REVIEW PAPER

The influence of cell penetrating peptides on efficiency of lipid nanoparticles containing chemotherapeutics

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ABSTRACT

Cell-penetrating peptides (CPPs) are a group of short peptides that easily pass through the cell membrane and are able to carry various types of cargoes, such as drugs, nucleic acids, and proteins, into cells. Therefore, CPPs are investigated with the aim of effective drug delivery to treat diseases such as cancer, diabetes and genetic disorders. CPPs have different applications in different fields.

CPPs have common functions and some structural features, such as a high content of positively charged amino acids, but their structural differences are in the high variety of elements in them. In this paper, the effect of cell penetrating peptides on the efficiency of lipid nanoparticles containing chemotherapeutics is reviewed. Various drug delivery systems such as liposomes, solid lipid nanoparticles and exosomes were considered. Both *in-vitro* and *in-vivo* delivery routes were discussed.

Keywords: *Cell-penetrating peptides, Drug delivery, Lipid nanoparticles, Cancer*

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Abbreviations

CPPs: Cell-penetrating peptides; ***PFV:*** Persistent fetal vasculature; ***siRNA:*** small interfering RNA; ***SLN:*** Solid Lipid Nanoparticles; ***TAT:*** transcribing protein; ***NLC:*** Nanostructured Lipid Carriers; ***HIV:*** Human immunodeficiency virus; ***MCAO:*** middle cerebral artery occlusion; ***PEG:*** polyethylene glycols; ***HA:*** hyaluronic acid; ***MCS:*** myocardium cells; ***BBB:*** blood-brain barrier.

INTRODUCTION

Cell-penetrating peptides (CPP) are a group of peptides that have the ability to pass through the cell membrane and transfer molecules such as DNA, protein, siRNA (small interfering RNA) and plasmid (1). The ability of CPPs to pass through the molecules has described this group of peptides as a promising candidate for drug delivery. CPPs are hydrophobic short lengths of amino acids and are usually considered sequences containing 5 to 30 amino acids. CPPs also known as protein transduction domains (2). Various factors such as temperature, cell type, size of the carrier and peptide concentration have different effects on the entry of CPPs into the cell (3). Most of CPPs are cationic and contain 5 positive charges. Direct penetration and endocytosis are the two main mechanisms for the entry of CPP peptides into cells, and these two mechanisms differ in the way of energy usage (4). In the direct permeation model, CPPs pass through lipid bilayer independently of energy and without the involvement of receptors (5). While in the process of endocytosis, CPPs enter the endosome or lysosome along with their therapeutic molecules with energy consumption. In addition, CPPs are classified into three main categories: spherical, natural and synthetic (6). Also, based on structural features, CPPs are divided into two main categories, including arginine-rich and amphipathic CPPs (3).

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In 1988, Frankel and Pabo discovered that the transcribing protein (TAT) from the HIV (Human immunodeficiency virus) can penetrate the cell membrane (7). This discovery was an introduction to the identification and characterization of different CPP peptides (8). TAT peptide has the ability to carry molecules with different molecular weight, including siRNA, antisense oligonucleotides and therapeutic agents (9). Recent studies show that CPPs can be considered as an appropriate candidate for cancer treatment (6). For example, Lim *et al* (2013) introduced a new CPP peptide named BR2, which showed the ability to interact with tumor cell membrane gangliosides and had a cytotoxic effect on HeLa, HCT116, and B16-F10 cancer cells (10). One of the important applications of CPPs is their use as carriers for the transfer of anticancer drugs (11). Although chemotherapy is considered as a treatment method for most cancers, drug resistance is one of the main problems of this treatment method. One of the important mechanisms of drug resistance is the reduction of membrane permeability and drug metabolism (12). It has been established that adding anticancer drugs to CPPs reduces this drug resistance (13). In recent years, drug delivery using CPPs has been considered for many diseases, including cancer (14). Evidence shows that CPPs easily transport cytotoxic drugs into tumor cells and induce apoptosis (15). It was also shown that the use of CPPs in combination with silver nanoparticles has stronger effects in killing MCF-7 cancer cells by increasing the penetration of silver nanoparticles in cancer cells compared to silver nanoparticles alone (16).

CPPs can be classified into two main categories based on the interaction between drug and CPP, the first category requires chemical bonding with the drug and the second group includes the formation of stable, non-covalent complexes with the drug. In recent years, many studies have investigated CPPs conjugated to small molecules and macromolecules in order to treat cancer (17).

Today, cancer is one of the main causes of death in the world. The common method of cancer treatment, chemotherapy, in most patients has drug resistance and lack of specific action on tumors. Therefore, it is very important to develop new methods. CPPs with features such as small size, easy synthesis, high activity and specificity, and biodiversity have become the target of many researchers' studies (18). In recent years, anti-cancer cationic peptides and cell-permeable peptides have been used to treat cancer. In this research, a number of studies conducted in this field, especially in the field of anti-cancer peptides and permeable peptides, are given. The results of various studies indicate that antimicrobial peptides with anticancer properties act against cancer cells and tumors using membrane and non-membrane mechanisms (19). Also, cell permeable peptides conjugated with therapeutic agents are considered as an effective mechanism in cancer treatment by overcoming drug resistance. In addition, anticancer and cell-permeable peptides can be proposed as a successful method for cancer treatment due to factors such as low toxicity, mode of action, and the ability to penetrate the cell membrane. However, further studies are needed to understand the mechanism of action of these peptides with therapeutic potential (20, 21).

In a study, several cell-penetrating peptides were designed and made from the sequence related to RGD. These analogs were designed to interact with integrins to increase entry into the cell through these receptors, and due to their amphipathic and basic properties, they can facilitate endosomal escape (5). In another study conducted on four cell lines (A549, NCIH322, NCIH460, NIH3T3), cell penetrating peptides derived from RGD and its analogs were tested. These peptides including PL, PD1, PD2, PE1, PE2) were mounted on liposomes and each of them was tested on the cell groups mentioned above. A group of liposomes without peptides was considered as a control group; the result of penetration in cell lines was that the control group did not show any penetration into any of the cell lines. The cell penetration of PE1, PE2 was more than that of PD1, and the penetration of PD2 was much higher than that of PD1 (22).

In this study, we investigated the effect of cell penetrating peptides on the efficiency of lipid nanoparticles containing chemotherapeutics. In the present paper, the effect of cell penetrating peptides on the efficiency of lipid and polymeric nanoparticles will be reviewed. All data has been summarized in Table 1.

Table 1. Some studies have been conducted on the effect of cell penetrating peptides on efficiency of lipid nanoparticles in cancer treatment.

Nanoparticles	CPP Type	Active Ingredient	Results	*in vitro / in vivo*	Ref.
Liposome	histidine amino acids	Paclitaxel	Better inhibition against tumor cell growth	*in vitro* and *in vivo*	(59)
Liposome	17-amino acid peptide (BR2)	2-aminoethyl dihydrogen phosphate (2-AEH$_2$P)	Better cytotoxicity in tumor lines	*in vivo*	(60)
Liposome	TAT and PCM	Coumarin-6	Improvement of the myocardial targeting	*in vitro* and *in vivo*	(68)
Liposomes	H16	alpha-galactosidase A (GLA)	Specific lysosomal delivery into the lysosome		(69)
Liposomes	photolabile-caged cell-penetrating peptide and asparagine-glycine-arginine peptide	siRNA	Selective targeted delivery of siRNA	*in vivo*	(71)
Liposome	RLYMRYYSPTTRRYG	Gossypol	Better cytotoxic effects on the MCF-7 cells	*in vitro*	(74)
Liposome	C-terminal domain of the cationic antimicrobial peptide CAP18	Actinomycin D	High cytotoxic activity against cancer cells	*in vivo*	(76)
Liposome	TAT and PEN	Paclitaxel and doxorubicin	Enhanced targeting efficiency and increased therapeutic efficacy	*in vitro* and *in vivo*	(79)
SLN	TAT	Paclitaxel and TOS-cisplatin	Synergistic effect in the suppression of cervical tumor cell growth and superior antitumor efficiency	*in vivo*	(87)
SLN	octaarginine	Paclitaxel	Enhanced cytotoxicity in A549 cells	*in vitro*	(107)

Characterization and classification of CPPs

CPPs are usually cationic or amphipathic peptides with 5-30 amino acids and soluble in water, which can be extracted from natural sources or designed (23). Compared to traditional methods such as microinjection and electroporation, CPPs have the ability to enter living cells in a non-invasive manner and without destroying the integrity of the cell membrane, and therefore are safe and very efficient (24). Recent studies have shown that CPPs pass a wide range of active pharmaceutical molecules, nanoparticles, liposomes, micelles, nucleic acids, proteins and peptides with high efficiency through the cell membranes of plants, bacteria and mammals (25).

In 1988, CPP derived from TAT protein of HIV-1 virus with amino acid sequence RKKRRQRRR was discovered and introduced for the first time (26). Related studies showed that TAT effectively crosses the cell membrane of cultured mammalian cells and accumulates in the nucleus and followed by other CPPs of natural origin such as VP22 derived from herpes simplex virus (HSV) and antennapedia or penetratin derived from *Drosophila melanogaster* or of synthetic origin such as transportan, all of which were able to pass through the cell membrane either individually or attached to cargo molecules were identified (26). CPPs, even in concentrations less than micromolar, are able to bind to glycosaminoglycans on the cell surface and enter the cell through the endocytic pathway without destroying the membrane structure (27). Of course, the exact mechanism of the entry of CPPs from the membrane into the cell is still under discussion, however, during the last decade, much evidence has been presented for their entry through endocytic mechanisms, especially endocytosis by receptors and micropinocytosis (28). In Figure 1. The mechanism of CPP penetrating to cell membranes is shown.

Direct energy-independent permeation occurs at low temperatures and using endocytosis inhibitors. As shown in Figure 1, positively charged CPPs interact with negatively charged cell membranes, causing membrane instability. Researchers have proposed three proposed mechanisms for this phenomenon: pore formation, carpet-like model, and reverse micelle model. In these mechanisms, small, hydrophobic CPP cargoes can permeate via energy-dependent pathways, while large, hydrophilic cargoes preferentially act as direct permeation (Figure 1) (29). So far, the exact mechanism by which CPPs cross cell membranes has not been elucidated. CPPs can enter cells in different ways under different conditions. Studies by researchers indicate that at low concentrations, arginine-rich CPPs are predominantly endocytosed, while at higher concentrations, they rapidly enter the cytoplasm (29).

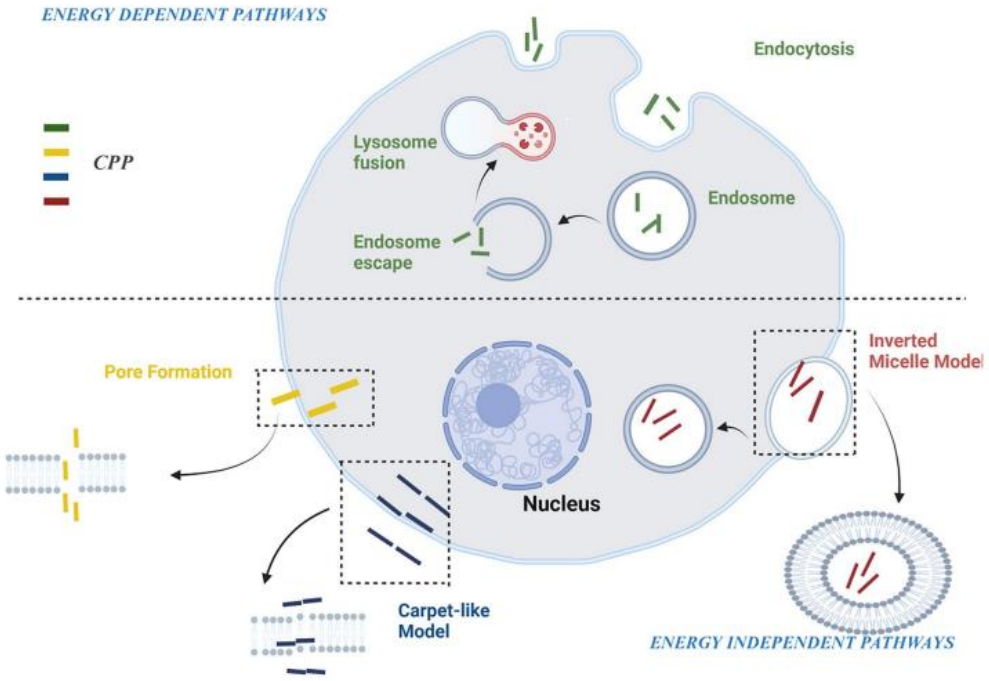

Fig. 1. Mechanism of CPP penetrating cell membranes (29).

Classification of CPPs

CPPs can be classified into different groups based on the physicochemical characteristics and based on the origin from the CPPs derived from proteins, natural peptides (28).

Classification of CPPs based on physicochemical characteristics

Based on the physicochemical characteristics of the overall net charge, CPPs can be classified into three hydrophobic, amphipathic and cationic groups (30). Anionic CPPs are not placed in a separate group and based on their characteristics they are placed in the group of amphipathic and hydrophobic CPPs. Cationic CPPs have a short amino acid sequence and are usually made of lysine, histidine and arginine amino acids and do not have any amphipathic helix in their three-dimensional structure (28). As mentioned above, the first discovered cationic CPP is the TAT peptide. Also, various studies on peptides based on the number of arginine amino acids from 3 to 12 have shown that the minimum sequence required for the cellular uptake of these peptides is octa-arginine (R8), so that the increase in the number of arginine increases the rate of cellular uptake. According to the studies, the presence of at least eight basic amino acids is necessary for efficient absorption of cationic CCPs (31). Although positively charged residues play an important role for the absorption of cationic CPPs, other residues are also important, for example, the W14F mutation in penetration with the amino acid sequence RQIKIWFQNRRMKWKK disrupts the absorption of this peptide into the cell (32).

Some cationic CPPs are Nuclear Localization Sequences (NLS) which are short peptides based on lysine-arginine or proline rich motifs. NLSs can enter the nucleus through the nuclear pore complex. NLSs are divided into two groups: monopartite signals (one cluster of four or more basic amino acids) and bipartite signals (two clusters of four or more basic amino acids) (33). For example, nucleoplasmin is a bipartite NLS with the amino acid sequence KRPAATKKAGQAKKKL, while the NLS derived from simian virus 40 (SV40) with the sequence PKKKRKV is a monopartite NLS (34). Among the NLSs, some peptides are not suitable for use as CPP, such as: NF-κB with the sequence TFIIE-beta VKQKLMP with the sequence SKKKKTKV and 6-Oct with the sequence GRKRKKRT because the number of charged amino acids in most NLSs is less than eight; However, they can be covalently attached to hydrophobic peptide sequences to produce hydrophobic CPPs with high cell absorption capability (35). Amphipathic CPPs are composed of both cationic and anionic peptide groups and four group of primary amphipathic peptides (primary amphipathic CPPS) and secondary amphipathic alpha-helix peptides (36).

β-Sheet amphipathic peptides are divided to β-Sheet amphipathic CPP and amphipathic peptides

amphipathic peptides rich in proline of amphipathic CPPs (37). Several types of primary amphipathic peptides are derived as chimeric peptides from the coupling of the covalent NLS to a hydrophobic domain, for example by fusing the NLS sequence of the SV40 virus (hydrophilic part PKKRKV) to the glycoprotein 1-HIV fusion sequence GALFGLGAAGSTMGA by the chimeric and amphipathic CPP WSQP linker (38).

Other primary amphipathic peptides are completely derived from natural proteins (39). Proteins are available from plants, animals and cells of microorganisms. Abundant economic proteins can be obtained from plant seeds. Natural proteins can be isolated by separation methods based on the physicochemical properties of the proteins. Worldwide, plant protein is of great importance because it contains essential amino acids to meet human physiological needs. However, many diverse plant proteins are used as pharmaceutical agents, which are produced using molecular tools of biotechnology. Examples such as pVEC, ARF, and BPrPp are derived from natural proteins. These peptides possess both hydrophilic and hydrophobic regions, enabling interaction with both aqueous environments and cell membranes (40).

In secondary amphipathic alpha-helix peptides, hydrophilic and hydrophobic amino acids are located on both sides of the helix, so that the hydrophilic side consists of anionic or polar cationic amino acids and interacts with the cell membrane; while the hydrophobic side is responsible for disruption and penetration across the membrane. Hydrophobic CPPs can be peptides based on natural amino acids and chemically modified peptides, which include pepducin peptides (41). Pepducins are produced by connecting palmitoyl or other fatty acids or steroid groups to the amine end of peptides. These types of peptides are able to pass through the plasma membrane and enter the cytosol part of a large number of transmembrane proteins such as GPRS (G) protein binding receptors and adrenergic receptors (24). Pepducins can be used to control various physiological processes, such as platelet-dependent homeostasis and thrombosis (42), tumor growth, invasion and angiogenesis, stabilization of the peptide in the alpha-helix conformation by covalent binding of adjacent two compounds with a hydrophobic chemical linker, and proteolytic stability (43). It should be noted that by being stapled, there is no guarantee that the peptide will become a CPP; however, most of the peptides have this ability. Not only the cationic stapled alpha-helix peptides, but also the uncharged and anionic types are converted into CPPs, and some impenetrable anionic peptides have the ability to enter the cell after replacing the charged amino acids. Negative effects are obtained by neutral and cationic types (44). Among these peptides, 8-AH and MTide (45), which are able to activate the tumor suppressor protein P53 by inhibiting Mdm2. Based on studies, prenylation of peptides by adding farnesyl (Cis) or geranylgeranyl (C20) gives them the ability to enter the cell in an ATP-independent and endocytic manner (46).

Classification of CPPs based on origin

CPPs can be classified into three groups based on their origin: CPPs derived from proteins, natural peptides, synthetically designed CPPs, and chimeric CPPs. CPPs derived from proteins, natural peptides with diverse motifs, are able to enter cells and are classified into several groups based on the protein from which they are derived (2). Cationic CPPs are endocytosed, for example, DPV3 and DPV3/10 from superoxide desmutase, DPV6 from platelet-derived growth factor, DPV7 from epidermal-like growth factors, DPV10 and DPV10/6 are derived from rhododend mucin (47). CPPs derived from proteins that bind to DNA, RNA contains highly cationic motifs so that they bind to nucleic acids after entering the nucleus. CPPs derived from DNA-binding proteins include human cJun and cFos, as well as GCN4 transcription factor in yeast (48). Homeoproteins are a special class of DNA-binding proteins that have a conserved homeodomain motif for DNA binding. In addition to human cJun and cFos, as well as GCN4 in yeast, this class includes penetratin and 1-PDX (49).

Lipid-based delivery systems

Liposomes

Liposomes can be used to deliver drugs, nutrients, and to prepare carriers such as lipid nanoparticles in mRNA vaccines and DNA vaccines (50). To prepare liposomes, the method of disrupting biological membranes using processes such as ultrasound is often used. If compatible with the lipid bilayer structure, fats such as egg phosphatidylethanolamine can also be used in the liposome structure. Sometimes surface ligands are also used for the construction and design of liposome to attach to unhealthy tissue (51).

Liposomes are one of the lipid-based drug delivery systems, the most common of which have a simple emulsion in which there is a simultaneous combination of oil and water. Despite the knowledge of basic emulsions for many years, with the introduction of synthetic surfactants such as polyethylene glycols (PEG), complex emulsions with

a high application scope in the field of drug delivery were developed (52). Incorporation of high-performance material processing methods for particle size distribution, such as sonication and homogenization, resulted in dispersed droplets with nanoscale sizes of 10-200 nm. As a result, micelles and nanoemulsions (particles with an oily core surrounded by a monolayer of surfactants) and subsequently species such as solid lipid nanoparticles (particles with a lipid core or solid wax and monolayer or surfactants) and nanostructures (lipid carriers) were created. The possibility of preparing particles with a core of a mixture of solid and liquid lipids with a size of less than 100 nm made it possible for the Brownian motion (intrinsic random vibration of the particles) to overcome the gravitational force that pushes oils and other heavy substances upwards. This results in a higher stability and longer shelf life of the prepared compositions and produces a characteristic transparency (particles smaller than visible light wavelengths), while suspensions of larger particles are opaque or milky (53).

In a study, liposomes treated with transferrin and TAT, which contained the drug doxorubicin, were tested on U87 cells, and the rate of crossing the blood-brain barrier was also evaluated in rats. This study showed the effective role of these peptides in crossing the blood-brain barrier and it also showed a significant inhibition of U87 cell line proliferation. This formulation was effective against glioma (54).

The use of CPPs in *in vivo* models is problematic due to their loss in non-target tissues and enzymatic degradation. This failure requires a strategy to camouflage these peptides and protect them before they reach the target tissue. For this purpose, heat-sensitive liposomes containing the NGR sequence were used. CPPs were hidden in the heat-sensitive liposomes and were made available in the target tissue by the application of heat and also the NGR sequence (55). Liposomes containing doxorubicin were used against HT-1080 fibrosarcoma cells and MCF-7 cell line which increased penetration into cell line compared to heat-sensitive liposomes containing NGR. This new formula was able to inhibit tumor growth in nude mice Xenograft HT-1080 tumors and did not damage other body tissues (56).

Cell-penetrating peptides were used to increase the penetration of lipopeptide vaccines. Lipopeptide vaccines were administered to Swiss mice by CPP-treated liposomes (57). Various CPPs were used, including Tat 47-57, an effective combination for stimulation of immune responses. The immunogenic potential of other types of CPPs was investigated after intranasal administration. Among them, Tat and KALA induced the highest antibody titer. Therefore, the use of cell-penetrating peptides in liposomes containing vaccines is a promising strategy for the development of liposome-based vaccines (57).

In one study, the TAT peptide was attached to the surface of the liposome by a UV-sensitive linker, and when exposed to UV light, breaking the link, the peptide TAT is exposed to cells and leads to increased cellular uptake and penetration (58). In another study, TH peptide was used. This peptide is sensitive to pH changes. Because of the neutral pH in blood and other healthy tissues, the ability of this peptide to penetrate into the cell is preserved, and consequently, due to the acidic pH of the target tissue, histidine amino acids are protonated and the surface charge of liposomes containing this peptide changes from negative to positive. Because of this, the amount of cellular absorption increases and when this formulation, along with paclitaxel, was used in an animal model, it inhibited tumor growth by 86.3% (59). In one study, BR2 peptide was used to modify the surface of the liposome, and the drug cantharidin was loaded inside the liposome to be used for the treatment of liver cancer. The results of cell uptake on MIHA cell line as a control group as well as HepG 2 cells, showed that liposomes modified with BR2 peptide containing cantharidin drug had better anti-cancer effects. *In vivo* studies show the accumulation of liposomes modified with BR2 at the tumor site and the reduction of tumor growth (60).

Liposomes are among the systems of interest to researchers for lipid delivery (61). In these drug delivery systems, phospholipids are placed in spherical fat layers like the cell membrane, and their hydrophilic heads face the aqueous environment and their fatty tails face each other. In the interior of these structures, water and water-soluble compounds are trapped, which have the potential to protect them from hostile digestive conditions and facilitate possible gastrointestinal absorption. Also, the bilayer hydrophobic fatty acid core hosts hydrophobic compounds and forms a small spherical package that can carry hydrophilic and hydrophobic compounds. The interactions between the liposome membrane and the cell membrane make this idea more attractive and increase cellular uptake through endosomal mechanisms (62). Liposomes have been a very attractive drug delivery technology since day one, but until the late 90s, there was no news of using this technology. From the late 1990s to today,

successful commercial products have been developed based on liposomal drug delivery technology. Examples of drugs are liposomal carriers of doxorubicin, amphotericin B, nystatin, and vincristine (63).

It was shown that the penetration of pH-sensitive liposomes containing doxorubicin modified by CPP into the cytosol was 1.6 times higher compared to pH-sensitive liposomes. But at pH below 7, no significant difference was observed between pH-sensitive liposomes modified with CPP and liposomes modified with CPP. In pharmacokinetic studies using Tc-99 and gamma counter, it was shown that pH-sensitive liposomes modified with CPP can circulate in the body as long as pegylated liposomes. In biodistribution studies, it was found that the highest accumulation rate of pH-sensitive liposomes modified with CPP was in the liver and to a lesser extent in the spleen, which was compared to pegylated liposomes. But in heart, brain, lung and kidney, they did not differ much (64).

In a study that was conducted in order to investigate the crossing of the blood-brain barrier and access to the central nervous system, three different types of CPP were used, and these peptides along with transferrin were placed on the surface of liposomes containing the active ingredient (65). This study, which was conducted on the End3 cell line, primary striatal cells and primary neurons, showed an increase in the transfer of modified liposomes. In an *in vitro* model in which the blood-brain barrier was simulated, it was observed that these modified liposomes have a greater ability to cross this barrier. Biodistribution studies showed that liposomes modified with KFGF peptide in intravenous injection had a greater ability to cross the blood-brain barrier and were more present in the brain of mice (66).

In another study, conventional liposome, liposome modified with TAT, liposome with Tat /PCM (Pericardial Mesothelium) and liposome with PCM were investigated in order to pass through the myocardium (67). The study conducted on myocardium cells (MCS) showed that liposomes modified by Tat and PCM have a great ability to drug transportation (68). In another study, a new CPP containing 16 histidine amino acids called H16 was used. Thus, when this CPP was placed on the desired liposomes, this peptide could increase the penetration of the liposome into the lysosome. This formulation can be used to target the intracellular lysosome and treat diseases related to lysosome dysfunction (69).

In another study, light-sensitive CPP was used in this way that ultraviolet light was previously used to release cell-penetrating peptides, but in this study, infrared radiation was used, because its power to penetrate tissues is greater and it does not cause the destruction of healthy tissues. In this study, NGR sequence was used along with CPP involved in the hydrophobic layer of liposome. The NGR sequence causes liposomes to actively bind to tumor cells. Then, with Non-ionizing radiation (NIR) radiation, the peptides in the liposome come into contact with the surface of the tumor cells, and in this way, the entry into the cell was increased (70). The proof of this claim is the entry of the active substance inside the liposome into the HT-1080 cell line and the silencing of the relevant gene and the reduction of tumor growth (71).

In an animal study, the researchers used liposomes modified with CPP containing doxorubicin with pH-sensitive pegylated groups on their surface in terms of pharmacokinetics, biocompatibility and pharmacodynamics. This comparison was made with conventional pegylated liposomes containing doxorubicin. It was found that pH-sensitive pegylated liposomes modified with cell-penetrating peptides had 1.9 times more selectivity and effectiveness than conventional pegylated liposomes. Also, histological studies showed that no necrosis and inflammation were observed in healthy tissues (72).

Artemisinin is a well-known drug in the treatment of malaria and its anti-tumor effects have been reported in in vitro studies. The disadvantage of artemisinin is its low solubility in water. In order to increase the solubility of artemisinin and its cytotoxic effect in the target tissue, this drug was loaded into nanocarriers. To increase the permeability of liposomes containing artemisinin, these liposomes were modified by HER6 CPPs. In this study, which was carried out both in vivo and ex vivo, it was shown that in different pH conditions, these liposomes have a slow release and in acidic pH conditions, liposomes modified with CPP have a higher rate of entering the cell than conventional liposomes, and as a result, they have more cytotoxic effects. It was also found that the residence time of CPP-modified liposomes in tumor tissue was longer than conventional liposomes, which increases efficiency and effectiveness (73).

In a study, two biological and physical factors were used together to increase the effectiveness of the drug doxorubicin. CPP was considered as the biological agent and the physical agent of the magnetic field was considered. The biological factor

increases the cell penetration and the physical factor increases the drug release by increasing the temperature. In the *in vitro* study, the cytotoxic property was effective on the MCF-7 cell line, and it has also been successful in the *in vivo* study, both in the stage of targeted drug delivery and antitumor effects on the MCF-7 xenograft mice (74).

A study was conducted in order to investigate the effectiveness of various CPPs in crossing the cell membrane and delivering the drug doxorubicin into the cell. In this study, liposomes containing transferrin and CPP were used to determine the efficiency of each of these CPPs. The passage barrier for these liposomes was considered the brain endothelial barrier, which was evaluated both *in vivo* and *in vitro*. In this evaluation, it was found that the release of doxorubicin using liposomes modified by transferrin and CPP was improved compared to single-ligand liposomes (75). Among the liposomes containing transferrin and CPP, the liposome containing penetratin was able to show the highest rate of passing through the endothelial barrier, approximately 15% of the *in vitro* model and only 4% of the *in vivo* model. Penetratin-containing liposomes as well as TAT-containing liposomes have excellent biocompatibility and did not show any hemolytic activity up to a concentration of 200 nanomolar. The three types of CPP studied include TAT, Penetratin, and mastoparan (24).

Also, liposomes modified by Persistent fetal vasculature (PFV) peptide were loaded with paclitaxel. It was shown that the presence of hydrophobic CPP can better interact with MCF-7 cell membrane. The better interaction of the surface of the nanoparticle with the surface of the cell membrane facilitates the entry of the nanoparticle into the cell. By increasing the entry of liposomes modified by PFV, more paclitaxel enters the cell, as a result, the effectiveness of the drug increases and its toxicity to other organs decreases. This study, which was carried out both *in vivo* and *in vitro*, tried to simulate the breast cancer model in mice and MCF-7 cell line was used (76).

Two CPP-type peptides named TAT and PEN were separately mounted on liposome. The kinetics of cellular uptake depend on the type of cell as well as the peptide. Intracellular accumulation of TAT-modified liposomes increases with time (77), but PEN-modified liposomes reach maximum intracellular accumulation in the first hour (78). TAT-modified liposomes containing doxorubicin could increase the absorption of the drug by 12 times by affecting the A431 cell line within two hours. Binding of CPP to the cell surface does not affect the rate of drug release inside the cell, and an additional approach is needed to release the drug inside the cell. In a study conducted on glioma tumor cells, a new peptide named CB5005 was loaded onto liposomes containing doxorubicin. The reason for choosing this peptide was due to its double effect, firstly, this peptide is a CPP. And secondly, it is a disruptor of NF-κB, since the level of NF-κB activity increases in cancer cells, this peptide inhibits the growth of cancer cells through disruption of NF-κB activity (79).

Solid lipid nanoparticles

Colloidal drug carrier have received a lot of attention from researchers in the fields of medicine and pharmacy in recent years. In addition to overcoming many problems due to the low solubility of hydrophobic drugs, the attention of many researchers was drawn to the transport of various drugs, especially lipophilic drugs, from these carriers (80). Lipid nanoparticles are very important as drug carriers. These nanoparticles have been successfully used to deliver lipophilic drugs and sometimes hydrophilic drugs (81). The use of two types of solid and liquid lipids during the preparation of these nanoparticles has created two different forms of these particles with the titles of solid lipid nanoparticles and nanostructured lipid carriers. Applications of Solid Lipid Nanoparticles (SLN) or Nanostructured Lipid Carriers (NLC) have been studied as carrier systems (82). SLNs contain pure solid fats, while NLCs are composed of a solid matrix in which nanoparticles are trapped in liquid fats. Many drugs with diverse applications have been placed in NLCs and SLNs successfully (83).

The initial studies in this field were conducted by three groups of researchers named Muller, Kasko and Westsen. SLN is a colloidal carrier characterized by the delivery of drugs with limited solubility. To prepare SLN, the oily phase of the w/o emulsion is replaced with a solid oil or a mixture of solid oils, i.e. a mixture of lipid matrix particles that are solid at room temperature and in the body (84). In the structure of SLN, 0.1 to 30% of solid fat is dispersed in the liquid phase and 0.5 to 5% of surfactant is formed (85). The average size of SLN particles ranges from 40 to 1000 nm. It has been reported in studies that the physicochemical properties and stability of drugs loaded in SLN depend on the properties of drugs and components used in it (86).

In a study to investigate the combined effect of paclitaxel and cisplatin in the treatment of ovarian cancer, these two drugs were loaded into solid lipid particles. In order to increase penetration into the

cell by the carrier system, the surface of SLN was modified by TAT peptide. Modified SLNs containing two combination drugs of cisplatin as prodrug and paclitaxel were evaluated in the treatment of mice infected with ovarian tumor by HeLa cells. The modified SLN system was able to increase intracellular accumulation by 80% within 4 hours after injection. This carrier system, together with two drugs, cisplatin and paclitaxel, was able to reduce the tumor growth rate by 72.2%. The combined treatment system is an effective method to treat ovarian cancer, it is also probably effective for the treatment of other cancers as well (87).

The size of SLN particles is a function of the proper selection of lipids, surfactants, compounds and their amounts, these parameters can affect the long-term stability during drug storage, load and release. Therefore, for each drug, a specific formulation for SLN is required (88). The use of SLN is limited due to problems such as drug loading limitation, irregular drug release, and drug excretion during storage in SLN preparation. In the late 1990s, NLCs were introduced due to their ability to load more drugs and their favorable stability compared to SLNs (89). Three methods have been proposed to produce NLCs:

In the first method, fats such as glycerides, which are composed of different fatty acids, are mixed together. The use of different lipids leads to a greater distance between the fatty acid chains of the crystallized glycerides and thus creates more space for the entry of guest molecules (90). A mixture of solid lipids and a small amount of liquid lipids increases the ability of the drug to enter the matrix, which is called the incomplete NLC model (91).

In the second method, to prepare different types of nanostructures, a large amount of oil is mixed with solid fat. Therefore, the solubility of oil molecules in solid fat increases and leads to phase separation and the formation of oily nanostructures inside the solid fat matrix (91). Research has shown that many drugs are more soluble in oils than in solid fats, which helps to dissolve them in the oil and prevent it from being excreted by the surrounding solid fats. This model is called multiple NLC and is very similar to w/o/w emulsions, as both oil in solid and fat in water are dispersed here (92). The addition of liquid lipids leads to the formation of colonies of small particles that contribute to the dynamics of the matrix.

In the third method, which is known as the amorphous type of SLN and in which solid particles are also used, by mixing certain fats (such as hydroxyoctacosanyl hydroxystearate and isopropyl myristate) crystals are created that are formed by cooling (93).

The solid fats that are used in the production of NLCs are: terpalmitin, glyceryl bihanate (Campritol), glyceryl distearate (Percyrol) and cetyl palmitate. In order to prepare the particle matrix, solid fats are mixed with liquid fats in the ratio of 70 to 30 or 1.99 to 0.1 (94). Due to the presence of oil in these matrices, the melting point of these compounds is lower than pure solid fats. Although the resulting mixture will be solid at room temperature and body temperature (95).

The solid content of NLC can increase up to 95%. Tri-palmetin is a triglyceride that is generally used as a solid fat in SLNs and NLCs to facilitate emulsification and the formation of solid nanoparticles. For its compatibility and stability, phospholipids that are naturally derived from it are used as the main emulsifiers in the preparation of injection emulsions (96). Gelucire is a multi-purpose fat additive consisting of mono, deuteryglycerides and mono, diesters of fatty acids of polyethylene glycol. Glucyric acid is a fat additive that is obtained by mixing polyethylene glycol fatty acid esters and glycerides (97). The existence of a special combination made with surfactants, surfactants and fatty phase for its production has been considered as an emulsifier, drug solubility enhancer and granule formation. Their entry into lipid nanostructures may be useful in increasing drug loading in hydrophilic components (98). Gelucire can be used as a surfactant, co-surfactant and lipid matrix in drug delivery systems. Vitamin E (tocopherol) has recently been suggested as a drug delivery agent (99).

Exosomes

Surface functionalization of exosomes (or exosome-like vesicles) with cell-penetrating peptides (CPPs) involves modifying the outer membrane of these vesicles to enhance their ability to deliver therapies into cells. Exosomes are small vesicles naturally released from cells, playing a crucial role in intercellular communication and transport of biomolecules. When exosomes are functionalized with CPPs, these peptides facilitate the uptake of the exosomes by target cells. CPPs are short sequences of amino acids that can penetrate cell membranes and promote the internalization of the attached exosomes, potentially delivering drugs, proteins, or genetic material more effectively. This process can improve the therapeutic efficacy of exosome-based treatments and is an area of significant interest in drug delivery and biomedical applications. By using CPPs,

researchers aim to enhance the targeting and delivery capabilities of exosomes, making them more effective carriers in various therapeutic contexts (100).

Extracellular vesicles (EVs), particularly exosomes, are gaining attention in biomedical applications due to their biocompatibility, suitable size, and low immunogenicity, which enhance their circulation time. Exosomes, formed from endosomal multivesicular bodies, range from 40 to 120 nm in size and can interact with their microenvironment after being released from cells. They serve as effective drug delivery systems capable of encapsulating proteins and genetic material, with advantages such as enhanced permeability and retention in vivo. Recent advancements include modifying exosome surfaces and creating hybrid nanovesicles to improve anticancer therapy and drug delivery (101).

The safe and effective delivery of drugs for ischemic stroke treatment is challenging. Exosomes are promising as an endogenous drug delivery system due to their low immunogenicity, stability, high delivery efficiency, and ability to cross the blood-brain barrier. However, their limited targeting capability restricts clinical use. This study demonstrates the conjugation of the c(RGDyK) peptide onto exosomes using a quick and bio-orthogonal chemistry approach. In a transient middle cerebral artery occlusion (MCAO) mouse model, the engineered c(RGDyK)-conjugated exosomes (cRGD-Exo) effectively targeted ischemic brain lesions after intravenous administration. Additionally, curcumin was loaded onto these exosomes, leading to significant suppression of inflammatory responses and cellular apoptosis in the affected areas. The findings indicate that cRGD-Exo can serve as targeted delivery vehicles for cerebral ischemia and highlight a strategy for the efficient production of functionalized exosomes (102).

Exosomes are cell-derived extracellular vesicles with significant potential for diagnostic and therapeutic applications. The surface characteristics of exosomes are crucial for their biological behavior; however, existing surface modification methods are often limited or complex. Wang et al. present a straightforward and rapid approach to modify exosome surfaces using polydopamine coating. This coating enables customizable functionalization through subsequent reactions, thereby facilitating new possibilities for the application of exosomes in various biomedical fields (103).

In vivo delivery

Cell-penetrating peptides (CPPs) are known for their ability to rapidly permeate cell membranes without causing cytotoxicity, with the HIV-1 TAT protein being a notable example that can enter cell nuclei. Modified TAT peptides, such as CG-TAT-GC, show improved penetration but lack specificity. As common lipid nanoparticles (NPs) often lack specific targeting capabilities, developing targeted NPs is crucial for effective molecular imaging and therapy. Targeting strategies include ligand-mediated active targeting and ultrasound or radiofrequency-mediated passive targeting. Specifically, the cluster of differentiation CD44, overexpressed in human hepatoma SMMC-7721 cells, serves as a potential target for liver cancer treatment since it binds hyaluronic acid (HA), a biocompatible and biodegradable polysaccharide. HA-enhanced delivery systems can improve drug targeting to tumor sites, minimize toxicity to normal cells, and boost therapeutic efficacy. Additionally, the presence of hyaluronidase in tumor microenvironments can expose CPPs in HA-coated NPs, enhancing their effectiveness as drug carriers for cancer chemotherapy (104).

Despite significant advancements in nanomedicine, challenges remain, including off-targeting, poor endosomal escape efficiency, and clearance by the liver and kidneys. Additionally, barriers like the blood-brain barrier (BBB), skin, and mucosal barriers hinder access to affected tissues and organs. Liposomes and lipid nanoparticles (LNPs) have emerged as effective and biocompatible delivery systems, with PEGylation enhancing their circulation times. While improvements in endosomal escape have facilitated pharmaceutical applications such as Doxil, Onpattro, and Comirnaty, the endosomal uptake pathway limits the full therapeutic potential of these formulations. To address these issues, researchers are exploring ways to bypass the endosomal pathway and enhance the targeting of lipid-based particles through functionalization with CPPs and cell-targeting peptides (CTPs). Various nanoparticle types, including liposomes, LNPs, polymeric nanoparticles, gold/metal nanoparticles, and silica quantum dots, are being modified to overcome physiological and cellular barriers for more effective drug delivery (105, 106).

Recent research has demonstrated the successful internalization of lipid nanoparticles (LNPs) through membrane fusion using coiled-coil-forming lipopeptides, CPE and CPK. In this study, LNPs encapsulating EGFP-mRNA were functionalized with either 1 mol % CPE3 or CPE4

and tested on HeLa cells pretreated with CPK. Consistent with previous findings regarding liposomes, the combination of CPK4 and CPE4 resulted in significantly enhanced cellular uptake compared to the CPK3/CPE3 pair. Additionally, the physicochemical analysis of LNP formulations revealed no major differences between those with CPE4 functionalization and those without, indicating that the lipopeptide modification effectively improved uptake without altering the LNP characteristics significantly. The schematic representation illustrates nonviral lipid nanoparticles (LNPs) that effectively deliver mRNA into cells when modified with fusogenic coiled-coil peptides. These peptides enhance the nanoparticles' ability to fuse with cell membranes, facilitating the release of mRNA into the cytoplasm for efficient intracellular delivery (Figure 2) (105).

Future outlook and limitations

Cell-penetrating peptides (CPPs) have shown significant promise in macromolecular drug delivery and cancer treatment, enhancing the efficacy of therapies like botulinum toxin and irinotecan while reducing gastrointestinal toxicity. Their ability to facilitate intracellular transport positions CPPs as valuable tools for delivering anticancer drugs, genes, and imaging agents. However, challenges such as lack of specificity for tumor cells, poor stability, and rapid elimination from the body hinder their effectiveness. Ongoing research focuses on modifying CPPs for targeted delivery, improving their pharmacological properties, and enhancing endosomal escape mechanisms. Despite advancements in tumor immunotherapy, the integration of CPPs with existing therapies presents a novel strategy to improve treatment outcomes, although overcoming their inherent limitations remains essential for future clinical applications (29).

CPPs offer several advantages in drug delivery, but they also face significant limitations, including low cell selectivity, penetrating efficacy, and in vivo stability. Many CPPs exhibit low selectivity due to their chemical properties, necessitating direct administration to target tumors to minimize adverse effects. Additionally, immunogenicity can restrict their clinical applications, emphasizing the need for thorough immunogenicity assessments for CPPs used as delivery carriers. Endosomal uptake and escape are critical for enhancing efficacy; strategies such as incorporating membrane-disrupting peptides and chemical modifications to stabilize CPPs against protease inactivation are being explored. Recent advancements in peptide chemistry aim to address these challenges and develop the next generation of CPPs (2).

CONCLUSION

The ability of CPPs to pass molecules has caused this group of peptides to be used as a promising candidate for drug delivery. Antimicrobial peptides with anticancer properties act against cancer cells and tumors through membrane and non-membrane mechanisms. Also, cell permeable peptides conjugated to therapeutic agents through overcoming drug resistance are considered as an effective mechanism in cancer treatment. In addition, anticancer and cell-permeable peptides can be suggested as a favorable candidate in cancer treatment due to factors such as low toxicity, mode of action, and the ability to penetrate the cell membrane. Evidence shows that CPPs can be used as a drug delivery method. The main problem of using these peptides is the lack of selectivity and specificity against cancer cells and tumors.

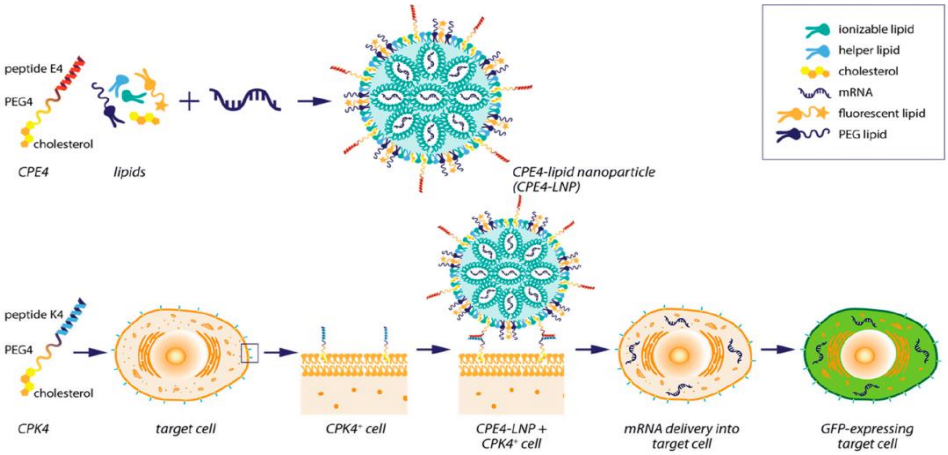

Fig. 2. Nonviral lipid nanoparticles (LNPs) that induce efficient mRNA delivery (105).

Therapeutic peptides are CPPs that are used in cancer treatment through covalent or non-covalent binding with small and macromolecules and entering cells. The use of lipid nanoparticles attached to CPPs has improved the performance of transferring substances into the membrane. Although CPPs have many advantages, limitations such as high cost and lack of specificity have been described for them. However, scientists have proposed various methods to overcome these problems. Therefore, the use of CPPs peptides along with lipid nanoparticles is suggested as a promising method in cancer treatment. However, more extensive studies are needed in order to use these therapeutic peptides in the clinical phase and to understand their mechanism.

AUTHOR CONTRIBUTION

Behrad Khoshbin: Writing original draft; **Zahra Khalili Azimi**: Writing original draft; **Mahmoud Reza Jaafari**: Reviewing and editing; **Bizhan Malaekeh-Nikouei**: Reviewing and editing.

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CONFLICTS OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Ghosh A, Sharma M, Zhao Y. Cell-penetrating protein-recognizing polymeric nanoparticles through dynamic covalent chemistry and double imprinting. Nat Commun. 2024;15(1):3731.
2. Bottens RA, Yamada T. Cell-penetrating peptides (CPPs) as therapeutic and diagnostic agents for cancer. Cancers. 2022;14(22):5546.
3. Milletti F. Cell-penetrating peptides: classes, origin, and current landscape. Drug Discov. 012;17(15-16):850-860.
4. Lundberg P, Langel Ü. A brief introduction to cell-penetrating peptides. J Mol Recognit. 2003;16(5):227-233.
5. Copolovici DM, Langel K, Eriste E, Langel U. Cell-penetrating peptides: design, synthesis, and applications. ACS nano. 2014;8(3):1972-1994.
6. Langel Ü. Cell-penetrating peptides: Springer; 2011.
7. Frankel AD, Pabo CO. Cellular uptake of the tat protein from human immunodeficiency virus. Cell. 1988;55(6):1189-1193.
8. Gautam A, Sharma M, Vir P, Chaudhary K, Kapoor P, Kumar R, et al. Identification and characterization of novel protein-derived arginine-rich cell-penetrating peptides. Eur J Pharm Biopharm. 2015;89:93-106.
9. Torchilin VP. Tat peptide-mediated intracellular delivery of pharmaceutical nanocarriers. Adv Drug Deliv. 2008;60(4-5):548-558.
10. Lim KJ, Sung BH, Shin JR, Lee YW, Kim DJ, Yang KS, Kim SC. A cancer specific cell-penetrating peptide, BR2, for the efficient delivery of an scFv into cancer cells. PloS one. 2013;8(6):e66084.
11. Borrelli A, Tornesello AL, Tornesello ML, Buonaguro FM. Cell penetrating peptides as molecular carriers for anti-cancer agents. Molecules. 2018;23(2):295.
12. Duan Z, Chen C, Qin J, Liu Q, Wang Q, Xu X, Wang J. Cell-penetrating peptide conjugates to enhance the antitumor effect of paclitaxel on drug-resistant lung cancer. Drug Deliv. 2017;24(1):752-764.
13. Klimpel A, Luetzenburg T, Neundorf I. Recent advances of anti-cancer therapies including the use of cell-penetrating peptides. Curr Opin Pharmacol. 2019; 47:8-13.
14. Pescina S, Ostacolo C, Gomez-Monterrey I, Sala M, Bertamino A, Sonvico F, et al. Cell penetrating peptides in ocular drug delivery: State of the art. J Control Release. 2018;284:84-102.
15. Javadzadeh Y, Bahari LA. Chapter 8-Therapeutic nanostructures for dermal and transdermal drug delivery. Nano- and Microscale Drug Delivery Systems. Elsevier. 2017; 131-146.
16. Kovács D, Igaz N, Gopisetty MK, Kiricsi M. Cancer therapy by silver nanoparticles: fiction or reality? Int J Mol Sci. 2022; 23(2):839.
17. Gessner I, Neundorf I. Nanoparticles modified with cell-penetrating peptides: Conjugation mechanisms, physicochemical properties, and application in cancer diagnosis and therapy. Int J Mol Sci. 2020;21(7):2536.
18. Kroemer G, Chan TA, Eggermont AM, Galluzzi L. Immunosurveillance in clinical cancer management. CA Cancer J Clin. 2024;74(2):187-202.
19. Chen P, Ye T, Li C, Praveen P, Hu Z, Li W, Shang C. Embracing the era of antimicrobial peptides with marine organisms. Nat Prod Rep. 2024; 41(3):331-346.
20. Zhang L-j, Gallo RL. Antimicrobial peptides. Curr Biol. 2016; 26(1):R14-R19.
21. Lei J, Sun L, Huang S, Zhu C, Li P, He J, et al. The antimicrobial peptides and their potential clinical applications. Am J Transl Res. 2019;11(7):3919.
22. Patel SG, Sayers EJ, He L, Narayan R, Williams TL, Mills EM, et al. Cell-penetrating peptide sequence and modification dependent uptake and subcellular distribution of green florescent protein in different cell lines. Sci Rep. 2019;9(1):6298.
23. Heitz F, Morris MC, Divita G. Twenty years of cell-penetrating peptides: from molecular mechanisms to therapeutics. Br J Pharmacol. 2009;157(2):195-206.
24. Langel Ü. CPP, cell-penetrating peptides: Springer; 2019.
25. Nakase I, Akita H, Kogure K, Graslund A, Langel U, Harashima H, Futaki S. Efficient intracellular delivery of nucleic acid pharmaceuticals using cell-penetrating peptides. Acc Chem Res. 2012;45(7):1132-1139.

26. Rizzuti M, Nizzardo M, Zanetta C, Ramirez A, Corti S. Therapeutic applications of the cell-penetrating HIV-1 Tat peptide. Drug Discov Today. 2015;20(1):76-85.
27. Pae J, Liivamägi L, Lubenets D, Arukuusk P, Langel Ü, Pooga M. Glycosaminoglycans are required for translocation of amphipathic cell-penetrating peptides across membranes. Biochim Biophys Acta. 2016;1858(8):1860-1867.
28. Desale K, Kuche K, Jain S. Cell-penetrating peptides (CPPs): An overview of applications for improving the potential of nanotherapeutics. Biomater Sci. 2021;9(4):1153-1188.
29. Yang D, Liu B, Sha H. Advances and prospects of cell-penetrating peptides in tumor immunotherapy. Sci Rep. 2025;15(1):3392.
30. Madani F, Lindberg S, Langel Ü, Futaki S, Gräslund A. Mechanisms of cellular uptake of cell-penetrating peptides. Biophys J. 2011;2011.
31. Holm T, Johansson H, Lundberg P, Pooga M, Lindgren M, Langel Ü. Studying the uptake of cell-penetrating peptides. Nat Protoc. 2006;1(2):1001-1005.
32. Delgado EM. Chemical Approaches to Improve the Manufacturability of Disulfide-Rich Peptide Toxins: Engineering a Stable and Selective Peptide Blocker of the K Ca 1.1 Channel: University of Hawai'i at Manoa; 2022.
33. Li J, Li S, Du M, Song Z, Han H. Nuclear Delivery of Exogenous Gene in Mature Plants Using Nuclear Location Signal and Cell-Penetrating Peptide Nanocomplex. ACS Appl Nano Mater. 2022;6(1):160-170.
34. Ohshima K, Takeda S, Hirose M, Akiyama Y, Iguchi K, Hoshino M, et al. Structure-function relationship of the nuclear localization signal sequence of parathyroid hormone-related protein. Biomed Res. 2012;33(3):191-199.
35. Chen H-CG, Chiou S-T, Zheng J-Y, Yang S-H, Lai S-S, Kuo T-Y. The nuclear localization signal sequence of porcine circovirus type 2 ORF2 enhances intracellular delivery of plasmid DNA. Arch Virol. 2011;156:803-815.
36. Deshayes S, Morris MC, Divita G, Heitz F. Interactions of amphipathic CPPs with model membranes. BBA Biomembranes. 2006;1758(3):328-335.
37. Oba M. Chapter 5- Amphipathic peptide. Cell-penetrating peptides; Design, development and applications. Wiely. 2023:57-67.
38. Dariushnejad H, Karimitabar F, Hamidi M, Ahmadi NA. Cell-penetrating peptides (CPPs): A tool in modern biotechnology. J Paramed Sci. 2014; 5(3): 108-113.
39. Johansson HJ, El-Andaloussi S, Holm T, Mäe M, Jänes J, Maimets T, Langel Ü. Characterization of a novel cytotoxic cell-penetrating peptide derived from p14ARF protein. Mol Ther. 2008;16(1):115-123.
40. Nehete JY, Bhambar RS, Narkhede MR, Gawali SR. Natural proteins: Sources, isolation, characterization and applications. Phcog Rev. 2013;7(14):107.
41. Song J, Qian Z, Sahni A, Chen K, Pei D. Cyclic cell-penetrating peptides with single hydrophobic groups. Chembiochem. 2019;20(16):2085-2088.
42. Freedman JE. Molecular regulation of platelet-dependent thrombosis. Circulation. 2005;112(17):2725-2734.
43. Lai Z, Yuan X, Chen H, Zhu Y, Dong N, Shan A. Strategies employed in the design of antimicrobial peptides with enhanced proteolytic stability. Biotechnol Adv. 2022;59:107962.
44. Mello LR, Hamley IW, Castelletto V, Garcia BB, Lourenço TC, Vassiliades SV, et al. Self-assembly and intracellular delivery of DNA by a truncated fragment derived from the Trojan peptide Penetratin. Soft Matter. 2020;16(20):4746-4755.
45. Varnamkhasti BS, Jafari S, Taghavi F, Alaei L, Izadi Z, Lotfabadi A, et al. Cell-penetrating peptides: As a promising theranostics strategy to circumvent the blood-brain barrier for CNS diseases. Curr Drug Deliv. 2020;17(5):375-386.
46. Qian Y, Wang X, Liu Y, Li Y, Colvin RA, Tong L, et al. Extracellular ATP is internalized by macropinocytosis and induces intracellular ATP increase and drug resistance in cancer cells. Cancer Lett. 2014;351(2):242-251.
47. Tiwari MG. From inception of herbal medicine to an ideal perception of therapeutic agent: Rhododendron as a therapeutic agent–A review. IJMSCR. 2020;3(6):147-162.
48. Greene LA, Zhou Q, Siegelin MD, Angelastro JM. Targeting transcription factors ATF5, CEBPB and CEBPD with cell-penetrating peptides to treat brain and other cancers. Cells. 2023;12(4):581.
49. Zhang X, Liu E, Song Y, Yu P, Redkar S, Yu G-L. Dependence of EGFR-mutant NSCLC on MET as demonstrated by vebreltinib, a novel and selective brain-penetrating MET kinase inhibitor. Cancer Res. 2024;84(6_Supplement):6500.
50. Filipczak N, Pan J, Yalamarty SSK, Torchilin VP. Recent advancements in liposome technology. Adv. Drug Deliv Rev. 2020;156:4-22.
51. Nsairat H, Khater D, Sayed U, Odeh F, Al Bawab A, Alshaer W. Liposomes: Structure, composition, types, and clinical applications. Heliyon. 2022;8(5): e09394.
52. Bozzuto G, Molinari A. Liposomes as nanomedical devices. Int J Nanomedicine. 2015:975-999.
53. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. Adv. Drug Deliv Rev. 2013;65(1):36-48.
54. Mojarad-Jabali S, Mahdinloo S, Farshbaf M, Sarfraz M, Fatahi Y, Atyabi F, Valizadeh H. Transferrin receptor-mediated liposomal drug delivery: Recent trends in targeted therapy of cancer. Expert Opin Drug Deliv. 2022;19(6):685-705.
55. Sauer WH, Steiger NA, Tzou WS, Schuller JL, Zheng L, Nguyen DT. Facilitated myocardial ablation using heat-sensitive liposomes containing doxorubicin: A proof-of-concept preclinical study. JACC Clin Electrophysiol. 2023; 9(8 Pt 1):1404-1408.
56. Xu J, Khan AR, Fu M, Wang R, Ji J, Zhai G. Cell-penetrating peptide: a means of breaking through the physiological barriers of different tissues and organs. J Control Release. 2019;309:106-124.

57. Hamley IW. Lipopeptides for vaccine development. Bioconjug Chem. 2021;32(8):1472-1490.
58. de Jong H, Bonger KM, Löwik DW. Activatable cell-penetrating peptides: 15 years of research. RSC Chem Biol. 2020;1(4):192-203.
59. Zhang Q, Tang J, Fu L, Ran R, Liu Y, Yuan M, He Q. A pH-responsive α-helical cell penetrating peptide-mediated liposomal delivery system. Biomater. 2013;34(32):7980-7993.
60. de Sousa Cabral LG, Hesse H, Freire KA, de Oliveira CS, Pedron CN, Alves MG, et al. The BR2 peptide associated with 2-aminoethyl dihydrogen phosphate is a formulation with antiproliferative potential for a triple-negative breast cancer model. Biomed Pharmacother. 2022;153:113398.
61. Saraf S, Jain A, Tiwari A, Verma A, Panda PK, Jain SK. Advances in liposomal drug delivery to cancer: An overview. J Drug Deliv Sci Technol. 2020;56:101549.
62. Fan Y, Marioli M, Zhang K. Analytical characterization of liposomes and other lipid nanoparticles for drug delivery. J Pharm Biomed Anal. 2021;192:113642.
63. Nekkanti V, Kalepu S. Recent advances in liposomal drug delivery: a review. Pharm Nanotechnol. 2015;3(1):35-55.
64. Reveret L, Leclerc M, Morin F, Émond V, Calon F. Pharmacokinetics, biodistribution and toxicology of novel cell-penetrating peptides. Sci Rep.2023;13(1):11081.
65. Dymek M, Sikora E. Liposomes as biocompatible and smart delivery systems–the current state. Acta Odontol Scand. 2022;309:102757.
66. Yengopal V, Mickenautsch S. Caries preventive effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP): a meta-analysis. Acta Odontologica Scandinavica. 2009;67(6):321-332.
67. Liu X, Zhang L, Jiang W, Yang Z, Gan Z, Yu C, et al. In vitro and in vivo evaluation of liposomes modified with polypeptides and red cell membrane as a novel drug delivery system for myocardium targeting. Drug Deliv. 2020;27(1):599-606.
68. Wang X, Huang H, Zhang L, Bai Y, Chen H. PCM and TAT co-modified liposome with improved myocardium delivery: in vitro and in vivo evaluations. Drug Deliv. 2017;24(1):339-345.
69. Hayashi T, Shinagawa M, Kawano T, Iwasaki T. Drug delivery using polyhistidine peptide-modified liposomes that target endogenous lysosome. Biochem Biophys Res Commun. 2018;501(3):648-653.
70. Mazhar A, El-Hansi N, Shafaa MW, Shalaby M. Radiation sterilization of liposomes: A literature review. Radiat Phys Chem. 2024:111592.
71. Yang Y, Yang Y, Xie X, Wang Z, Gong W, Zhang H, et al. Dual-modified liposomes with a two-photon-sensitive cell penetrating peptide and NGR ligand for siRNA targeting delivery. Biomater. 2015;48:84-96.
72. Cho HJ, Jung JI, Lim DY, Kwon GT, Her S, Park JH, Park JHY. Bone marrow-derived, alternatively activated macrophages enhance solid tumor growth and lung metastasis of mammary carcinoma cells in a Balb/C mouse orthotopic model. Breast Cancer Res. 2012;14:1-12.
73. Ding Y, Cui W, Sun D, Wang G-L, Hei Y, Meng S, et al. In vivo study of doxorubicin-loaded cell-penetrating peptide-modified pH-sensitive liposomes: biocompatibility, bio-distribution, and pharmacodynamics in BALB/c nude mice bearing human breast tumors. Drug Des Devel Ther. 2017:3105-3117.
74. Maity SK, Stahl P, Hensel A, Knauer S, Hirschhäuser C, Schmuck C. Cancer-cell-specific drug delivery by a tumor-homing CPP-gossypol conjugate employing a tracelessly cleavable linker. Chem Eur J. 2020;26(14):3010-3015.
75. dos Santos Rodrigues B, Kanekiyo T, Singh J. In vitro and in vivo characterization of CPP and transferrin modified liposomes encapsulating pDNA. Nanomedicine: NBM. 2020;28:102225.
76. Gronewold A, Horn M, Ranđelović I, Tóvári J, Muñoz Vázquez S, Schomäcker K, Neundorf I. Characterization of a cell-penetrating peptide with potential anticancer activity. Chem Med Chem. 2017;12(1):42-49.
77. Shi J, Guo S, Wu Y, Chen G, Lai J, Xu X. Behaviour of cell penetrating peptide TAT-modified liposomes loaded with salvianolic acid B on the migration, proliferation, and survival of human skin fibroblasts. J. Liposome Res. 2020;30(1):93-106.
78. Kawak P, Sawaftah NMA, Pitt WG, Husseini GA. Transferrin-targeted liposomes in glioblastoma therapy: a review. Int J Mol Sci. 2023;24(17):13262.
79. Yuan M, Qiu Y, Zhang L, Gao H, He Q. Targeted delivery of transferrin and TAT co-modified liposomes encapsulating both paclitaxel and doxorubicin for melanoma. Drug Deliv. 2016;23(4):1171-1183.
80. Paliwal R, Paliwal SR, Kenwat R, Kurmi BD, Sahu MK. Solid lipid nanoparticles: A review on recent perspectives and patents. Opin Ther Pat. 2020;30(3):179-194.
81. Mirchandani Y, Patravale VB, Brijesh S. Solid lipid nanoparticles for hydrophilic drugs. J Control Release. 2021;335:457-464.
82. Mendoza-Munoz N, Urbán-Morlán Z, Leyva-Gómez G, de la Luz Zambrano-Zaragoza M, Quintanar-Guerrero D. Solid lipid nanoparticles: an approach to improve oral drug delivery. J Pharm Pharm Sci. 2021;24:509-532.
83. Scioli Montoto S, Muraca G, Ruiz ME. Solid lipid nanoparticles for drug delivery: pharmacological and biopharmaceutical aspects. Front Mol Biosci. 2020;7:319.
84. Salah E, Abouelfetouh MM, Pan Y, Chen D, Xie S. Solid lipid nanoparticles for enhanced oral absorption: A review. Colloids Surf B Biointerfaces. 2020;196:111305.
85. Khairnar SV, Pagare P, Thakre A, Nambiar AR, Junnuthula V, Abraham MC, et al. Review on the scale-up methods for the preparation of solid lipid nanoparticles. Pharmaceutics. 2022;14(9):1886.

86. Satapathy MK, Yen T-L, Jan J-S, Tang R-D, Wang J-Y, Taliyan R, Yang C-H. Solid lipid nanoparticles (SLNs): an advanced drug delivery system targeting brain through BBB. Pharmaceutics. 2021;13(8):1183.
87. Liu B, Han L, Liu J, Han S, Chen Z, Jiang L. Co-delivery of paclitaxel and TOS-cisplatin via TAT-targeted solid lipid nanoparticles with synergistic antitumor activity against cervical cancer. Int J Nanomedicine. 2017:955-968.
88. Costa CP, Moreira JN, Lobo JMS, Silva AC. Intranasal delivery of nanostructured lipid carriers, solid lipid nanoparticles and nanoemulsions: A current overview of in vivo studies. Acta Pharm Sin B. 2021;11(4):925-940.
89. Ganesan P, Ramalingam P, Karthivashan G, Ko YT, Choi D-K. Recent developments in solid lipid nanoparticle and surface-modified solid lipid nanoparticle delivery systems for oral delivery of phyto-bioactive compounds in various chronic diseases. Int J Nanomedicine. 2018:1569-1583.
90. Garcês A, Amaral M, Lobo JS, Silva AC. Formulations based on solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for cutaneous use: A review. Eur J Pharm Sci. 2018;112:159-167.
91. Weber S, Zimmer A, Pardeike J. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for pulmonary application: a review of the state of the art. Eur J Pharm Sci. 2014;86(1):7-22.
92. Pimentel-Moral S, Teixeira M, Fernandes A, Borrás-Linares I, Arráez-Román D, Martínez-Férez A, et al. Polyphenols-enriched Hibiscus sabdariffa extract-loaded nanostructured lipid carriers (NLC): Optimization by multi-response surface methodology. J Drug Deliv Sci Technol. 2019;49:660-667.
93. Assefi M, Ataeinaeini M, Nazari A, Gholipour A, Vertiz-Osores JJ, Calla-Vásquez KM, et al. A state-of-the-art review on solid lipid nanoparticles as a nanovaccines delivery system. J Drug Deliv Sci Technol. 2023:104623.
94. Subroto E, Andoyo R, Indiarto R. Solid lipid nanoparticles: Review of the current research on encapsulation and delivery systems for active and antioxidant compounds. Antioxid. 2023;12(3):633.
95. Mohammed HA, Khan RA, Singh V, Yusuf M, Akhtar N, Sulaiman GM, et al. Solid lipid nanoparticles for targeted natural and synthetic drugs delivery in high-incidence cancers, and other diseases: Roles of preparation methods, lipid composition, transitional stability, and release profiles in nanocarriers' development. Nanotechnol Rev. 2023;12(1):20220517.
96. López KL, Ravasio A, González-Aramundiz JV, Zacconi FC. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) prepared by microwave and ultrasound-assisted synthesis: Promising green strategies for the nanoworld. Pharmaceutics. 2023;15(5):1333.
97. Harish V, Mohd S, Tewari D, Pandey NK, Vishwas S, Babu MR, et al. Unravelling the role of solid lipid nanoparticles in drug delivery: Journey from laboratory to clinical trial. J Drug Deliv Sci Technol. 2023:104616.
98. Salminen H, Kasapoğlu KN, Özçelik B, & Weiss J. Stabilization of solid lipid nanoparticles with glycyrrhizin. Eur Food Res Technol. 2023;249(3):787-798.
99. Chauhan I, Singh L. A comprehensive literature review of lipids used in the formulation of lipid nanoparticles. Curr Nanosci. 2023;8(2):126-152.
100. Salunkhe S, Basak M, Chitkara D, Mittal A. Surface functionalization of exosomes for target-specific delivery and in vivo imaging & tracking: Strategies and significance. J Control Release. 2020; 326:599-614.
101. Mondal J, Pillarisetti S, Junnuthula V, Saha M, Hwang SR, Park I-k, Lee Y-k. Hybrid exosomes, exosome-like nanovesicles and engineered exosomes for therapeutic applications. J Control Release. 2023; 353:1127-1149.
102. Tian T, Zhang H-X, He C-P, Fan S, Zhu Y-L, Qi C, et al. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. Biomater. 2018;150:137-149.
103. Wang C, Kimura K, Li J, Richardson JJ, Naito M, Miyata K, et al. Polydopamine-Mediated Surface Functionalization of Exosomes. Chem Nano Mat. 2021;7(6):592-595.
104. Zhao H, Wu M, Zhu L, Tian Y, Wu M, Li Y, et al. Cell-penetrating peptide-modified targeted drug-loaded phase-transformation lipid nanoparticles combined with low-intensity focused ultrasound for precision theranostics against hepatocellular carcinoma. Theranostics. 2018;8(7):1892.
105. Aschmann D, Knol RA, Kros A. Lipid-based nanoparticle functionalization with coiled-coil peptides for in vitro and in vivo drug delivery. Acc Chem Res. 2024;57(8):1098-1110.
106. Qin J, Xue L, Gong N, Zhang H, Shepherd SJ, Haley RM, et al. RGD peptide-based lipids for targeted mRNA delivery and gene editing applications. RSC Advances. 2022;12(39):25397-25404.
107. Zhang Y-L, Zhang Z-H, Jiang T-Y, Lv H-X, Zhou J-P. Cell uptake of paclitaxel solid lipid nanoparticles modified by cell-penetrating peptides in A549 cells. Pharmazie. 2013;68(1):47-53.