Advances in Liposomal Drug Delivery System in the Field of Chemotherapy

Ying-Jie Hu[1#], Fan Zeng[2#], Rui-Jun Ju[3] and Wan-Liang Lu[1]*

[1]Beijing Key Laboratory of Molecular Pharmaceutics and New Drug System, State Key Laboratory of Natural and Biomimetic Drugs, and School of Pharmaceutical Sciences, Peking University, China

[2]Beijing Neurosurgical Institute, China

[3]Department of Pharmaceutical Engineering, Beijing Institute of Petrochemical Technology, China

[#]Authors contribute to this paper equally

Abstract

Liposomal drug delivery system has experienced nearly 50 years from laboratory research to clinical application. It has made evident breakthrough and innovation in the fields of anticancer, anti-infection and pain management. The objectives of this study were to briefly summarize and review the major fabrication approaches, progresses in the early studies, and clinical application of drug liposomes for chemotherapy. The manufacturing technology of drug liposomes is becoming mature now, consisting of film dispersion, reverse-phase evaporation, chemical gradient loading and the other encapsulation methods. A number of liposomal strategies have been developed to improve efficiency and function of drug delivery through passive, active and physicochemical targeting approaches. Some new liposomal formulations are undertaking laboratory evaluations. With regard to clinical application, tens of drug liposomes have been approved for clinical use meanwhile a number of drug liposomes are undergoing clinical trial evaluations. During clinical trials and uses, the liposomes have been evidenced having an optimal drug delivery efficiency and better efficacy, despite the anticancer drug liposomes may lead to new side effects like hand-foot syndrome. The drug liposomes can be enriched into the tumor site, hence demonstrating a better efficacy and a reduced adverse reaction such as cardiotoxicity. Besides, the liposomal formulations are capable of potentiating efficacy of anticancer drugs by circumventing multidrug resistance of cancers and cancer stem cells, and by transferring drug across the blood-brain barrier (BBB). These new functions have been evidenced in laboratory observations but await for clinical confirmation. The review demonstrates that the liposomes are useful and promising drug delivery systems in the field of chemotherapy.

Keywords: Liposomes; Drug delivery; Advance; Chemotherapy; Review

OPEN ACCESS

Introduction

During the latest decades, the comprehensive strategy which includes surgery, chemotherapy, radiotherapy, and immunotherapy has been used in treatment of various cancers. The combination of treatment plays an important role in enhancing the recovery rate and life quality of patients suffered with this severe illness. However, there exist in scientific and technical issues during treatment. The relapse and metastasis occur in the most of clinical cases. These are because the surgery is unable to remove all cancer cells while the radiation treatment faces the same situation. In addition, in case of the deteriorated health status, immunotherapy has a limited efficacy in improving the immune system of patients in a short term.

As a fundamental approach, chemotherapy still plays a crucial role in eliminating cancer cells. However, there have a number of obstacles to acquire a successful chemotherapy. On the first hand, an anticancer drug has difficulty in maintaining an effective therapeutic concentration in the tumor site but widely distributes in systemic organs and tissues, hence leading to severe damages to healthy tissues and immune system. On the other hand, the multidrug resistance has been experienced in clinical chemotherapy, and the resistance remains to a major problem in eliminating cancer and cancer stem cells, resulting in a poor clinical prognosis. Furthermore, the physical and chemical properties of anticancer drugs significantly affect the treatment outcomes. Consequently, the development of suitable drug delivery systems is a pressing mission, and the significance for studying new drug delivery strategies are no less favorable than that for hunting high performance
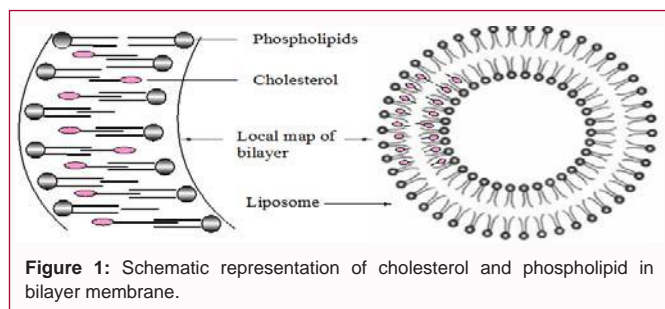
Figure 1: Schematic representation of cholesterol and phospholipid in bilayer membrane.

of new drug chemical entities.

As drug carriers, liposomes have been demonstrated to be the useful delivery systems in improving unfavorable pharmacokinetics, enhancing efficacy for removing cancer and cancer stem cells, and reducing systemic side effects [1].

Liposomes are drug-loaded tiny capsules with bilayer membrane structure, which are mainly made up of phospholipids and cholesterol [2]. When amphipathic molecules phospholipids are dispersed in water, the hydrophobic parts of molecules tend to gather together, while the hydrophilic parts expose towards water, thereby forming the round-shape vesicles with bilayer structure (Figure 1). This construct can be used for encapsulation of pharmaceutical agents, which a hydrophilic agent is entrapped into the aqueous vesicle core of liposomes, while a hydrophobic agent inserted into the lipid bilayers.

As drug delivery vehicles, liposomes are able to avoid the direct exposure of drug, hence lowering drug degradation and blood toxicity. Furthermore, the pegylated liposomes provide a prolonged circulation in blood system through avoiding the rapid clearance of reticuloendothelial system (RES) [3], and accumulate more into cancer tissue by the enhanced permeability and retention (EPR) effects [4,5]. Besides, a varied particle sizes of the liposomes change the distribution of drug in tissues by a physical retention due to the difference in compact degrees of each organ tissue. As a result, liposomes are able to reduce the cardiotoxicity of doxorubicin through this mechanism [6]. In addition to these favorable features, more and more the modified liposomes have been fabricated to reach unique actions for the purposes of transferring drug across the physiological barrier, enhancing the uptake of drug-resistant cancer cells, killing the "dormant" cancer stem cells, and interfering the critical life signaling pathways or targets, thus not only eradicating cancer cells themselves but also recovering the "soil" for growth of health body cells.

Up to date, a dozen of liposomal drug formulations have been applied to clinical treatments of cancer and other diseases, such as Doxil for treatment of cancer [7], Depodur for relief of pain [8], and AmBisome for treatment of fungal infection [9], etc. Moreover, the laboratory studies show that the nanostructured liposomal drug delivery system provides a promising strategy in cancer treatments. In this review, the advances in drug liposomes are briefly summarized and commented aiming at retrospection and prospection of the development in this field.

Fabrication of Drug Liposomes

Film dispersion method

Film disperse method is also known as the Bangham method or thin film hydration method and it is one of the most widely used techniques for the formation of liposomes [10,11]. Film disperse method is a fabrication process in which phospholipids with lipophilic drugs are dissolved in appropriate amount of chloroform or other solvents, and then the solvent is evaporated to form a lipid film. A buffer solution containing water soluble drug is added to the lipid film with shaking, yielding drug-loaded liposomes with a particle size range of 1-5 μm. The liposome suspension needs to be further treated by ultrasound or through the membrane extrusion to make the particle size of liposomes uniform. To lower down and homogenize the particle size of liposomes, several methods can be selected, including ultrasonic method, film extrusion, and French film extrusion method, etc.

Reverse-phase evaporation method

The reverse-phase evaporation process was first described by Szoka and Papahadjopoulos, and it is based on the formation of drops of water that are surrounded by lipid and dispersed in an organic solvent, referred to as inverted micelles [12]. The method is a preparation process in which phospholipid membrane material is dissolved in an organic solvent (such as chloroform and ether), and then aqueous drug solution is added to form W/O emulsions under ultrasonic treatment. The organic solvent is removed vacuum evaporation to yield liposomes. The liposomes prepared by reverse-phase evaporation method are usually large unilamellar liposomes. The problem of residual organic solvents can be solved by using supercritical CO_2, instead of organic solvents, known as supercritical reverse evaporation method [13,14].

Chemical gradient methods

pH gradient method: pH gradient method is an active encapsulation method. In this method, blank liposomes are firstly prepared by film dispersion method, then the pH value of the aqueous phase of liposome vesicles are adjusted to form a pH gradient difference between internal and external vesicles, and the weak acid or alkaline agents may be encapsulated in the internal phase of liposomes in the form of ions by using the pH gradient (Figure 2). This method makes it possible for preparing liposomes with high drug entrapment efficiency. However, as the method is dependent on drug structure, it cannot be applied to the drugs with arbitrary structures. The active encapsulation methods are also known as the remote loading methods, consisting of pH gradient method for alkaline drugs, ammonium sulfate transmembrane gradient method for alkaline drugs, and calcium acetate gradient method for weak acidic drugs.
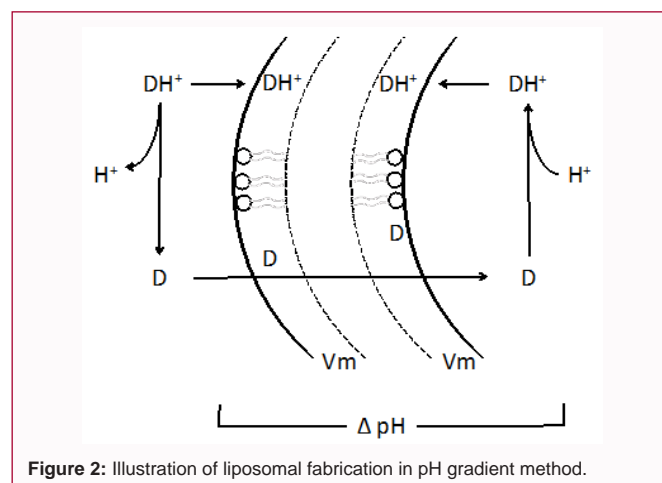

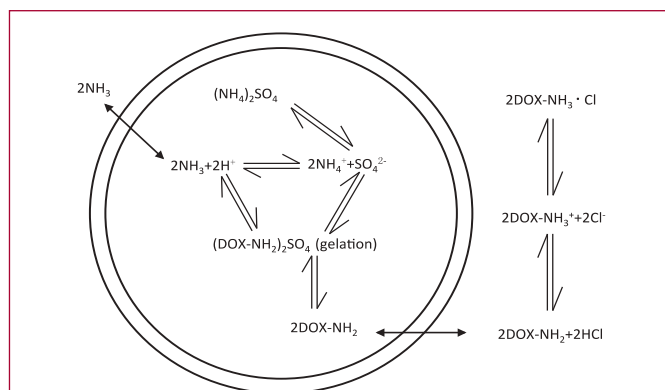**Figure 2:** Illustration of liposomal fabrication in pH gradient method.

Figure 3: Illustration of liposomal fabrication in ammonium sulfate gradient method.

Ammonium sulfate gradient method: Ammonium sulfate gradient method is designed according to the principle of chemical equilibrium, and also an active encapsulation method. The general process of the method could be described as the following procedures (Figure 3). Firstly, blank liposomes are prepared by using film dispersion method with ammonium sulfate solution as the hydration solution. Secondly, the blank liposomes are dialyzed in the dialysis tubing with phosphate buffered saline to remove the ammonium sulfate outside, thus forming an ammonium sulfate gradient between two sides of liposome vesicles, namely, the inside has a high concentration of ammonium sulfate while the outside has lower one. Finally, active encapsulation is achieved by incubation with amphipathic weak bases solution in water bath at 60°C with continually shaking for 20 min [15]. In this method, the stability of the ammonium ion gradient is related to the low permeability of its counterion, the sulfate, which also stabilizes anthracycline accumulation for prolonged storage periods (> 6 months) due to the aggregation and gelation of anthracycline sulfate salt [16].

Calcium acetate gradient method: The fabrication of calcium acetate gradient method is briefly described as the following procedures. Blank liposomes containing calcium acetate solution are prepared by thin film dispersion method, and then a concentration gradient is formed by removing the external calcium acetate of liposomes. The calcium acetate concentration gradient that the internal concentration is higher than the external one is produced via the transmembrane movement of calcium acetate so that a large number of protons transfer from the liposome interior to the outside, forming a pH gradient. The method is suitable for encapsulating weak acidic drugs. The drugs can bind with the liposome internal calcium ion to form less soluble calcium salt, and prevent the drugs from passing through the phospholipid bilayer, thereby improving the entrapment efficiency and reducing drug leakage [17-19].

Additional methods

There are many other methods in the preparation of liposomes. The ethanol and ether injection method can be used for dissolving the lipids into an organic phase, followed by the injection of the lipid solution into aqueous media, hence forming liposomes [20,21]. Besides, the heating method has been developed to produce blank liposomes by hydration of phospholipids in an aqueous solution containing 3% glycerol through raising the temperature to 60°C or 120°C [22]. During preparation, the drug was incorporated into blank liposomes using the heating method by addition of drug to the solution at different temperature stages, including the beginning,

above the transition temperature of the lipids and the ambient temperature. In addition, the freeze-drying of mono-phase solution method for encapsulation of heat sensitive drugs such as DNA, and the produced drug liposomes can be stored for a long time in a sealed container [23].

Advance In Anticancer Drug Liposomes

Liposomes have demonstrated to be an excellent carrier system for a variety of applications and are particularly ideal for anticancer drug delivery because of the similarity to natural cells, and multiple potentials in cancer chemotherapy.

Passive targeting drug liposomes

The passive targeting drug liposomes are the drug liposomes with a mechanism by which drug liposomes can be preferentially delivered to target cells in vivo, especially cells in cancer tissue. Based on their physicochemical properties, such as suitable particle size, drug liposomes can escape from nonspecific trapping by other tissues but passively accumulate in target tissues with the circulation of blood. Because cancer tissues are characterized by a high interstitial pressure, enhanced vascular permeability and retention (EPR effect, Figure 4), and the lack of functional lymphatic drainage, the passive targeting has become a useful approach for drug delivery.

Regular drug liposomes: The regular drug liposomes are mainly composed of phospholipid and cholesterol, without any modification. After injection, the regular drug liposomes are mainly concentrated in the liver, spleen, lung, lymph node, bone marrow and other reticuloendothelial rich locations. In addition, they also aggregated in the locations of inflammation, infection and vasculatures of solid tumors, exhibiting passive targeting effect. Based on laboratory evaluations, regular drug liposomes have shown multi-potentials in the prevention and treatment of cancers.

Imanaka et al. [24] reported that the beta-sitosterol liposomes exhibited the chemopreventive effect of tumor metastasis by oral delivery. Their results showed that the amount of immune response cytokines, such as IL-12 and IL-18, were increased in the small intestine after the intake of liposomes. After administration of the liposomes for 7 days, natural killer cell activity in the mice was increased, suggesting that the immune surveillance activity of mice was enhanced by the intake of beta-sitosterol liposomes. Furthermore, daily intake of beta-sitosterol liposomes prevents the metastasis of tumor.

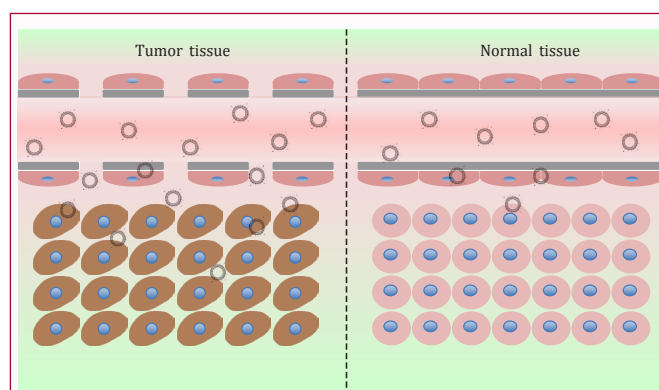Igarashi et al. [25] demonstrated that the liposomalization of

Figure 4: EPR effect of nanostructured liposomes in the fenestrated tumor tissue.

photosensitizer could be used to enhance the therapeutic efficacy of photodynamic therapy for gastrointestinal tumors. The results showed that the volume of necrotic tumor tissue was significantly higher in the group of photofrin liposomes group than that in the group of free photofrin. Moreover, the apoptotic index of the tumor was also significantly higher after treatment with photofrin liposomes. Their results revealed that the liposomalization of photosensitizer were able to increase accumulation of the liposomes in tumor site, with a resultant enhancement effect of photodynamic therapy.

Long-circulating drug liposomes: Long-circulating liposomes can be prepared by coating liposome surface with a hydrophilic layer of oligosaccharides, glycoproteins, polysaccharides and synthetic polymers in order to achieve a continuous action on the tumor tissue. Long circulating liposomes are also called stealth liposomes or sterically stabilized liposomes [26]. When modified with ganglioside, phosphatidylinositol or polyethylene glycol lipid derivatives, the liposomes will be densely covered by the conformational clouds around the surface of liposomes, hence playing a protective action for liposomes. This steric protection is associated with the polymer flexibility, steric hindrance and hydrophilicity, and it can prevent the recognition of liposomes by opsonin and reduce the rapid clearance of the liposomes by reticuloendothelial system [27,28]. Therefore, the modified liposomes can last for a longer time in the circulation system, and extend action time of drug during therapy.

Cogswell et al. [29] investigated that the long-circulating econazole liposomes had a superior efficacy in treatment of breast cancer by parenteral administration. In the study, long-circulating econazole liposomes were prepared by a novel micelle exchange technique in incorporating drug into the lipid bilayer of pre-formed liposomes using a polyethylene glycol-linked phospholipid, distearoylphosphatidyl ethanolamine (DSPE-PEG). This method allowed for stable and efficient drug incorporation. Results showed that the liposomes had a long-circulating effect and a better efficacy but did not induce significant liver toxicity, renal toxicity or weight loss in human breast cancer MCF-7 cells xenografted model in mice.

Furthermore, Fanciullino et al. [30] developed a kind of pegylated liposomes of 2'-deoxyinosine (d-Ino), which was used as a 5-fluorouracil (5-Fu) modulator, and evaluated its efficacy *in vitro* and in tumor-bearing mice and its pharmacokinetics in rats. The deoxyinosine liposomes exhibited a strong potentiation effect in a combination use of 5-Fu *in vitro*, and displayed a 7-fold long-circulating effect in animals. In tumor-bearing mice, the combination of deoxyinosine liposomes with 5-fu led to 70 % of tumor reduction with a doubling median survival time as compared to the control. In addition to the long-circulating effect, the deoxyinosine liposomes had demonstrated a capability to reverse the 5-Fu resistant colon cancer SW620 cells.

In the latest ten years, the long-circulating liposomes have been further assigned to be multifunctional long circulating liposomes, such as pH sensitive or temperature sensitive long -circulating liposomes, as discussed below.

Active targeting drug liposomes

Liposomes with passive targeting alone cannot reach the selectivity of tumor tissues. Combining passive targeting with active targeting brought a new strategy for chemotherapy, which could lead to promoting tumor specificity as well as diminished systemic adverse effects. Recently, efforts of scientists have been made on the development of active targeting liposomes, which could target tumor cells and cellular organelles. Enhanced intracellular uptake of the active targeting liposomes is usually achieved by interaction of the overexpressed receptors with ligands or the specific tumor antigens with monoclonal antibodies.

Ligand-mediated liposomes: There are many receptors that are overexpressed on the tumor cells but less expressed or non-expressed on the normal body cells, such as integrin receptors, transferrin receptors, folate receptors, lectin receptors, and low density lipoprotein receptors, etc. To selectively target tumor cell, special ligands that could specifically bind to these receptors are modified on the surface of liposomes. For instance, folate receptor is overexpressed on many epithelial cancers, and it has been exploited as the action target on cancer cells. As an example, folate receptor is overexpressed on ovarian cancer cells, thus allowing the binding of folate with folate receptor, and enhancing the internalization of folate through folate receptor-mediated endocytosis. Accordingly, liposomes modified with the folate demonstrated an active targeting behavior on the ovarian cancer cells.

Zeng et al. [31] developed a kind of functional vincristine plus dasatinib liposomes modified with a targeting molecule DSPE-PEG2000-c (RGD$_y$K) for eradicating triple-negative breast cancer (TNBC). C (RGD$_y$K) is a cyclic peptide that has a specific affinity with integrin receptor. It is found that the integrin receptor is overexpressed on many malignant cancer cells, including TNBC cells. Consequently, this cyclic peptide acts as a targeting molecule in modifying drug liposomes for binding with integrin receptor on the cancer cells.

Apart from the single modification, there were also multiple modifications with more than one ligand, which could avoid the heterogeneity of cancer cells. For example, Sriraman et al. [32] developed a kind of pegylated doxorubicin liposomes modified with folate (F), transferrin (Tf) or both (F+Tf). The dual-targeted liposomes (F+Tf) showed a 7-fold increase in cell association compared to either of the single-ligand targeted ones in human cervical carcinoma (HeLa) cells.

Antibody-mediated liposomes: Monoclonal antibodies (mAbs) and their derivatives are often used as the targeting molecules for preparing the active targeting liposomes. The liposomes modified with mAbs or their derivatives are defined as immunoliposomes, which can be designed to improve pharmacological properties of conventional anticancer drugs. The intracellular transport of immunoliposomes could be increased by the antibody-antigen interaction. To some extent, immunoliposomes can be described as a kind of novel targeting liposomes with high active affinity to specific cancer cells. A number of methods have been reported for modifying antibodies onto the surface of the drug liposomes.

Huwyler et al. [33] developed a kind of daunorubicin immunoliposomes by introducing anti-transferrin receptor OX26 antibody with PEG$_{2000}$ on the liposomes, [34] which successfully resulted in an enhanced accumulation at brain glioma sites. Besides, Loureiro et al. [35] designed and prepared dual targeting immounliposomes by modification with two antibodies, namely the OX26 antibody and the anti-amyloid beta peptide antibody (19B8MAb), as nanocarriers of drugs for Alzheimer's disease therapy. Results showed that the established immounliposomes could effectively cross the blood brain barrier (BBB) (Figure 5) and
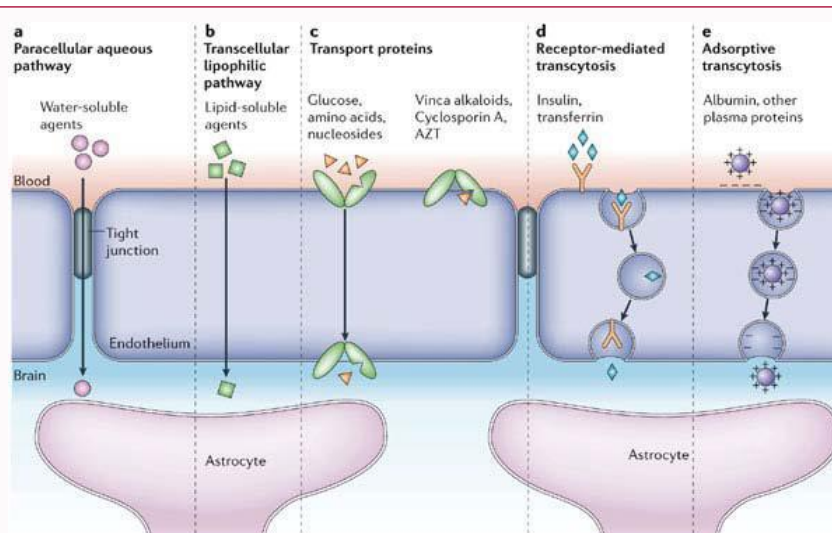
Figure 5: Pathways across the blood–brain barrier [34].

concentrate at the Alzheimer area.

Physicochemical targeting liposomes

Although liposomal formulations of chemotherapy demonstrated a significant reduction in systemic toxicity, the enhancement of therapeutic efficacy has not fully reached. Many advanced drug release strategies have been investigated ever since the liposomes were introduced as drug delivery carriers. Such strategies include utilization of pH, temperature, and enzyme sensitive systems, etc.

pH sensitive liposomes: In order to improve the anticancer efficacy of liposomes, several passive targeting, active targeting methods, and stimulus-responsive methods, have been developed [36]. Among these stimulus-responsive systems, pH-sensitive liposomes have attracted much interest [37]. Tumor microenvironment has been confirmed to have mildly acidic (pH 6.0-7.0) condition due to the glycolytic metabolism of glucose to lactate in tumor tissues, and this lowered pH value from that of normal tissues (pH 7.4) has been used for constructing pH sensitive drug liposomes [38].

Wang et al. [39] developed a pH sensitive drug delivery system, octylamine-graft-PASP modified liposomes (OPLPs). The OPLPs sustained a slow and steady release in the physiological pH 7.4 environment, while provided a fast release in sub-acid environment (pH 6.0 of resembled tumor tissues). The in vitro tumor cytotoxicity studies revealed that the tumor cells treated with OPLPs survived only 35.0% after 48 h whereas normal cells survived 100 % in the same condition. Júnior et al. [40] evaluated the tissue distribution of stealth pH sensitive liposomes containing cisplatin (SpHL-CDDP), compared with free cisplatin (CDDP), in solid Ehrlich tumor-bearing mice. The longer circulation of SpHL-CDDP led to a higher blood drug exposure and a higher accumulation of CDDP in tumor.

However, after internalization by tumor cells, the pH sensitive liposomes would be captured by tumor cellular endosomes, in which more acidic microenvironment in endosomes (pH 4.5-5.5) may lead to degradation of the liposomes by endosomal enzyme and hydrolase. For this reason, endosomal pH sensitive liposomes have been formulated for endosomal escape. Moku et al. [41] reported a kind of endosomal pH sensitive liposomes in which glutamic acid backbone-based cationic amphiphiles play multifunctional roles in enhancing

cellular uptake by guanidine moiety and improving the endosomal escape by histidine moiety. The endosomal pH-sensitive liposomal drug carriers not only effectively deliver anti-cancer drugs to mouse tumor, but also significantly contribute to enhancing anticancer efficacy.

Temperature-sensitive liposomes: Tumor tissues are usually exhibiting hyperthermia due to the rapid metabolism, and accordingly, the fever is often occurred in the tumor sites similar to the inflammatory response. This phenomenon inspired the development of temperature-sensitive liposomes. In case of temperature rising, the temperature-sensitive liposomes can release anticancer drug at tumor sites under the condition of pathological hyperthermia or external warming by which solid tumors can also be heated by a controlled device with an external energy source, such as infrared ray irradiation. This is because temperature-sensitive liposomes are composed of lipids that could undergo a gel-to-liquid phase transition at a critical temperature (transition temperature, Tm). Afterwards, double molecular chain of phospholipids would gain a higher degree of disorder and activity along with the increase of temperature, hence resulting in the release of drug from the liposome vesicles.

Yatvin et al. [42] described the temperature-sensitive liposomes which were able to release a hydrophilic drug when the temperature was increased a few of degrees above physiological temperature. The lipid materials for fabricating liposomes were based on 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). Similarly, Kakinuma et al. [43] developed a kind of temperature-sensitive liposomes containing cis-platinum for treatment of brain glioma-bearing rats. And the results showed a significantly increased concentration of cis-platinum in the brain glioma sites.

Enzyme-sensitive liposomes: Recently, the over-expressed enzyme systems in tumor microenvironment have been utilized to trigger the release of anticancer drug from liposomes, such as matrix metalloproteinases. Mura et al. [44] established enzyme-sensitive liposomes were by coupling a monoclonal antibody 2C5 with the polyethylene glycol chain via an MMP2-cleavable linker. Similarly, the over-secretion of phospholipase A2 (sPLA2) has been found in tumor site and can be used to initiate drug release of enzyme-sensitive
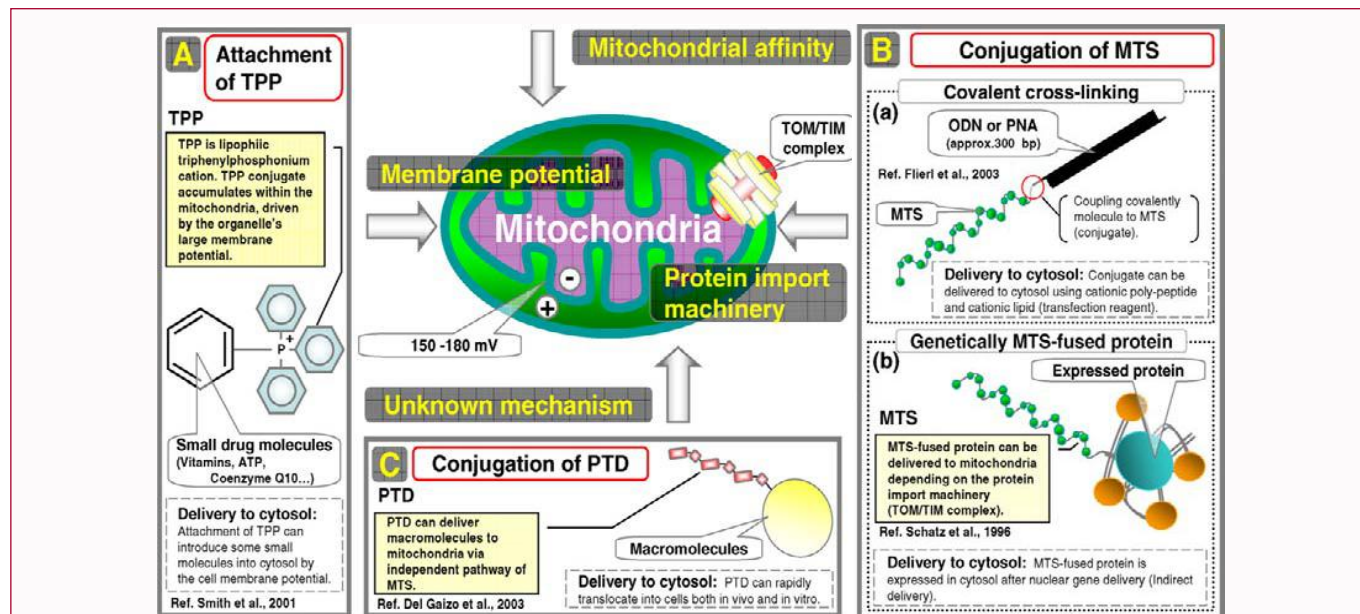
Figure 6: Covalently coupling conjugate for mitochondrial drug delivery [46-52].
Notes: A. The attachment of a lipophilic cation such as triphenyl phosphonium (TPP) improves the uptake of an attached molecule into mitochondria driven by the large membrane potential across the mitochondrial membrane. B. The attachment of a mitochondrial targeting signal peptide (MTS) to ODN or PNA allows mitochondrial delivery via TOM/TIM complex. C. A protein conjugated with protein transduction domain peptide (PTD) is delivered to mitochondria without passing through the classical protein import pathway.
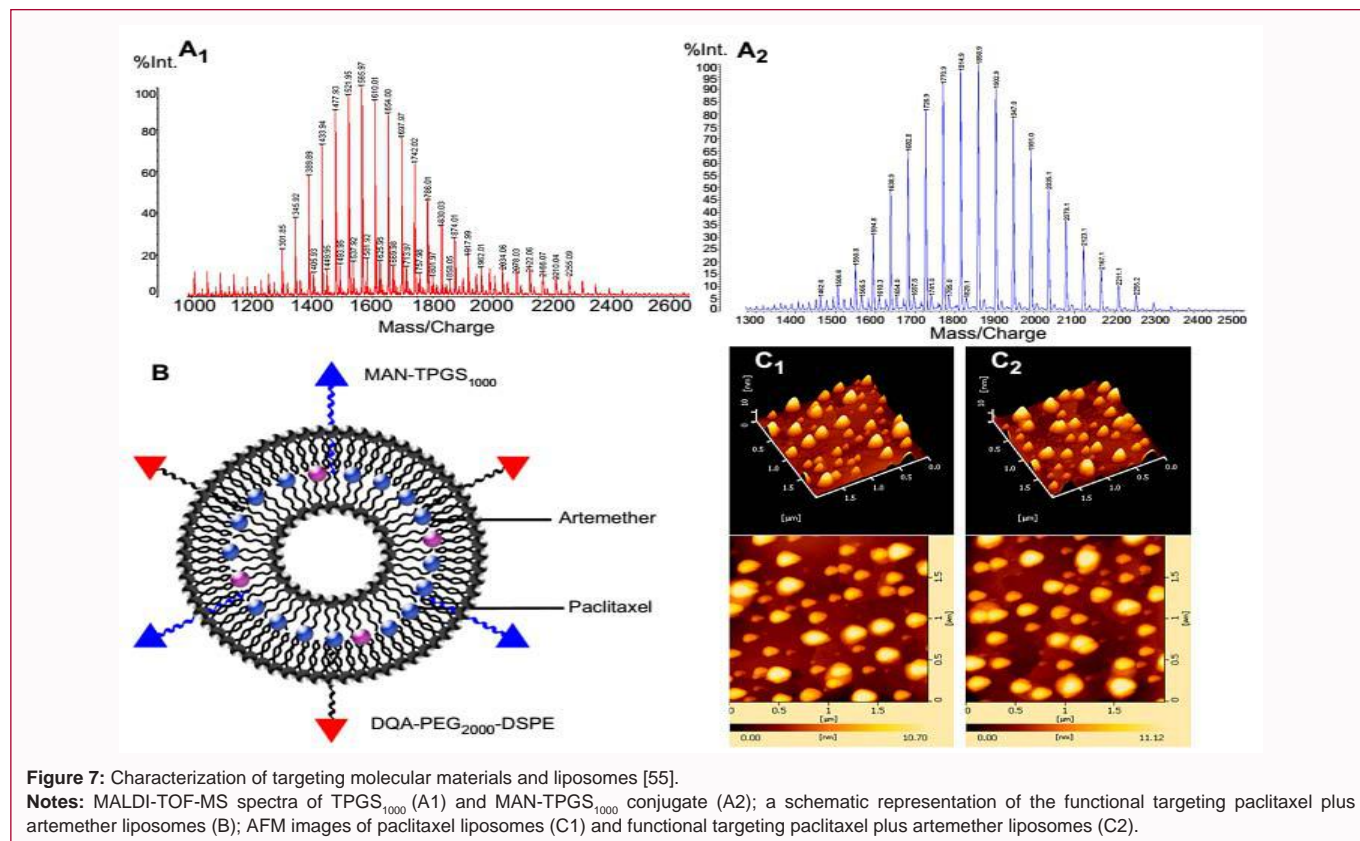

Figure 7: Characterization of targeting molecular materials and liposomes [55].
Notes: MALDI-TOF-MS spectra of $TPGS_{1000}$ (A1) and MAN-$TPGS_{1000}$ conjugate (A2); a schematic representation of the functional targeting paclitaxel plus artemether liposomes (B); AFM images of paclitaxel liposomes (C1) and functional targeting paclitaxel plus artemether liposomes (C2).

liposomes. Hansen et al. [45] evidenced that the activity of human sPLA2 was highly sensitive to phospholipid acyl-chain length and negative surface charge density of the liposomes, thereby triggering drug release of enzyme-sensitive liposomes.

Physical adsorption-mediated liposomes: To adsorb onto the membrane of cancer cells, a targeting effect can be achieved by physical adsorption-mediated liposomes, which utilizes cationic material to modify the surface of liposomes to produce a kind of positively charged liposomes. The electropositive liposomes are able to strongly adsorb onto the electronegative cell membrane of cancer cells. Furthermore, after uptake by cancer cells, the cationic liposomes can further accumulate into mitochondria of living cells in response

to mitochondrial membrane potential (Figure 6) [46-52].

Wang et al. developed mitochondrial targeting resveratrol liposomes by modifying a conjugate of dequalinium (DQA) with polyethylene glycol distearoylphosphatidylethanolamine (PEG_{2000}-DSPE). The results exhibited a significant antitumor efficacy in either cancer cells or drug resistant cancer cells [53]. In addition, Ma et al. developed mitochondrial targeting berberine liposomes by modifying DQA-PEG_{2000}-DSPE [54]. The mitochondrial targeting berberine liposomes could transport across cancer stem cell membrane, and selectively accumulate into the mitochondria of cancer cells. When co-treatment with paclitaxel liposomes, mitochondrial targeting berberine liposomes significantly potentiated the anticancer efficacy in human breast cancer stem cells xenografts in nude mice.

Multifunctional liposomes: Multifunctional liposomes have been developed in the latest years to achieve multiple purposes by which one liposome formulation is able to reach a comprehensive objective by combing passive, active and physicochemical targeting effects. For example, Li et al. developed a kind of multifunctional targeting paclitaxel plus artemether liposomes for treatment of brain glioma [55]. In this construct, paclitaxel was used as the anticancer drug and artemether used as the regulator of apoptosis and inhibitor of vasculogenic mimicry channels. Two functional materials, mannose-vitamin E derivative conjugate (MAN-$TPGS_{1000}$) and dequalinium-lipid derivative conjugate (DQA-PEG_{2000}-DSPE), were used to enhance the capabilities of liposomes in transferring drug across blood-brain barrier (BBB), eliminating brain glioma stem cells and destroying vasculogenic mimicry channels (Figure 7). The transport mechanism of the liposomes across the BBB was associated with receptor-mediated endocytosis by MAN-$TPGS_{1000}$ conjugate *via* glucose transporters, and adsorption-mediated endocytosis by DQA-PEG_{2000}-DSPE conjugate *via* electric charge-based interaction.

Furthermore, Kono et al. [56] designed a kind of multifunctional liposomes which combined the properties of active targeting and temperature-sensitive liposomes, and were used for treatment and diagnosis of human epidermal growth factor 2 (Her-2) positive cancer by imaging, such as ovarian cancer and breast cancer. The liposomes were functionalized with thermosensitive poly[2-(2-ethoxy)ethoxyethyl vinyl ether] chains for triggering drug release of liposomes (approximately 38°C), with conjugation of antibody trastuzumab for targeting Her-2 positive cancer, and with entrapment of indocyanine green for diagnosis by near-infrared fluorescence imaging. The liposomes could retain drug under physiological temperature while release drug immediately at a slightly higher temperature in tumor, and exhibited significant ability in targeting Her-2 positive cancer cells.

Application of Drug Liposomes

Liposomes were first proposed by Bangham et al. [57] in 1965. The study showed that the multilayer vesicles were spontaneously formed with onion-like structure when phospholipids were dispersed in water. This experiment makes people aware of the biodegradability and biocompatibility of liposomes, and lays a foundation for the liposomes to be used as drug carriers. In 1971, Gregoriadis et al. [58] firstly encapsulated amyloglucosidase and ^{131}I-albumin into liposomes. After intravenous injection into rats, the liposomes were mainly distributed in liver and spleen tissues. It indicated that the liposomes could be used for loading bioactive substances, and had a distinctive distribution in specific tissues, i.e., a targeting potential.

With laboratory study in-depth for five decades, a variety of liposomes have been successfully used in the clinical practice, and there are still many liposomes are undergoing clinical trial evaluations.

In 1995, doxorubicin liposomes were approved by Food and Drug Administration (FDA) for treatment of various types of cancer [7]. It is a kind of decorated liposomes with hydrophilic polymer, which is conjugated by polyethylene glycol with distearoylphosphatidyl enthanolamine (PEG_{2000}-DSPE). The use of PEG_{2000}-DSPE conjugate is used to prevent the adsorption of plasma proteins onto liposomes or to prevent opsonization, thereby avoiding the rapid clearance of liposomes by reticuloendothelial system. The pegylated liposomes are able to extend the circulating time in blood circulation and to accumulate more into the specific tissues such as solid tumor tissue. Because of the decreased distribution in heart tissue, doxorubicin liposomes could evidently lower the cardiotoxicity after uses. Nevertheless, an unexpectedly hand-foot syndrome (HFS) has been experienced during clinical application of doxorubicin liposomes. Actually, the pegylated doxorubicin liposomes have become one of the most common causes of HFS. The risk of developing HFS appears to be doxorubicin dose-dependent. Drug formulations that prolong serum drug levels or that concentrate drug at affected sites have higher rates. This may be one reason why doxorubicin liposomes are associated with a higher HFS incidence than the standard, nonencapsulated formulation [59]. In spite of this, pegylated doxorubicin liposomes are still considered as an efficient drug in tumor therapy.

The success of Doxil® has inspired the research and development of liposomal drug delivery systems (Table 1) [7,60-77]. With the gradual study of the liposomes in-depth, a number of drug liposomes have been approved for clinical uses, such as daunorubicin liposomes (DaunoXome®) [60], cytarabine liposome (Depocyt®) [61], vincristine sulfate liposomes (Marqibo®) [64] and nonpegylated doxorubicin liposomes (Myocet®) [65].

Meanwhile, several regular liposomes in clinical trials are expected to be approved by drug administration authority soon. A phase 2 clinical trial showed that the single-agent nanomolecular liposomal annamycin appeared to be well tolerated, and exhibited a significant clinical activity as a single agent in treatment of the refractory adult acute lymphoblastic leukemia [73]. Another phase 2 study indicated that L-NDDP (Aroplatin), a liposomal formulation of a structural analogue of oxaliplatin, was well tolerated in treatment of the refractory patients with advanced colorectal cancer, and demonstrated a positive anti-tumor activity. Further studies of L-NDDP, preferably in combination with other agents such as fluoropyrimidines, are warranted [70].

In addition to these, several active targeting liposomes are undergoing clinical evaluation. MBP-426, a transferrin-mediated liposomes containing oxaliplatin, is now in phase I trial as second line treatment for gastric, gastroesophageal and esophageal adenocarcinomas [72]. The other transferrin-mediated liposomes that contain P53 plasmid DNA is now in phase Ib trial to treat solid tumor [77]. Besides, immunoliposomes also have made a lot progress. MCC-465, a type of pegylated doxorubicin liposomes functionalized with the F(ab)2 of GAH antibody that show a significant anticancer activity against GAH-positive colorectal and gastric cancer cells, is progressed to phase I clinical trial [76].

However, clinical trials also revealed some unexpected adverse events and results. According to early results of an ongoing phase

Table 1: Liposomal anticancer drugs used in clinical uses or in clinical trials.

Drug liposomes	Main materials	Anticancer spectrum	Research unit	Current state
Doxorubicin long circulating liposome(Doxil®)	PEG-DSPE/ phospholipids	Various cancers	Ortho Biotech; Zeneus Pharm	1995 sale in the USA [7]
Daunorubicin liposome (DaunoXome®)	Phospholipids	Various cancers	Gilead	1996 sale in the USA [60]
Cytarabine liposome (Depocyt®)	Phospholipids	Meningeal lymphoma	Enzon	1999 sale in the USA and Canada [61]
Paclitaxel liposome (Lipusu)	Phospholipids	Ovarian cancer	Luye Pharma Group	2004 sale in China [62]
Doxorubicin long circulating liposome(LipoDox)	PEG-DSPE/ phospholipids	Various cancers	Fudan Zhangjiang biomedical	2009 sale in China [63]
Vincristine sulfate liposomes (Marqibo®)	Phospholipids	Hodgkin's lymphoma and leukemia	Talon	2012 sale in the USA [64]
Doxorubicin liposomes (Myocet®)	Phospholipids	Metastatic breast cancer	Elan	2000 sale in EU [65]
Thermosensitive liposomes (ThermoDox)	Phospholipids	Hepatocellular Carcinoma	Celsion	USA III clinical trials [66]
Cisplatin liposomes(SPI-77)	PEG-DSPE/ phospholipids	Head and neck cancer, non-small cell lung cancer	ALZA	USA II clinical trials [67,68]
Vincristine liposomes (Onco TCS)	Phospholipids	Non-Hodgkin's lymphoma	Inex Pharmaceuticals	USA II clinical trials [69]
Oxaliplatin analogue liposomes (Aroplatin/L-NDDP)	Phospholipids	Colorectal cancer	Aronex Pharmaceuticals	USA II clinical trials [70]
Lurtotecan liposomes (OSI-211)	Phospholipids	Various cancers	OSI Pharmaceuticals	USA II clinical trials [71]
Oxaliplatin liposomes (MBP-426)	Transferrin/ phospholipids	Various cancers	Mebiopharm Co., Ltd	USA II clinical trials [72]
Annamycin liposomes (L-annamycin)	Phospholipids	Acute lymphoblastic leukemia	Callisto Pharmaceuticals	USA I/II clinical trials [73]
C-Raf AON cationic liposomes(LErafAON-ETU)	Phospholipids	Solid tumors	INSYS Therapeutics Inc	USA I clinical trials [74]
Sirna liposomes (CALAA-01)	Transferrin/ phospholipids	Solid tumors	Calando Pharmaceuticals	USA I clinical trials [75]
Doxorubicin liposomes (MCC-465)	Mcab-GAH- phospholipids	Gastric carcinoma	Mitsubishi	Japan I clinical trials [76]
P53 plasmid DNA liposomes (SGT-53)	Transferrin/ phospholipids	Solid tumors	SynerGene Therapeutics, Inc.	USA I clinical trials [77]

II trial, liposomal vincristine (Onco-TCS) was active and well tolerated in this heavily pretreated population with relapsed non-Hodgkin's lymphomas, but was neurotoxic in a fraction of patients heavily exposed to prior neurotoxic agents [69]. The clinical study of pegylated liposomal cisplatin (SPI-077) showed that SPI-077 was essentially inactive against squamous cancers of head and neck and only modestly active in patients with non-small-cell lung cancer [67,68]. ThermoDox, a thermally sensitive liposomal doxorubicin, was considered as an efficient agent against liver cancer. However it was announced that ThermoDox failed in the phase 3 study, and it was unable to demonstrate a significant improvement in progression-free survival. Nonetheless, ThermoDox is not given up and a phase 3 study (OPTIMA) is conducted now to determine whether ThermoDox is effective in the treatment of non-resectable hepatocellular carcinoma when used in conjunction with standardized radiofrequency ablation (sRFA).

Concluding Remarks

Up to date, the manufacturing methods of liposomes are becoming more and more mature, including film dispersion, reverse-phase evaporation, chemical gradient loading and the other encapsulation approaches. A variety of liposomal strategies have been developed to improve efficiency and functions of drug delivery through passive, active and physicochemical targeting methods. These new liposomal formulations are undertaking laboratory evaluations, exhibiting a promising and broad prospect, especially in the field of chemotherapy of cancers. In clinical application, tens of drug liposomes have been approved for clinical use meanwhile plenty of drug liposomes are undergoing clinical trial evaluations. During clinical trials and uses, the liposomes have been evidenced having an optimal drug delivery efficiency and a better efficacy, despite the anticancer drug liposomes may lead to new side effect like hand-foot syndrome induced by the pegylated doxorubicin liposomes. The drug liposomes can be effectively accumulated into the lesion or tumor site, demonstrate a better efficacy and a reduced adverse reaction such as cardiotoxicity of doxorubicin. Varying liposomal formulations are useful in improving the property of chemical drug itself. As a typical example, paclitaxel liposomes that are approved by State Food and Drug Administration of China (SFDA) in the 2004 are able to diminish the severe allergy as compared to the regular paclitaxel injection in which an allergic surfactant Cremophor is used as a solubilizer of insoluble paclitaxel. Besides, the liposomal formulations are capable of potentiating the efficacy of anticancer drugs by circumventing multidrug resistance of cancer and cancer stem cells, and by penetrating across biological barriers (the BBB barrier). These new functions have been evidenced in laboratory observations while await for clinical evaluations. It is believed that the liposomal drug delivery systems would have more applications in the field of chemotherapy with the progress of science and the development of pharmaceutical technology.

Acknowledgment

References

1. Eloy JO, Claro de Souza M, Petrilli R, Barcellos JP, Lee RJ, Marchetti JM. Liposomes as carriers of hydrophilic small molecule drugs: strategies to enhance encapsulation and delivery. Colloids Surf B Biointerfaces. 2014; 123: 345-63.

2. Vijayakumar MR, Vajanthri KY, Balavigneswaran CK, Mahto SK, Mishra N, Muthu MS, et al. Pharmacokinetics, biodistribution, *in vitro* cytotoxicity and biocompatibility of Vitamin E TPGS coated trans resveratrol liposomes. Colloids Surf B Biointerfaces. 2016; 145: 479-491.

3. Woodle MC, Lasic DD. Sterically Stabilized Liposomes. Biochim Biophys Acta. 1992; 1113: 171-199.

4. Zucker D, Barenholz Y. Optimization of vincristine-topotecan combination--paving the way for improved chemotherapy regimens by nanoliposomes. J Control Release. 2010; 146: 326-333.

5. Qiao Y, Huang X, Nimmagadda S, Bai R, Staedtke V, Foss CA, et al. A robust approach to enhance tumor-selective accumulation of nanoparticles. Oncotarget. 2011; 2: 59-68.

6. Forssen EA, Tokes ZA. Use of Anionic Liposomes for the Reduction of Chronic Doxorubicin-Induced Cardiotoxicity. P Natl Acad Sci-Biol. 1981; 78: 1873-1877.

7. Barenholz Y. Doxil°-the first FDA-approved nano-drug: lessons learned. J Control Release. 2012; 160: 117-134.

8. Chopra R, Blair S, Strang J, Cervi P, Patterson KG, Goldstone AH. Liposomal amphotericin B (AmBisome) in the treatment of fungal infections in neutropenic patients. J Antimicrob Chemother. 1991; 28: 93-104.

9. Gambling D, Hughes T, Martin G, Horton W, Manvelian G. A comparison of Depodur, a novel, single-dose extended-release epidural morphine, with standard epidural morphine for pain relief after lower abdominal surgery. Anesth Analg. 2005; 100: 1065-1074.

10. Bangham AD, Standish MM, Weissmann G. The action of steroids and streptolysin S on the permeability of phospholipid structures to cations. J Mol Biol. 1965; 13: 253-259.

11. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol. 1965; 13: 238-252.

12. Szoka F Jr, Papahadjopoulos D. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. Proc Natl Acad Sci U S A. 1978; 75: 4194-4198.

13. Sakai H, Gotoh T, Imura T, Sakai K, Otake K, Abe M. Preparation and properties of liposomes composed of various phospholipids with different hydrophobic chains using a supercritical reverse phase evaporation method. J Oleo Sci. 2008; 57: 613-621.

14. Garg M, Garg BR, Jain S, Mishra P, Sharma RK, Mishra AK, et al. Radiolabeling, pharmacoscintigraphic evaluation and antiretroviral efficacy of stavudine loaded 99mTc labeled galactosylated liposomes. Eur J Pharm Sci. 2008; 33: 271-281.

15. Men Y, Wang XX, Li RJ, Zhang Y, Tian W, Yao HJ, et al. The efficacy of mitochondrial targeting antiresistant epirubicin liposomes in treating resistant leukemia in animals. Int J Nanomedicine. 2011; 6: 3125-3137.

16. Haran G, Cohen R, Bar LK, Barenholz Y. Transmembrane ammonium sulfate gradients in liposomes produce efficient and stable entrapment of amphipathic weak bases. Biochim Biophys Acta. 1993; 1151: 201-215.

17. Qin J, Chen D, Lu W, Xu H, Yan C, Hu H, et al. Preparation, characterization, and evaluation of liposomal ferulic acid *in vitro* and *in vivo*. Drug Dev Ind Pharm. 2008; 34: 602-608.

18. Kheirolomoom A, Kruse DE, Qin S, Watson KE, Lai CY, Young LJ, et al. Enhanced *in vivo* bioluminescence imaging using liposomal luciferin delivery system. J Control Release. 2010; 141: 128-136.

19. Li N, Zhuang C, Wang M, Sun X, Nie S, Pan W. Liposome coated with low molecular weight chitosan and its potenial use in ocular drug delivery. Int J Pharm. 2009; 379: 131-138.

20. Batzri S, Korn ED. Single bilayer liposomes prepared without sonication. Biochim Biophys Acta. 1973; 298: 1015-1019.

21. Deamer DW. Preparation and properties of ether-injection liposomes. Ann N Y Acad Sci. 1978; 308: 250-258.

22. Mozafari MR. Liposomes: an overview of manufacturing techniques. Cell Mol Biol Lett. 2005; 10: 711-719.

23. Li C, Deng Y. A novel method for the preparation of liposomes: freeze drying of monophase solutions. J Pharm Sci. 2004; 93: 1403-1414.

24. Imanaka H, Koide H, Shimizu K, Asai T, Kinouchi Shimizu N, Ishikado A, et al. Chemoprevention of tumor metastasis by liposomal beta-sitosterol intake. Biol Pharm Bull. 2008; 31: 400-404.

25. Igarashi A, Konno H, Tanaka T, Nakamura S, Sadzuka Y, Hirano T, et al. Liposomal photofrin enhances therapeutic efficacy of photodynamic therapy against the human gastric cancer. Toxicol Lett. 2003; 145: 133-141.

26. Awasthi VD, Garcia D, Goins BA, Phillips WT. Circulation and biodistribution profiles of long-circulating PEG-liposomes of various sizes in rabbits. Int J Pharm. 2003; 253: 121-132.

27. Crosasso P, Ceruti M, Brusa P, Arpicco S, Dosio F, Cattel L. Preparation, characterization and properties of sterically stabilized paclitaxel-containing liposomes. J Control Release. 2000; 63: 19-30.

28. Yokoe J, Sakuragi S, Yamamoto K, Teragaki T, Ogawara K, Higaki K, et al. Albumin-conjugated PEG liposome enhances tumor distribution of liposomal doxorubicin in rats. Int J Pharm. 2008; 353: 28-34.

29. Cogswell S, Berger S, Waterhouse D, Bally MB, Wasan EK. A parenteral econazole formulation using a novel micelle-to-liposome transfer method: *in vitro* characterization and tumor growth delay in a breast cancer xenograft model. Pharm Res. 2006; 23: 2575-2585.

30. Fanciullino R, Giacometti S, Aubert C, Fina F, Martin PM, Piccerelle P, et al. Development of stealth liposome formulation of 2'-deoxyinosine as 5-fluorouracil modulator: *in vitro* and *in vivo* study. Pharm Res. 2005; 22: 2051-2057.

31. Zeng F, Ju R J, Liu L, Xie HJ, Mu LM, Zhao Y, et al. Application of functional vincristine plus dasatinib liposomes to deletion of vasculogenic mimicry channels in triple-negative breast cancer. Oncotarget. 2015; 6: 36625-36642.

32. Sriraman SK, Salzano G, Sarisozen C, Torchilin V. Anti-cancer activity of doxorubicin-loaded liposomes co-modified with transferrin and folic acid. eur j pharm biopharm. 2016; 105: 40-49.

33. Huwyler J, Wu D, Pardridge WM. Brain drug delivery of small molecules using immunoliposomes. Proc Natl Acad Sci U S A. 1996; 93: 14164-14169.

34. Abbott N J, Rönnbäck L, Hansson E. Astrocyte–endothelial interactions at the blood–brain barrier. Nat Rev Neurosci. 2006; 7: 41-53.

35. Loureiro JA, Gomes B, Fricker G, Cardoso I, Ribeiro CA, Gaiteiro C, et al. Dual ligand immunoliposomes for drug delivery to the brain. Colloids Surf B Biointerfaces. 2015; 134: 213-219.

36. Deshpande PP, Biswas S, Torchilin VP. Current trends in the use of liposomes for tumor targeting. Nanomedicine (Lond). 2013; 8: 1509-1528.

37. Kanamala M, Wilson WR, Yang M, Palmer BD, Wu Z. Mechanisms and biomaterials in pH-responsive tumour targeted drug delivery: A review. Biomaterials. 2016; 85: 152-167.

38. Cardone RA, Casavola V, Reshkin SJ. The role of disturbed pH dynamics and the Na+/H+ exchanger in metastasis. Nat Rev Cancer. 2005; 5: 786-95.

39. Wang L, Geng D, Su H. Safe and efficient pH sensitive tumor targeting modified liposomes with minimal cytotoxicity. Colloids Surf B Biointerfaces. 2014; 123: 395-402.

40. Júnior AD, Mota LG, Nunan EA, Wainstein AJ, Wainstein AP, Leal AS, et al. Tissue distribution evaluation of stealth pH-sensitive liposomal cisplatin versus free cisplatin in Ehrlich tumor-bearing mice. Life Sci. 2007; 80: 659-64.

41. Moku G, Gulla SK, Nimmu NV, Khalid S, Chaudhuri A. Delivering anti-cancer drugs with endosomal pH-sensitive anti-cancer liposomes. Biomater Sci. 2016; 4: 627-638.

42. Yatvin MB, Weinstein JN, Dennis WH, Blumenthal R. Design of liposomes for enhanced local release of drugs by hyperthermia. Science. 1978; 202: 1290-1293.

43. Kakinuma K, Tanaka R, Takahashi H, Sekihara Y, Watanabe M, Kuroki M. Drug delivery to the brain using thermosensitive liposome and local hyperthermia. Int J Hyperthermia. 1996; 12:157-165.

44. Mura S, Nicolas J, Couvreur P. Stimuli-responsive nanocarriers for drug delivery. Nat Mater. 2013; 12: 991-1003.

45. Hansen AH, Mouritsen OG, Arouri A. Enzymatic action of phospholipase A₂ on liposomal drug delivery systems. Int J Pharm. 2015; 491: 49-57.

46. Yamada Y, Akita H, Kogure K, Kamiya H, Harashima H. Mitochondrial drug delivery and mitochondrial disease therapy--an approach to liposome-based delivery targeted to mitochondria. Mitochondrion. 2007; 7: 63-71.

47. Flierl A, Jackson C, Cottrell B, Murdock D, Seibel P, Wallace DC. Targeted delivery of DNA to the mitochondrial compartment via import sequence-conjugated peptide nucleic acid. Mol Ther. 2003; 7: 550-557.

48. Schatz G. The protein import system of mitochondria. J Biol Chem. 1996; 271: 31763-31766.

49. Del GV, Payne RM. A novel TAT-mitochondrial signal sequence fusion protein is processed, stays in mitochondria, and crosses the placenta. Mol Ther. 2003; 7: 720-730.

50. Smith RA, Porteous CM, Coulter CV, Murphy MP. Selective targeting of an antioxidant to mitochondria. Eur J Biochem. 1999; 263: 709-716.

51. Weissig V, Torchilin VP. Cationic bolasomes with delocalized charge centers as mitochondria-specific DNA delivery systems. Adv Drug Deliv Rev. 2001; 49: 127-149.

52. Shokolenko IN, Alexeyev MF, LeDoux SP, Wilson GL. TAT-mediated protein transduction and targeted delivery of fusion proteins into mitochondria of breast cancer cells. DNA Repair (Amst). 2005; 4: 511-518.

53. Wang XX, Li YB, Yao HJ, Ju RJ, Zhang Y, Li RJ, et al. The use of mitochondrial targeting resveratrol liposomes modified with a dequalinium polyethylene glycol-distearoylphosphatidyl ethanolamine conjugate to induce apoptosis in resistant lung cancer cells. Biomaterials. 2011; 32: 5673-5687.

54. Ma X, Zhou J, Zhang CX, Li XY, Li N, Ju RJ, et al. Modulation of drug-resistant membrane and apoptosis proteins of breast cancer stem cells by targeting berberine liposomes. Biomaterials. 2013; 34: 4452-4465.

55. Li XY, Zhao Y, Sun MG, Shi JF, Ju RJ, Zhang CX, et al. Multifunctional liposomes loaded with paclitaxel and artemether for treatment of invasive brain glioma. Biomaterials. 2014; 35: 5591-604.

56. Kono K, Takashima M, Yuba E, Harada A, Hiramatsu Y, Kitagawa H, et al. Multifunctional liposomes having target specificity, temperature-triggered release, and near-infrared fluorescence imaging for tumor-specific chemotherapy. J Control Release. 2015; 216: 69-77.

57. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol. 1965; 13: 238-252.

58. Gregoriadis G, Ryman BE. Liposomes as carriers of enzymes or drugs: a new approach to the treatment of storage diseases. Biochem J. 1971; 124: 58.

59. Miller KK, Gorcey L, McLellan BN. Chemotherapy–induced hand-foot syndrome and nail changes A review of clinical presentation, etiology, pathogenesis, and management. J Am Acad Dermatol. 2014; 71: 787-794.

60. Pratt G, Wiles ME, Rawstron AC, Davies FE, Fenton JA, Proffitt JA, et al. Liposomal daunorubicin: *in vitro* and *in vivo* efficacy in multiple myeloma. Hematol Oncol. 1998; 16: 47-55.

61. Benesch M, Urban C. Liposomal cytarabine for leukemic and lymphomatous meningitis: recent developments. Expert Opin Pharmacother. 2008; 9: 301-309.

62. Zhang Q, Huang XE, Gao LL. A clinical study on the premedication of paclitaxel liposome in the treatment of solid tumors. Biomed Pharmacother. 2009; 63: 603-607.

63. Taiwanese Gynecologic Oncology Group, Chou HH, Wang KL, Chen CA, Wei LH, Lai CH, et al. Pegylated liposomal doxorubicin (Lipo-Dox) for platinum-resistant or refractory epithelial ovarian carcinoma: a Taiwanese gynecologic oncology group study with long-term follow-up. Gynecol Oncol. 2006; 101: 423-428.

64. Rodriguez MA, Pytlik R, Kozak T, Chhanabhai M, Gascoyne R, Lu B, et al. Vincristine sulfate liposomes injection (Marqibo) in heavily pretreated patients with refractory aggressive non-Hodgkin lymphoma: report of the pivotal phase 2 study. Cancer. 2009; 115: 3475-82.

65. CE Swenson, WR Perkins, P Roberts, AS Janoff. Liposome technology and the development of MyocetTM (liposomal doxorubicin citrate). The Breast. 2001; 10: 1-7.

66. Poon RT, Borys N. Lyso-thermosensitive liposomal doxorubicin: a novel approach to enhance efficacy of thermal ablation of liver cancer. Expert Opin Pharmacother. 2009; 10: 333-343.

67. Harrington KJ, Lewanski CR, Northcote AD, Whittaker J, Wellbank H, Vile RG, et al. Phase I-II study of pegylated liposomal cisplatin (SPI-077) in patients with inoperable head and neck cancer. Ann Oncol. 2001; 12: 493-496.

68. Seetharamu N, Kim E, Hochster H, Martin F, Muggia F. Phase II study of liposomal cisplatin (SPI-77) in platinum-sensitive recurrences of ovarian cancer. Anticancer Res. 2010; 30: 541-545.

69. Sarris AH, Hagemeister F, Romaguera J, Rodriguez MA, McLaughlin P, Tsimberidou AM, et al. Liposomal vincristine in relapsed non-Hodgkin's lymphomas: early results of an ongo-ing phase II trial. Ann Oncol. 2000; 11: 69-72.

70. Dragovich T, Mendelson D, Kurtin S, Richardson K, Von Hoff D, Hoos A. A Phase 2 trial of the liposomal DACH platinum L-NDDP in patients with therapy-refractory advanced colorectal cancer. Cancer Chemother Pharmacol. 2006; 58: 759-764.

71. Seiden MV, Muggia F, Astrow A, Matulonis U, Campos S, Roche M, et al. A phase II study of liposomal lurtotecan (OSI-211) in patients with topotecan resistant ovarian cancer. Gynecol Oncol. 2004; 93: 229-232.

72. Sankhala KK, Mita AC, Adinin R, Wood L, Beeram M, Bullock S, et al. A phase I pharmacokinetic (PK) study of MBP-426, a novel liposome encapsulated oxaliplatin. J Clin Oncol. 2009; 27: 2535.

73. Wetzler M, Thomas DA, Wang ES, Shepard R, Ford LA, Heffner TL, et al. Phase I/II trial of nanomolecular liposomal annamycin in adult patients with relapsed/refractory acute lymphoblastic leukemia. Clin Lymphoma Myeloma Leuk. 2013; 13: 430-434.

74. Rudin CM, Marshall JL, Huang CH, Kindler HL, Zhang C, Kumar D, et al. Delivery of a liposomal c-raf-1 antisense oligonucleotide by weekly bolus dosing in patients with advanced solid tumors: a phase I study. Clin Cancer Res. 2004; 10: 7244-7251.

75. Zuckerman JE, Gritli I, Tolcher A, Heidel JD, Lim D, Morgan R, et al. Correlating animal and human phase Ia/Ib clinical data with CALAA-01, a targeted, polymer-based nanoparticle containing siRNA. Proc Natl Acad Sci U S A. 2014; 111: 11449-11454.

76. Matsumura Y, Gotoh M, Muro K, Yamada Y, Shirao K, Shimada Y, et al. Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer. Ann Oncol. 2004; 15: 517-525.

77. Senzer N, Nemunaitis J, Nemunaitis D, Bedell C, Edelman G, Barve M, et al. Phase I study of a systemically delivered p53 nanoparticle in advanced solid tumors. Mol Ther. 2013; 21: 1096-1103.