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Review

Designing of 'intelligent' liposomes for efficient delivery of drugs

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Abstract

The liposome- vesicles made by a double phospholipidic layers which may encapsulate aqueous solutions- have been introduced as drug delivery vehicles due to their structural flexibility in size, composition and bilayer fluidity as well as their ability to incorporate a large variety of both hydrophilic and hydrophobic compounds. With time the liposome formulations have been perfected so as to serve certain purposes and this lead to the design of "intelligent" liposomes which can stand specifically induced modifications of the bilayers or can be surfaced with different ligands that guide them to the specific target sites. We present here a brief overview of the current strategies in the design of liposomes as drug delivery carriers and the medical applications of liposomes in humans.

Keywords: liposomes - drug delivery - gene delivery - targeted delivery - human therapy

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Introduction

Beginning with 1965, the liposome-vesicles formed by the self-assembly bilayers of phospholipid molecules in an aqueous environment- have constituted an experimental tool in a large number of laboratories throughout the world. The liposomes may be constructed so as to accommodate a large variety of both hydrophilic and hydrophobic agents and to protect the encapsulated agents from cellular metabolic processes. In addition, the lipid composition of the bilayers can be modified in order to obtain other desirable properties. Therefore, the attention was focused on using liposomes as delivery systems. For a successful therapy with liposome-incorporated drugs, they should be able to reach the accurate target site at the right time, correct concentration and at the proper rate. In pharmaceutical industry beside the synthesis and isolation of new potent compounds, a new approach is to develop efficient delivery vehicles for available drugs. The specific aim is either to modify the drug biodistribution within the body, or to improve the therapeutic efficiency. Starting with their discovery 40 years ago, the liposomes seemed to be attractive candidates as a drug delivery system. Liposomes resemble cell membrane in structure and composition. They are made of natural, biodegradable, nontoxic and nonimmunogenic lipid molecules and can encapsulate a large variety of both hydrophilic and hydrophobic compounds. Their utilization is based on their properties (dimensions, lamellarity, loading efficiency, surface properties, stability, which can be manipulated during the preparation process) as well as their biological interactions with the cells. The encapsulation or the association of drugs with liposomes alters drug pharmacokinetics, and this may be exploited to achieve targeted therapies. Coupling of different ligands on the liposome surface may be used to obtain a specific liposomal drug targeting. Thus, according to the purpose, the appropriate composition and preparation method should be employed in order to obtain a specific liposomal system.

Liposomes as drug-delivery vehicles

Subsequent to the first description of liposomes in the middle of 1960s (by AD Bangham) the use of liposomes as vehicles for selective delivery of drugs to specific tissues has received considerable attentions. By virtue of their biodegradable and nontoxic nature, liposomes can be safely administered without severe side-effects. The first results were rather disappointing because the first generation of liposomes, referred to as conventional liposomes (C-liposomes) were unstable in biological fluids and inefficient in drug

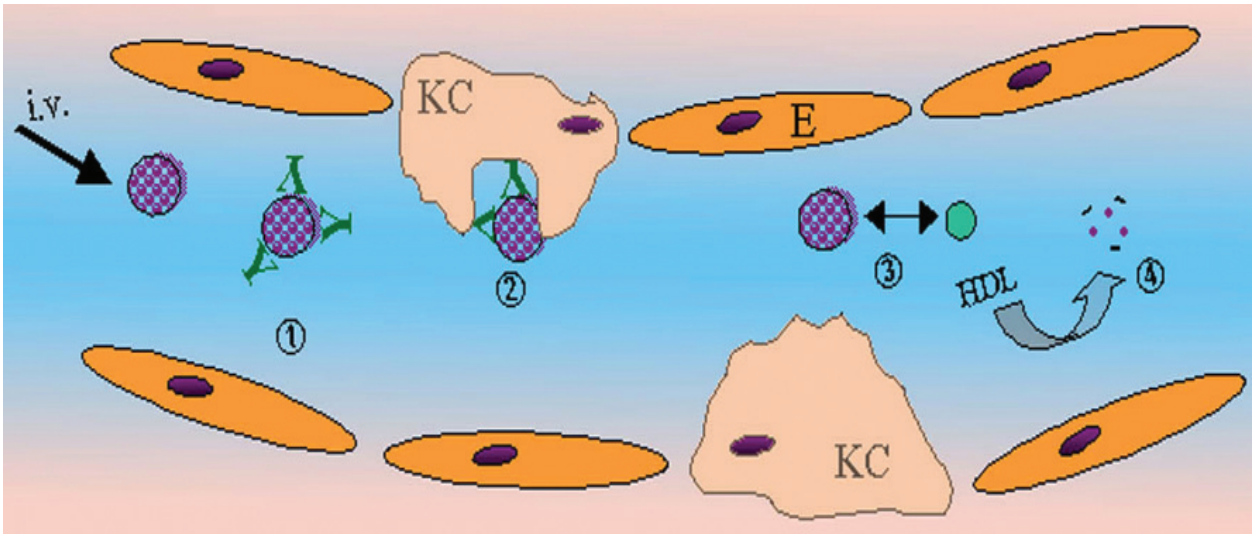

Fig. 1 Schematic diagram indicating the fate of intravenously (i.v.) administrated conventional liposomes. Upon interaction with the plasma proteins, liposomes may be covered with opsonins which mediate their endocytosis by Kupffer cells (KC) in the liver sinusoidal endothelia (E). Alternatively, an exchange of liposome lipids with lipoproteins, especially HDL can take place; the latter process leads to liposome destabilization and release of encapsulated molecules in the plasma.

loading. For drug delivery, liposomes can be administrated topically or parenterally. After systemic (usually intravenous) administration, which seems to be the most promising route for this carrier system, C-liposomes are recognized as foreign particles and are taken up by the cells of the mononuclear phagocytic system (MPS), mostly Kupffer cells in the liver and macrophages of the spleen. In addition, C-liposomes are highly unstable in biological fluids, leading to a rapid release of encapsulated molecules mainly due to the interactions with two distinct groups of plasma proteins, HDL and opsonins adsorbed onto liposome surface and mediating their endocytosis by MPS (Fig. 1). Therefore, the rate of liposome clearance from blood circulation depends on the ability of opsonins to bind to the liposome surface; nonetheless this can be manipulated through the appropriate selection of liposome characteristics. In addition, the clearance of liposomes from blood stream depends on the liposome properties such as bilayer fluidity, surface charge and vesicle size. The pronounced tendency of C-liposomes to be taken up, that is to target cells of MPS is very useful for delivering drugs to macrophages but restrain the *in vivo* use of liposomes for selective delivery of drugs to other sites. Thus, the C-liposome uptake by MPS cells has limited the development of liposomes as drug delivery systems for over 20 years.

After numerous and various studies, new formulations of liposomes with increased stability were designed; thus, liposomes that contain lipidic derivatives of polyethylene-glycol (PEG) which possess the properties to avoid MPS uptake and show increased times in blood circulation were found particularly appropriate [1]. Also, "smart" liposomes that can tolerate specifically induced modifications of the bilayers or can be covered with different molecules were constructed. These sort of liposomes include proteoliposomes containing fusogenic proteins [2,3], pH-sensitive liposomes (able to avoid lysosomal degradation [4-6]), cationic liposomes (form complexes with DNA [7-9]), target sensitive liposomes (disintegrate after binding to a target cell and release the content in the cell vicinity [10]) and immunoliposomes (directed toward specific sites by coupling antibodies to their surface [11,12]). At present, researchers in the liposome field are trying to reach the concept of "magic bullet" introduced by Paul Ehrich in 1906. Thus, "smart" liposomes capable to deliver specifically drugs or genes to a certain cell or tissue have been designed; still, it remains to be validated by *in vivo* and clinical studies.

Designing of "intelligent" liposomes

It is expected that liposomes can be widely used as drug delivery systems due to their structural versatility related to size, composition, bilayer fluidity and ability to incorporate a large variety of compounds. However, in order to use effectively liposomes as drug delivery vehicles in the treatment of a wide range of diseases involving cells other than MPS, "intelligent" liposomes which stay longer in the circulation, or can stand specifically induced modifications of the bilayers or can be covered with specific molecules (targeted liposomes) have been designed and prepared.

Long-circulating liposomes

As mentioned above, the rapid uptake of liposomes composed of natural phospholipids, referred as conventional liposomes (C-liposomes) by the MPS has limited their use for targeting other cells. As a result, the development of liposomes as drug delivery vehicles relied on attempts to construct vesicles that avoid the MPS, such as small, rigid, cholesterol-rich liposomes that exhibited increased stability in plasma [13]. Other methods of extending the liposome blood circulation time include the incorporation into liposomes of polyvinyl-pyrrolidone polyacrylamide lipids [14], glucoronic acid lipids [15] or the high phase transition temperature phospholipid distearoyl phosphatidylcholine [16]. Also, coating of liposomes with proteins, polysaccharides and glycolipids of red blood cells confer the ability to increase their circulation time [17]. Some success has been achieved using liposomes coated with ganglioside G_{M1} and hydrogenated phosphatidyl inositol (HPI) considered as the component responsible for their increased circulation time and referred to as Stealth^R liposome [18]. Unfortunately, the effect of G_{M1} on the increasing circulation time occurs only in the mouse model and not in rat or rabbit models because the serum of the latter contains anti-G_{M1} antibodies which enhance rapid clearance of G_{M1} containing liposomes [19]. A better formulation which displays a longer circulation time

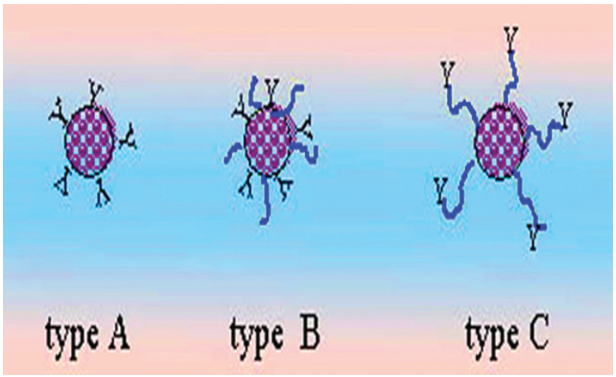

Fig. 2 Classes of immunoliposomes: type A: an antibody is coupled to an anchor inserted into the bilayer of conventional liposome; type B: an antibody is coupled to an anchor inserted into the bilayer of sterically stabilized liposome; type C: an antibody is coupled to the distal end of the polyethylene glycol (PEG) grafted to the liposome surface.

in the blood stream is that containing a relatively inexpensive and easily prepared phospholipid conjugated with a synthetic hydrophilic polymer, polyethylene glycol, PEG [1]. These liposomes, referred to as sterically stabilized liposomes (SS-liposomes) have been found to have the best capabilities to reduce MPS uptake. The PEG polymers are believed to sterically hinder the interaction of serum proteins with the liposome surface by virtue of their hydrophobicity and flexibility, resulting in a reduced uptake of liposomes by the cells of MPS [20]. This last finding restored much of the original promise of liposomes as "magic bullet" that can deliver selectively drugs to specific sites.

Targeted liposomes

The most promising way for selective targeting of liposomes to specific sites is the attachment on their surface of ligands, which recognize specific molecules. Antibodies or other ligands such as folate [21], transferrin [22] anionized albumin [23], dextran [24] which bind to receptors that are upregulated on the surface of a target cell, can be attached onto the liposome surface using various anchors or to the terminus PEG, which is inserted into the liposome bilayer by a phospholipidic derivative [25-29] (Fig. 2).

Immunoliposomes, that are liposomes bearing on the surface covalently coupled antibodies, have been designed so as to secure a targeted delivery of drugs to specific surface antigens. Promising results were obtained *in vitro* [26,27,30] and *in vivo* on animal models [31-33] and despite of the use of humanized antibodies for cancer treatment [34] they have not been widely introduced in clinic, yet. Also, numerous molecules have been identified on the surface of cells in pathological conditions; thus the immunoliposomes are a promising tool as diagnostic or therapeutically agents. However, the specific binding of liposomes to a target cell (mediated by an antibody directed against a certain molecule on the cell's surface) does not lead always to an efficient drug delivery. Targeting antigens that are internalized by the cells could mediate an efficient intracellular drug delivery. Therefore, the strategy in constructing immunoliposomes should be optimized so as that internalization and intracellular drug delivery will take place (Fig. 3). Thus, targeting of the liposomes to receptors that are known to be internalized is the most attractive approach in future research because this would allow the intracellular delivery of the liposome content and increases the therapeutic benefit. The efficient delivery associated with the receptor-mediated endocytosis, or surface antigens - where liposomes are transferred to endosomal-lysosomal compartment may be useful only for drugs that effectively resist degradation into these compartments.

"Sensitive" liposomes : pH-, temperature-, target- sensitive liposomes

To avoid lysosomal degradation, ***pH-sensitive liposomes*****,** which destabilize and become fusogenic at a pH ~ 6, have been designed. This type of liposomes are composed by a mixture of phosphatidyl ethanolamine (PE) with an acidic phospholipid. At pH< 6.5, after protonation of the bilayers, PE undergo a transition from the bilayer phase to a hexagonal phase, destabilize, becomes fusogenic and the liposomal content reaches the citosol (figure 3). The pH-sensitive liposomes have been successfully used for nucleic acids delivery [35,36].

Another distinct type of liposomes is the ***temperature-sensitive liposomes*** that are prepared from phospholipids with a phase transition temperature of around 40ºC. Heating the sites where the liposomes have been accumulated is assumed to induce a rapid release of the liposomal content (figure 3). This class of liposomes have been successfully used *in vitro* and

in animal models [37] but have not been introduced in the clinic although the local hyperthermia is used as an anti-cancer treatment and temperatures over 40ºC are easily achieved in various tissues.

Target-sensitive liposomes have been obtained by stabilization of PE into bilayer with antibodies derivatives of fatty acids (usually palmitic acid). After binding to target cell's surface, concentration of immunoglobulins molecules at contact points, leads to destabilization of bilayers [10] (Fig. 3). At this site, the liposomal content will be released in the cell's vicinity. This technique has been used for delivery of antiviral agents [38].

Liposomes in human therapy

Despite of the good and encouraging results obtained using liposomes as vehicles for drugs in numerous diseased animal models, in human therapy, the use of liposomes is restricted to systemic fungal infections and cancer therapy, only. However, liposomes based vaccines show great promise and a vaccine against hepatitis A is already on the market.

Liposomes in anticancer therapy

Based on the early studies that showed that encapsulation of a drug inside of liposomes reduces its toxic side effects, the liposomes were considered as attractive candidates for the delivery of anticancer agents. However, their use was hampered by the rapid uptake of conventional liposomes by MPS cells. The increase of *in vivo* circulation time of modified lipids (PEG polymerized lipids, gangliosides, shingomyelin etc.) restored the initial expectation of the advantages of liposomes. Intravenously administered stealth liposomes were passive targeted to solid tumors due to their extravasation in leaky blood vessels supporting the tumor [39].

The good results obtained with liposomal-encapsulated doxorubicin and daunorubicin have lead to two products licensed for use in the treatment of Kaposi' sarcoma, namely Doxil and Daunoxome

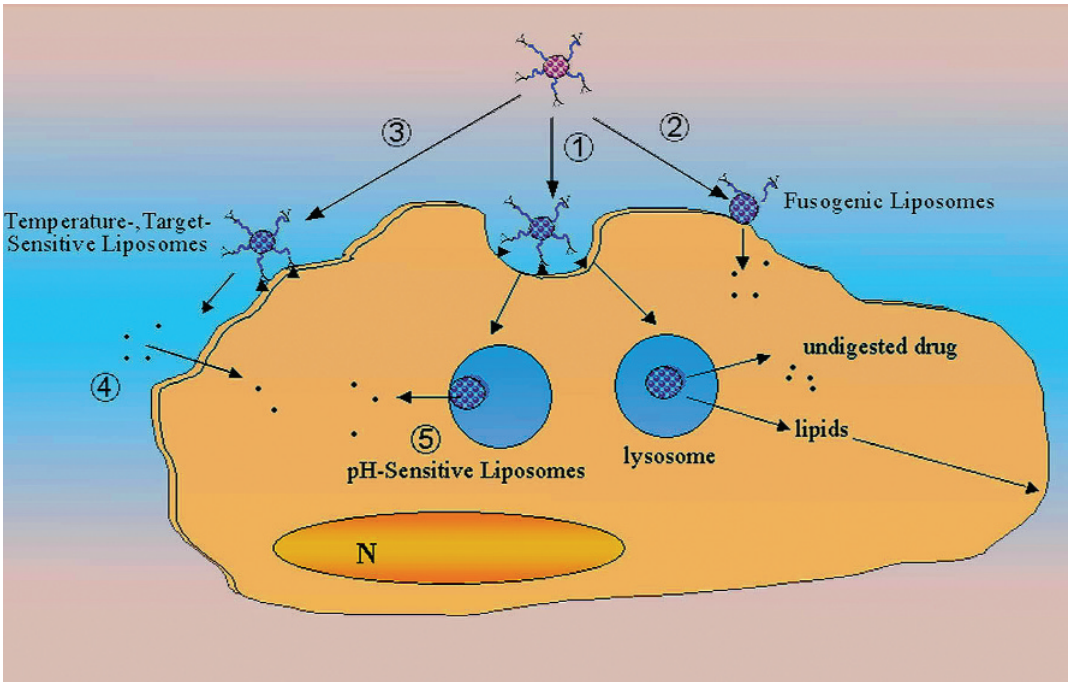

Fig. 3 Type of interactions between ligand-targeted liposomes with a cell. Upon binding to the cell surface, ligand-targeted liposomes can be either internalized by receptor mediated endocytosis, or can fuse with the cell membrane (fusogenic liposomes obtained by insertion of fusogenic viral proteins). For an effective drug delivery, temperature- and target-sensitive liposomes have been designed: the liposomes disintegrate and release in the vicinity of the cell their content, part of which permeate the plasma membrane. In addition, pH-sensitive liposomes are constructed so as to fuse with endosomes and release the content in citosol avoiding lysosomal degradation.

[40]. Doxil (commercialized by Sequus Pharmaceuticals, Menlo Park, USA) is a suspension of doxorubicin precipitated in 80-100 nm sterically stabilized liposomes. Daunoxome (commercialized by NeXstar Pharmaceuticals, Inc., Boulder, USA) is a small, rigid formulation of liposomes with daunorubicin. These liposomes circulate in the vasculature of patients for several days, and thus have increased chances of extravasating at sites of increased permeability.

Other approaches employed in liposomal anticancer therapy include the use of immunoliposomes [41,42] and termosensitive liposomes [43,44]. Although the use of immunoliposomes is an elegant strategy [45,46], their efficiency in anticancer drug delivery to solid tumors is not increased when comparing with stealth liposomes. This may be due to a high binding of immunoliposomes to the periphery of solid tumors that prevent their penetration into deeper layers [47]. The success achieved with anthracycline anticancer agents led to the development of other liposomal formulations that are in preclinical stages (5-fluorouracil lipid analogue [48], vincristine [49-51], a porphyrin derivative for use in combination with laser light irradiation [52], bleomycin [53], mitozantrone [54], paclitaxel [55], valinomycin in combination with cisplatin [56]) or clinical trials (muramyl tripeptide phosphatidylethanolamine, MTP-PE studies in USA and Europe sponsored by Ciba-Geiby, 6-aminochrysene studies in Belgium sponsored by Jules Bordet Institute, platinum studies in USA sponsored by The Liposome Company and MD Anderson Hospital [40] and cytarabine [57]).

Liposomes in infections treatment

Due to their uptake by the cells of the MPS, mainly Kupffer cells and spleen macrophages, conventional liposomes are useful in the treatment of parasitic infections of the MPS, such as leishmaniasis. Encapsulating the amphotericin B into liposomes reduces the renal and general toxicity, and the therapeutic efficiency is improved. Ambisome is a formulation of small, negatively charged liposomes with amphotericin B licensed for clinical use and commercialized by NeXstar, Pharmaceuticals Inc., Boulder, USA [40]. Now, the attention is focused on the encapsulation of more powerful antibiotics (that are exceedingly toxic in free form) and on the development of liposomal formulations for delivering the drugs to other sites than MPS. The encapsulation of the anti-tuberculosis drug rifampicin or isoniazid in liposomes targeted to lung improves the efficacy of the drug [58] and modulates toxicity [59] in mice. Also, good results in the treatment of infections in mice were obtained by incorporating immunomodulators (i.e. cytokines) in liposomes [60].

Liposomes as vaccine systems

Liposomes can be used as enhancers of the immunological response by incorporation of antigens [61], cytokines [62] or DNA sequences encoding an antigenic protein [63,64]. For this purpose the liposomes are administered intramusculary, a location where the encapsulated antigen is released slowly and accumulate passively within regional lymph nodes. To control the antigen release and to improve the antibody response, the liposomes encapsulating antigens are subsequently encapsulated into alginate lysine microcapsules [65]. At present, Epaxal, a liposome-based vaccine against hepatitis A was licensed for clinical use and was introduced on the market by Swiss Serum and Vaccine Institute, Bern, Switzerland [61]. This vaccine contains formalin inactivated hepatitis A virus particles attached to phospholipid vesicles together with influenza virus haemagglutinin. Hepatitis A virus incorporated into liposomes proved to be a suitable formulation in term of rapid seroconversion, high level of mean antibody content and low reactogenicity [66].

Also, there are in clinical trial vaccines against influenza, hepatitis B, diphteria, tetanus, E Coli infection [40].

Liposomes in gene delivery

Gene therapy is the process by which DNA sequences encoding specific altered genes are delivered to cells with the goal of treating or curing genetic diseases. Thus, instead of treating the symptoms of the disease, as in conventional medicines, gene therapy has the potential to correct the underlying cause of genetic diseases. While the idea of gene therapy is a simple concept, the delivery of genes to the diseased areas turned out to be a difficult task. The problems associated with the use of viral vectors for gene

therapy, lead to the search for less-hazardous, non-viral delivery systems. As an alternative to viral vectors, cationic liposomes have been developed for gene transfer since they have no limit for the size of the genes to be delivered and exhibit low immunogenicity. The efficacy of this system has been limited by the non-specific adherence to many cell types. In order to obtain an effective DNA transfer it is necessary to administer liposomes to a site near to the target area. The use of ligand-targeted liposomes will make possible to direct them precisely to diseased cells and not to other cells. Some pharmaceutical companies (Vical Company, San Diego, USA, Targeted Genetics Corporation, Seattle, USA, Valentis^{R} Burlingame, USA) are engaged in liposome-based gene delivery and have products in clinical trials. Vical Company (San Diego, USA) has two compounds on trial that are based on liposomes for gene delivery: (i) Allovectin-7: liposomes carrying a gene for HLA-B7 (a highly immunogenic molecule) that are injected into tumors: this is in phase III trial for metastatic melanoma and phase II trial for patients with head and neck squamous cell carcinoma and (ii) Leuvectin, a DNA/lipid complex containing gene for IL-2, a immunostimulatory cytokine, that is in phase II trial for patients with prostate cancer. Valentis^{R} Company (Burlingame, USA) has a liposome-based system in phase I trial for gene therapy with Del-1 (Developmentally Regulated Endothelial Locus-1, an extracellular matrix protein involved in the early growth and development of blood vessels and bone) for the treatment of peripheral arterial disease and ischemic heart disease.

A cationic liposome /E1A complex (a gene from common cold virus that acts as tumor inhibitor) that is injected intratumoral, is under investigation by Targeted Genetics Corporation (Seattle, USA); the liposome complex is in phase II study in the treatment of patients with recurrent head and neck squamous cell carcinoma [67] and in phase I in ovarian cancer in combination with paclitaxel (Taxol) and cisplatin chemotherapy [68]. Intratumoral injections of liposome/E1A complex were safe and well tolerated. The E1A gene expression was accompanied by HER-2/neu downregulation, increased apoptosis, and reduced proliferation.

Also in clinical trials, the UK CF Gene Therapy Consortium (London, United Kingdom) employs the complexes liposome/gene CFTR (cystic fibrosis transmembrane conductance regulator) that is delivered as an aerosol to the nose and lung of patients with cystic fibrosis [69-71].

Conclusions and perspectives

Liposomes are one of the most broadly studied modern drug delivery system. To overcome some difficulties with respect to the liposome stability and the MPS uptake, some “intelligent” liposomal systems able to deliver specifically and efficiently drugs or genes to appropriate tissues or cells have been developed. The next step is to validate the results obtained *in vitro* and on animal models by clinical trials. While effective anticancer and antifungic formulations have been completed after years of persistent research, progress in the field of gene delivery are anticipated to be the next largely developing area in molecular medicine. Thus, the designing of fusogenic peptides that mimic functions of SNARE proteins or fusogenic viral proteins will allow the development of liposomes that can deliver drugs and genes with high efficiency at the specific intracellular destination. The incorporation of translocation nuclear domains into modular fusion proteins provides new perspective in gene delivery. Also, the covering of liposomes with certain oligosaccharide sequences found on the surface of long-time circulating cells (such as erythrocytes) may increase the capacity of liposomes to circulate for days or weeks with minimal interactions outside the targeted tissue. Furthermore, the efficiency of liposome complexes will be improved due to the current progress in developing of new functionalized lipids to be used as anchors for attachment of proteins, peptides or drugs to the liposome surface.

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