD peptide	Curcumin (Cur)	Melanoma	Integrin αvβ3 and αvβ1	PLGA and Cholesterol	W/O/W double emulsification method	Cellular uptake of the CPP and hybrid system was improved along with tumor growth inhibition	[123]
3	mTAT (CHHHHHRKKRRQRRRRHHHHHC)	Docetaxel	Breast cancers	Her2/neu	PLGA and egg lecithin	Modified emulsification technique	Synergistic effect and superior therapeutic potency against the cancer cells	[127]
4	RGD peptide	Docetaxel	Glioblastoma multiforme	Integrin αvβ3	PLGA and soybean lecithin consisting of 90–95% phosphatidyl choline	Solvent extraction evaporation (W/O/W emulsion)	Enhanced tumor or penetration and improved targeting capacity and antiproliferative activity	[119]
5	TAT peptide	Docetaxel (DTX) and plasmid DNA (pDNA)	Castration-resistant prostate cancer (CRPC)	Cellular uptake of gene vectors	(PEI-PEG) [poly(ethylene imine) Polyethylene glycol] and Compritol1 888 ATO	Solvent evaporation method	Benefits include effective gene transport, *in vitro* anticancer impact, and *in vitro* transfection efficiency	[128]
6	iRGD peptide (CRGDK/RGPD/EC)	Doxorubicin (DOX) with sorafenib (SOR)	Hepatocellular carcinoma (HCC)	Integrin αvβ3 and αvβ1	PEG-DSPE and EPC	Not mentioned	The results demonstrated improved internalization, pro-apoptotic capacity, and synergistic cytotoxicity	[124]
7	iRGD peptide	Paclitaxel (PTX) and tetrandrine (TET)	Ovarian cancer	α $v\beta_{3/5}$ integrin receptors	PLGA and egg lecithin (EPC), cholesterol (CHOL)	Two synthesis steps	Enhanced cytotoxicity, apoptosis and cell cycle arrests. Promotes ROS production	[132]
8	iRGD peptide	Isoliquiritigenin (ISL)	Breast cancers	αvβ3 and αvβ5 integrin receptors	PLGA and lecithin	Modified single-step nanoprecipitation method	Higher cellular accumulation, cytotoxicity and cell apoptosis	[120]
9	RGD peptide	Paclitaxel (PTX)	Non-small cell lung cancer (NSCLC)	αvβ3 integrin receptors	PLGA and Soybean lecithin (SL)	Emulsification-sonication method	Enhanced antitumor activity o and synergistic effect	[121]
10	TAT peptide	Angiopep-2 peptide (Ang) and pDNA	Brain glioma	Rapid nuclear localization and endosomal or lysosomal escape for easy BBB penetration	PCL and DOTAP	Not mentioned	Enhanced penetration into BBB and improved targeting ability	[129]

Table III (continued)

Sl. No	CPP sequence	Drug	Cancer	Targeting/use of CPPs	Polymer and lipid	Preparation method	Outcome	Ref
11	cRGD (arginine–glycine–aspartic acid) and R9	Encoding mRNA from the suicide gene Bim	Colon cancer	Inhibiting Bcl-2 activity and triggering mitochondrial apoptosis	mPEG-PCL and DOTAP	Not mentioned	Inhibition of tumor progression with high safety	[130]
12	kNGR (Asn-Gly-Arg) peptide	Paclitaxel (PTX)	Human fibrosarcoma	CD13 receptors specific isoform	PLGA and lecithin	Modified nanoprecipitation process	The hybrid system with CPPs demonstrated substantial cytotoxicity, as evidenced by greater intracellular uptake and apoptosis, according to the results	[126]

Conclusion

PLNHs give excellent properties and greater advantages as a drug delivery vehicle for both hydrophilic and lipophilic drugs. The preparation method determines the type of PLHNs produced which can act either target the intestinal cells for the oral absorption or can also bio-mimic the systemic circulation in order to carry some specific drugs. CPPs when combined with the PLHNs not only aid their penetration, but also help in targeting several types of tumors, their microenvironment and the specific over expressed receptors. Therefore, we can conclude by saying that PLHNs conjugated with the CPPs act as a greater formulation overcoming several barriers.

Abbreviations DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid; SLN : Solid lipid nanoparticles; NLC: Nanostructured lipid carriers; RES: Reticuloendothelial system; PEG: Polyethylene glycol; PLGA: Poly(lactide-co-glycolide); FDA: Food and Drug Administration; PVP: Poly-vinyl pyrrolidone; PVA: Poly-vinyl alcohol; PG: Polyglycerol; PLD: Polyethylene glycol liposome doxorubicin; PLHNs: Polymer-lipid hybrid nanoparticles; PTDs: Protein-transduction domains; CPPs: Cell-penetrating peptides; MAPs: Membrane active peptides; HIV-1: Human immuno-deficiency virus-1; PCL: Polycaprolactone; PLA: Polylactic acid; PbAE: Poly(beta-amino-ester); siRNA: Small interfering RNAs; RBC: Red blood cells; ESE: Emulsification solvent evaporation; O/W: Oil in water; W/O: Water in oil; W/O/W: Water in oil in water; TAGs: Triacylglycerols; M cells: Microfold cells; GLAT: Gut-associated lymphoid tissue; DAGs: Diacylglycerols; MAG: Monoacylglycerols; MGATs: Monoacylglycerol acyltransferases; DGATs: Diacylglycerol acyltransferases; CoA: Coenzyme A; ACATs: Cholesterol acyltransferases; PCTVs: Pre-chylomicron transport vesicles; apoB-48: Apolipoprotein B-48; MTP: Microsomal triglyceride transfer protein; Cu: Curcumin; Tmx: Tamoxifen; LPHNP: Lipid-polymer hybrid nanoparticles; AO/PI: Acridine orange/propidium iodidestaining; P53: Tumor protein gene; MMP-2,9: Metalloproteinase gene; ERL: Erlotinib; BEV: Bevacizumab; NSCLC: Non-small cell lung cancer; HA: Hyaluronic acid; NaCl: Sodium chloride; DOX: Doxorubicin; EDL: Edelfosine; MTT: (3-(4, 5-Dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide); LPNs: Lipid-polymer hybrid nanoparticles; CBP: Carboplatin; PTX: Paclitaxel; MDR: Multidrug resistance; PSO: Psoralen; MCF-7: Michigan Cancer Foundation-7; HEPG2 cells: Hepatoblastoma cell line; ROS: Reactive oxygen species; NO: Nitric oxide; NOPD: Nitric oxide photodonor; NPs: Nanoparticles; PDT: Photodynamic treatment; pTHPP: 5,10,15,20-Tetrakis(4-hydroxy-phenyl)-21H,23H-porphine; Cas9/CRISPR-associated 9: Clustered regularly interspaced short palindromic repeats (CRISPR)–associated protein 9; TMZ: Temozolomide; RGD: Arginine-glycine-aspartic acid; cRGD: Cyclic arginine-glycine-aspartic acid; MGMT enzyme: O6-methylguanine-DNA methyltransferase (MGMT) enzyme; FUS: Focused ultrasound technique; MB: Microbubble; GBM: Glioblastoma; BBB: Blood-brain barrier; NIR: Near-infrared radiation; GSH- Pt (IV): Glutathione (GSH)-sensitive platinum (IV); Pt (IV): Platinum IV; SV40 protein: Simian Vacuolating Virus 40 protein; NMR: Nuclear magnetic resonance; CvME: Clathrin-mediated endocytosis (CME); iii) caveolae-mediated endocytosis; CME: Clathrin-mediated endocytosis; FPE: Fluid phase endocytosis; Src: Steroid receptor coactivator; PI3: Phosphatidylinositol (PI) 3-kinases; GTPase: Guanosine triphosphatease; LDL: Low-density lipoprotein; AP2: Adaptor protein-2; PI(4,5)P2: Phosphatidylinositol 4,5-biphosphate; EAPs: Endocytic accessory proteins; ATPase: Adenosine triphosphatase; Cav: Caveolae; CLICs: Clathrin-independent

carriers; GPI-APs: Glycosyl-phosphatidylinositol anchored proteins; GEECs: Enriched early endosomal compartments; SPPS: Solid-phase peptide synthesis; C-terminal: Carboxyl terminal; N-terminal: Amine terminal; T: Temporary protecting groups; Pn: Permanent protecting groups; DiR: 1,10-Dioctadecyl3,3,30,30-tetramethylindotricarb-ocyanine iodide; ISL: Isoliquiritigenin; Nrp1: Neuropilin 1; CDDP: Cispaltin; IC50: Half maximal inhibitory concentration; EPC: Egg lecithin; DSPE-PEG: 1, 2-Distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol); HCPT: 10-Hydroxycamptothecin; mPEG: Methoxy-polyethylene glycol; Chol: Cholesterol; SOR: Sorafenib; AHNP: Anti-HER2/neu peptide; mTAT: Modified TAT; DTX: Docetaxel; pDNA: Plasmid DNA; PEI-PEG: Poly (ethylene imine) polyethylene glycol; Ang: Angiopep2 peptide; Fe_3O_4 MNPs: Iron oxide magnetic nanoparticle; DOTAP: N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl; mRNA: Messenger ribonucleic acid; DMP: Dentin matrix acidic phosphoprotein; Bim: Bcl-2 interacting mediator of cell death; TET: Tetrandrine; P-gp: P-glycoprotein

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Declarations

Conflict of Interest The authors declare no competing interests.

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