Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 6, Issue 4, 2014

Review Article

EVOLUTION OF LIPOSOMAL CARRIERS INTENDED TO ANTICANCER DRUG DELIVERY: AN OVERVIEW

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Received: 14 August 2014, Revised and Accepted: 03 September 2014

ABSTRACT

Most current anticancer agents present lack of specificity, leading to systemic toxicity and adverse effects, and limiting the maximum dose of drug. Liposomes quickly passed from a simple scientific curiosity to "magic bullets" for the delivery of drugs. Liposomal formulations of anticancer drugs have been extensively evaluated, with notorious advances and the market introduction of some of them. In the last years the research under liposomes has been carried out to increase the circulation time and the specificity to cancer cells. The aim of this work was to make a review about the research carried out about the application of liposomes as carriers for anticancer drugs. Liposomal formulations of anticancer drugs have been extensively evaluated. However, many other liposome based carriers were studied, like immunoliposomes, thermossensitive liposomes, dual functional liposomes and crosslinked multifunctional liposomes, intended to increase drug specificity. Additionally, some special types of liposomes, like niosomes, transfersomes and ethosomes were also investigated as cytotoxic drug carriers.

Keywords: Cancer, Drug delivery, Liposomes, Liposomes derivatives, Special liposomes

INTRODUCTION

Cancer is a term used for a heterogeneous group of malignant diseases in which abnormal cells divide without control and are able to invade other tissues. A mass of cancerous cells is called tumor and an uncontrolled growth of a tumor results in destruction of health tissue around. The dissemination of tumor cells through blood or lymphatic stream leads to the formation of secondary tumors or metastasis in other organs or tissues. There are more than 100 different types of cancer [1]. Cancer is a multifunctional disease that can arise through the influence of environmental factors, and life style. Some knew risk factors include high-fat diet, smoking and excess alcohol intake, viral infections and immune system perturbations. All cancers begin in cells, which are the body's basic unit of life [1].

According to data of GLUBOCAN project from International Agency for Research on Cancer (IARC), World Health Organization (WHO), there were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012, worldwide. A percentage of 57% of the new cancer cases, 65% of the cancer deaths and 48% of the 5-year prevalent cancer cases occurred in the less developed regions. Cancer incidence rate is almost 25% higher in men than in women [2].

The main treatments for cancer are surgery and radiation, which are considered local treatments. Local control rates with radiation therapy alone, for patients treated with curative intent is about 50%, with some improvement seen when radiation therapy is combined with chemotherapy [3]. Chemotherapy finds its main use as an adjuvant to surgery and radiotherapy. Although the adverse effects of surgery and radiotherapy, chemotherapy is the third option in cancer management. This can be due to the fact that, although some excellent drugs are available, the efficacy of many existing chemotherapeutic drugs is limited by their inability to reach their therapeutic site of action in sufficient amounts to be efficacious [4]. In most cases only a small fraction of the administered drug reaches the site of action, whilst the rest of the drug is distributed throughout the body. Is this unavoidable distribution into healthy organs and tissues, and the depression of the immune system, that limits the dosage that can be given, and in turn, prevents these drugs from achieving the potential cures that they are clearly capable [5]. It is well recognized that when systemic chemotherapy is used in the treatment of solid tumors, it is almost impossible to achieve the therapeutic levels of drug at the tumor site without damaging healthy organs and tissues [6]. An inability to control the growth of primary (or regional) tumors, however, leads to fatality in a significant number of patients. In approximately one half of patients treated by local therapies, local control can be extremely difficult, the treatment fails, and the tumor grows back [5]. Because of the precarious blood supply and often high interstitial fluid pressure, many cancer chemotherapeutic agents are not-effectively delivered to the tumor region [6]. This situation is heightened by the need for almost 100% cell kill to obtain a cure. The tumor vessel wall represents a significant barrier for many therapeutic agents, and nonspecific delivery can lead to significant systemic toxicities and a low therapeutic index that is often seen with current cancer chemotherapeutics that use injected free drugs [7,8].

Role of carrier characteristics in drug delivery processes

Four key requirements of drug carrier design are essential to the overall function and performance of the carrier system. These requirements are: retain de drug; escape to the immune system, for example, extending the circulation time; target (passive) to the diseased site while avoiding most healthy organs; and release of the drug [9]. Drug delivery vehicles introduced in the bloodstream undergo a complex journey prior to arriving at the target site. The carriers need to circulate through the vasculature and interact with the reticuloendothelial system (RES). The RES is the body's primary mechanism of clearing and corresponds to the phagocytic cells, as macrophages [10]. Additionally, the carrier has to escape to the filtration that takes place in the spleen and in the kidney. If the carrier can overcome these cleaning mechanisms, it is able to adhere at the desired site in the vasculature or permeate through the vasculature into the desired tissue. This is followed by diffusion of the carrier through the interstitial space, attachment to the target cell membrane and endocytosis. The particle parameters that can influence these processes include size, shape, surface chemistry and mechanical flexibility [11]. These particle parameters and the processes that they influence are schematized in **Fig. 1**.

Particle size has a significant impact on the circulation time and for intravenous application the particles need to have a size intended to not clog the smallest capillaries [12]. Size has also impact on splenic and renal clearance of particles. Particles larger than 200 nm are susceptible to elimination through the splenic filtration while particles smaller than 10 nm are cleared by kidneys filtering systems [13]. Particle size influences the extent of cellular uptake by phagocytosis and endocytosis. Particles smaller than 500 nm are usually internalized by endocytosis whereas particles larger than 500 nm are believed to be internalized by phagocytosis [14]. Lavermanet al. have studied the effect of liposomes size on the removal rate, using liposomes with identical composition. These authors have observed

that liposomes with greater size were removed faster from the blood stream, with a half-life of 0.2 hours, comparatively with small vesicles that showed a half-life of 1.5 hours [15].

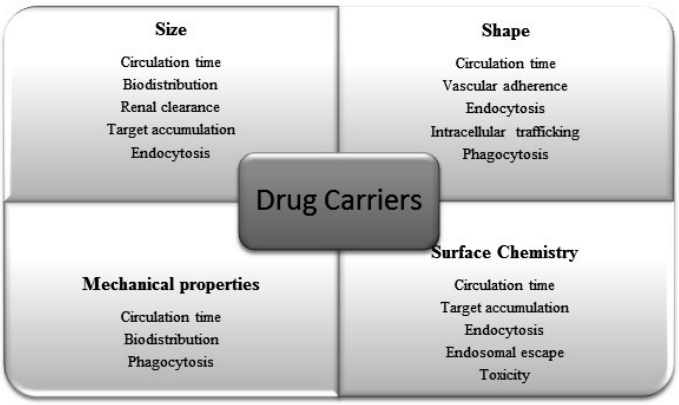

Fig. 1: Role of key carrier properties in drug delivery (adapted from 11)

Many studies have demonstrated the accumulation of nanocarriers in the abnormal tumor microenvironment through the enhanced permeability and retention (EPR) effect as an advantage of nanoparticle-based drugs[16-18]. The EPR effect results from the combination of the generally leaky microvasculature and missing or tight lymphatic capillary system [19].

Liposomes

Liposomes or phospholipid-based nanovesicles were first described in the mid-sixties, and have attracted attention because of their potential as drug delivery systems [20]. Following their discovery, liposomes were quickly developed for a range of potential uses and investigations which sought to exploit the capsular and biocompatibility properties of the lipid membrane in drug delivery applications [5,21]. These are small spherical vesicles with diameters varying from nanometers (> 25 nm) to few micrometers, essentially constituted by phospholipids and other constituents from cellular membrane, like cholesterol, which when in contact with water can originate double molecular layers, which, in turn form mono or poly-compartmental structures. **Fig. 2** illustrates the formation of mono or poly-compartmental vesicles.

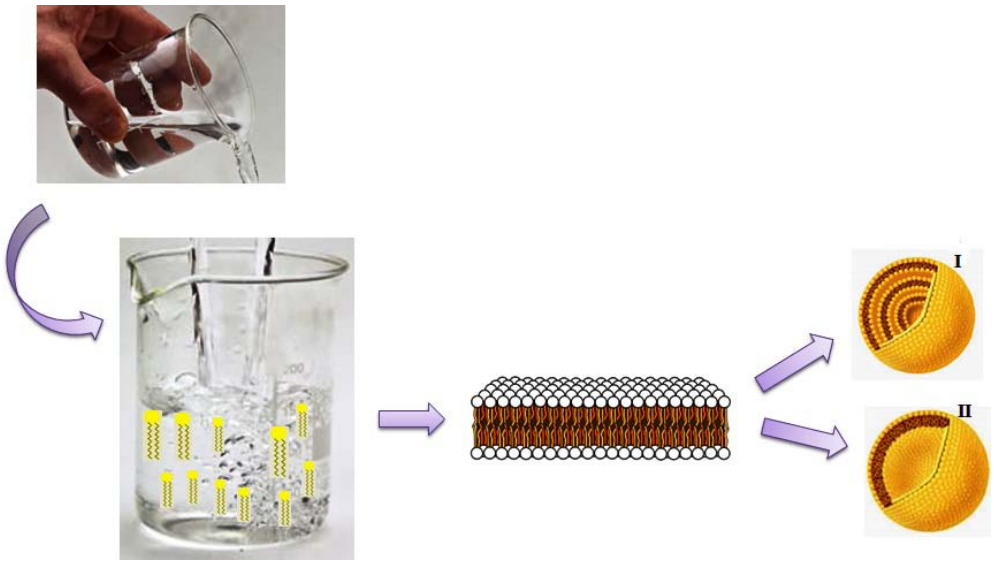

Fig. 2: Representation of liposomes formation. When in contact with water, phospholipids form a lipid bilayer that form poly-compartmental (I) or mono-compartmental vesicles (II).

Quickly, liposomes have passed from a simple scientific curiosity to "magic bullets" intended to carry drugs and can be considered one of the most popular nanocarriers for delivering many biologically active substances [22]. Liposomes can encapsulate hydrophilic and/or lipophilic drugs, wherein the first stay in the aqueous compartment and the seconds inserted or adsorbed in the membrane. Because they are biodegradable, biocompatible and no immunogenic, they are extremely versatile in therapeutics, research and analytical applications [23-25]. Liposomal formulations of anticancer drugs have been extensively evaluated for treating cancers [18,26,27]. In the last two decades, notorious advances occurred with the commercialization of pharmaceutical liposomal formulations as is the example of liposomal daunorubicin (DAU) (Daunoxome™, NeXtar, Inc.) and doxorubicin (DOX) (Doxil™, Sequus Pharmaceuticals), that showed more prolonged circulation times, greater release, an accumulation in the tumor tissue, increasing the drug efficacy and decreasing the side effects. The most recent examples of antineoplastic drugs encapsulated in liposomes, include Marqibo™ (Inex Pharmaceutical Corporation), a formulation containing vincristine sulfate, and DepoCyt™ a formulation for controlled released of cytarabine[28]. Additionally, other liposomal formulations are in clinical trials. **Table 1** presents some examples of liposomal platinum drugs under clinical trials [29].

Table 1: Liposomal platinum drugs under clinical trials [29].

Formulation	Incorporated drug	Approx. size	Clinical phase	Indication
Lipoplatin	Cisplatin	110 nm	Phase III	NSCLC, breast cancer, gastric cancer
SPI-77	Cisplatin	110 nm	Phase II	Advanced NSCLC, refractory ovarian cancer
Aroplatin(L-NDDP)	NDDP	1 µm	Phase II	Refractory colorectal cancer, malignant pleural mesothelioma
Lipoxal	Oxaliplatin	250 nm	Phase I	Advanced gastrointestinal cancer
MBP-426	Oxaliplatin	100 nm	Phase II	Gastric, gastroesophageal, esophageal adenocarcinomas

NSCLC: Non-small-cell lung carcinoma; NDDP: cis-bisneodecanoato-trans-R,R-1,2-diaminocyclohexane platinum, is a cisplatin analog.

By definition, liposomes are spherical vesicles formed by one or various lipid bilayers concentrically arranged, trapping therein one or more aqueous compartments. Their diameter and layers number depends, primarily, from the manufacture process [21]. According their dimensions and compartments, liposomes can be classified in three categories, namely, small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), multilamellar vesicles (MLV). In **Table 2** it is indicated the diameter and susceptible volume to be encapsulated by each liposome category [30].

Table 2: Susceptible volume to be encapsulated by each liposome category [30].

	Diameter (nm)	Encapsulated volume (µl/mg lipid)
SUV	20-100	0.5
LUV	100-800	13.7
MLV	500-5000	4.1

Liposomes are composed of substances with low intrinsic toxicity, and they can be formulated in a large range of sizes and chemical compositions. For clinical use, liposomes are commonly composed of neutral phospholipids and cholesterol and they have average diameters of 50 to 100 nm. The bilayer is impermeable to large molecules (such as proteins and enzymes) and has low permeability to charged molecules, including protons and other cations[20]. Because of the unique structural properties of liposomes, hydrophilic drugs can be entrapped in the aqueous interior of the liposomes and lipid soluble drugs can be incorporated into the hydrophobic core of the phospholipid bilayer. Even drugs of intermediate solubility such as DOX and DAU, can be stably associated with the liposome interior by manipulation of the internal liposomal pH or by addition of counter-ions to form molecular complexes of the drug within the liposome interior [20].

Although phospholipids are biodegradable and non-toxic amphiphiles, problems arise in practical applications of liposomes, because of the low physical and chemical stability of aqueous suspensions of this type of vesicles[31]. During a prolonged storage, liposomes can suffer various kinds of changes over time, such as physical, chemical and biological, that determine the liposomes half-life [21,32]. Regarding physical stability, depending on their composition and environment, liposomes can suffer aggregation, fusion, deposition, membrane rupture, or loss of content. Including a small proportion of charged lipids, the aggregation can be controlled by electrostatic repulsion. The permeability and diffusion of the encapsulated drug can be decreased by addition of cholesterol to the formulation [33]. Chemical instability depends on the liposomes composition, namely from the hydrolysis and oxidation of lipid bilayer constituents[34].

Liposomes derivatives

As previously mentioned, in order to achieve maximum targeting, liposomes should remain in the systemic circulation for a relatively long period of time. However, formulations of liposomes used in the past were rapidly removed from the circulation by the RES [35,36]. Based on this, several studies have been conducted in order to improve the characteristics of liposomes as carriers of antitumor drugs.

Pegylated liposomes

Many studies have focused on the use of liposomes with polyethylene glycol (PEG) attached in their surface. The presence of PEG reduces serum proteins binding (opsonins) and increases the circulation time to hours or days [37]. PEG also increases vascular permeability to liposomes, facilitating increased accumulation of drug containing liposomes in tumor tissues [38].

Doxil®or Caelyx® is the drug DOX encapsulated in PEG-liposome, approved in USA and Europe, respectively. Notably, the pegylated liposomes encapsulating DOX exhibited an improved safety profile by reducing cardiac toxicity and enhancing penetration and accumulation in solid tumors. Consequently, it has been used in the treatment of a wide range of cancers [39-41].

Indeed, such liposomal drug formulations do appear to improve accumulation of liposomes at the tumor site. However, slow and incomplete drug release could still lead to low drug bioavailability within tumor tissue, limiting, in turn, therapeutic activity [42-45]. Furthermore, a lack of controlled-release properties of encapsulated drug may lead to toxic side effects, such as palmar-plantar erythrodysesthesia that is thought to result from unwanted drug distribution to skin during prolonged circulation of liposomal DOX [46]. Efforts to design liposomes that are pH sensitive, temperature sensitive, antibody targeted, or fusogenic have all been pursued with various degrees of success [47].

Immunoliposomes

The first attempts to create the immune-conjugated liposomes were performed in the late 70s - early 80s. *In vivo* studies have revealed that coating liposomes with antibodies leads to enhanced uptake of immunoliposomes by the RES and the immunotargeting efficiency depends on the antibody density on the surface [48,49]. Given a suitable antibody with high specificity and affinity for the target antigen, the critical factor is the accessibility of target cells to immunoliposomes[37]. The earlier attempts to improve of the circulation longevity of immunoliposomes were performed by simple co-mobilization of an antibody and PEG on the surface of the same liposomal composition and a significant increase of their circulation time was achieved [50-52]. **Fig. 3** is an illustration of an immunoliposome, with the antibodies attached to liposome through a PEG chain.

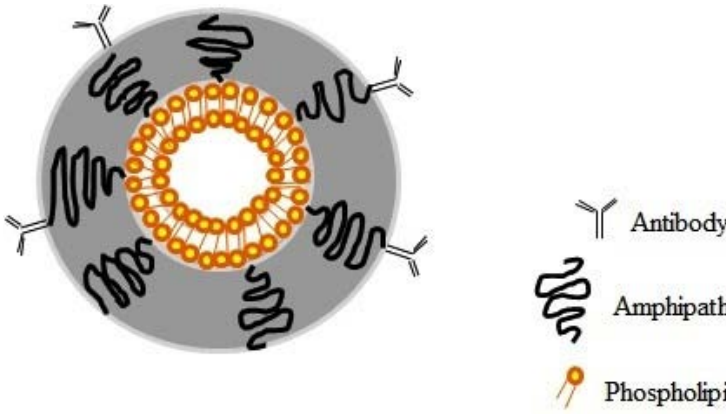

Fig. 3: Immunoliposome with co-mobilization of an antibody and PEG on the surface (adapted from 37)

The majority of long-circulating immunoliposomes are targeted for the delivery of anticancer drugs [53]. Thus, the clinical success of DOX-loaded long circulating pegylated liposomes (Doxyl®/Caelyx®) used in the treatment of metastatic breast cancer, progressive ovarian cancer, multiple myeloma and AIDS-related Kaposi's Sarcoma, stimulated numerous experimental attempts for the improvement of their targeting properties by surface immobilization of different antibodies or their fragments against specific tumor antigens [54,55].

As an example, the modification of pegylated DOX-liposomes with monoclonal antibodies or their Fab fragments against HER2, a member of the epidermal growth factor receptor (EGFR/ErbB) family frequently over-expressed on various cancer cells, successfully improved the tumor delivery and therapeutic efficiency of liposomal DOX in different HER2-overexpressing mouse xenograft models [56-59].

Another example is CD19, an internalizing receptor overexpressed in most types of B-lymphoid malignancies, is a promising targeting antigen. Introduction of anti-CD19 monoclonal antibodies or its Fab' fragments to PEG-liposomes loaded with DOX clearly enhanced targeting and therapeutic efficacy in mice bearing a human CD19+ B-lymphoma [60,61].

Pegylatedimmunoliposomes modified with anti-HER2 monoclonal antibody fragments are internalized much better by cancer cells compared to non-modified liposomes, which allows higher drug dose delivery inside cancer cells for, i. e., more efficient cancer cell killing [62].

The therapeutic effects are dependent on the type of the encapsulated drug and the rate of drug release from the immunoliposomes in the targeted areas [61,63]. The cytotoxic efficiency of immunoliposomes is also dependent on the surface density of the membrane antigen against which liposomes were targeted. It was estimated that about 4×10^4 antigen sites per single cell are required to exert the immunoliposomal targeting effect [64,65]. Another essential factor that determines the degree of immunoliposomal targeting is the extent of heterogeneous expression of antigens in the targeting area. It was suggested that a co-mobilization of antibodies against different antigens on a single immunoliposome will provide better and more uniform targeting of all cells within the tumor. Additionally, some cells can be killed by the "bystander effect", i. e. the drug released by the immunoliposome attached to a cancer cell expressing a specific antigen can act over the neighboring cancer cells devoid of a similar receptor [63,66].

Thermosensitive liposomes

In order to produce a more controlled, rapid and complete release of drug from a lipid carrier, local intervention techniques make use of temperature change (hyperthermia), light (photodynamic therapy) or mechanical disruption (e. g. by ultrasound), to initiate the breakdown or change the phase of the membrane capsule composed of specifically engineered lipids [5,67-70]. In the opinion of Koning*et al*., hyperthermia can be of great importance to achieve success in both suggested strategies [71]. In 1978, Yatvin*et al*. suggested the use of temperature sensitive liposomes to control and produce a burst release that can be an essential step to provide efficacious levels of drug in the tumor [72]. Hyperthermia can be applied to augment liposomal drug delivery by increasing tumor blood flow and microvascular permeability to liposomes [8,73].

Studies performed by Needham and collaborators goal to explore the use of hyperthermia, as a way to initiate a temperature-dependent change in the physical structure of a liposome membrane capsule that then might lead to an enhanced permeability of the encapsulating membrane and a rapid triggered release of the drug at clinically attainable temperatures [5]. One of the studies performed by the cited authors consisted in developing a new thermosensitive drug delivery system containing DOX that has been optimized for both, mild hyperthermic temperatures (39º to 40ºC) that are readily achievable in the clinic and rapid release times of drug (ten seconds). The liposomes were prepared by the lipid film hydration and extrusion method and DOX encapsulation into the liposomes was carried out using the pH gradient-driven loading protocol. This new thermosensitive liposomal formulation (lysolecithin containing thermosensitive liposome DOX) was composed by 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine, 1,2-distearoyl-*sn*-glycero- 3-phosphoethanolamine-*N*-polyethylene glycol 2000. This one differs from the traditional thermosensitive liposomes regarding the composition. The authors of this study have observed that the release of encapsulated DOX from the new thermosensitive liposomes were extremely fast (some seconds) upon heating the liposomes to 42ºC, compared with the other liposome formulations, including traditional thermosensitive liposomes. Studies carried out in a human squamous cell carcinoma tumor xenograft model showed that the tumor growth, during 60 days after treatment, is lower when heating at 42ºC and this is more evident, using thermosensitive liposomes than free DOX, non-thermosensitive and traditional thermosensitive liposomes [8,67].

Dual functional liposomes

The attachment of certain stimulus-sensitive moieties to the nanocarriers provides a novel strategy for the assembly of "smart" multifunctional nanocarriers, which function in response to intrinsic or externally applied stimuli in a coordinated manner to maximize the antitumor efficacy and minimize the drug side effects [74,75]. These multifunctionalities can be used to detach the long-circulating polymer (PEG) chains and release the nanocarrier contents at pathological areas, in the presence of certain intrinsic stimuli such as decreased pH, hyperthermia, altered enzyme levels or redox conditions characteristics of these zones [76].

In the case of pH-responsive systems, the pH-cleavable bond stabilize the nanocarrier in normal tissues and blood, but they disintegrate and release the drug load in areas with lowered pH, including neoplasic, ischemic and inflamed tissue, or endosomes or cell cytoplasm [54]. The long-circulating targeting and stimulus-responsive functions of nanocarriers can also be combined with certain cell penetrating proteins and peptides (CPPs), such as trans-activating transcriptional activator (TATp) for improved intracellular drug delivery [77,78]. These CPPs effectively translocate across the plasma membrane directly into cell cytoplasm, avoiding the endocytic pathway and so prevent lysosomal drug degradation and lead to increased cellular uptake [79,80]. **Fig. 4** represents a schematic mode of action of "smart" stimulus-sensitive long circulating immunoliposomes. Briefly, the mode of action of "smart" stimulus-sensitive long circulating immunoliposome include the shielding long-chain PEG with or without targeting monoclonal antibodies attached to the liposomal surface via low pH-degradable bonds. After the accumulation in the tumor due to passive-accumulation and/or active targeting, pH dependent de-shielding of the temporarily hidden cell-penetrating function allows for carrier penetration into tumor cells [54].

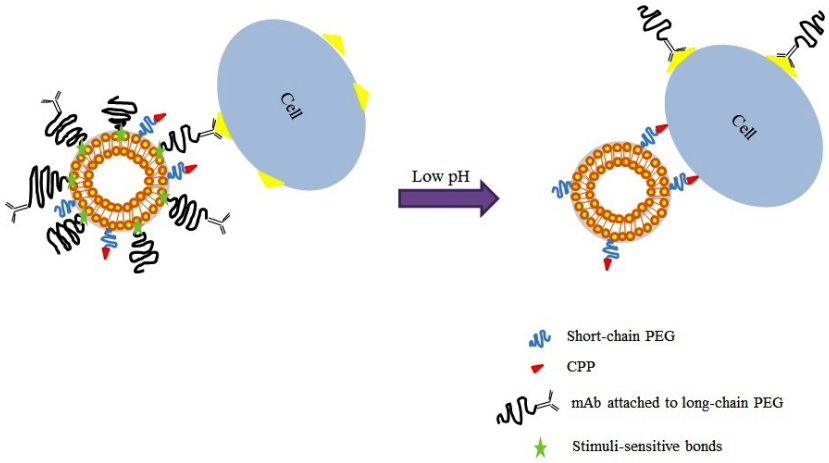

Fig. 4: Schematic mode of action of "smart" stimulus-sensitive long circulating immunoliposomes (adapted from 54).

Jiang *et al*., developed dual functional liposomes with pH-responsive CPP and active targeting hyaluronic acid (HA) for tumor target drug delivery [81]. CPPs, facilitating the cellular uptake of various cargos without causing any cellular injury, have been widely investigated in

the fields of gene and drug delivery for cancer therapy [82-84]. However, CPPs with effective tumor targeting are still lacking and remain highly desirable, which present more accumulations in tumor cells but less in normal cells. In the light of this, the pH gradient between the tumor milieu and physiological environment draws more attention to designing pH-responsive CPPs for tumor-targeted drug delivery, which can be used to conjugate drugs or modify nanocarriers[81]. Unfortunately, recent studies have suggested that CPPs on the surface of liposomes and micelles are susceptible to enzymatic cleavage by enzymes present in human plasma [85]. Additionally, for intravenous injection, positively charged nanoparticles, including cationic CPP-modified liposomes, cause severe toxicity, instability and a rapid clearance from the blood compartment, thereby limiting their applications *in vivo* [86-88]. To address this dilemma, surface pegylation of CPP-modified nanoparticles (CPP-NPs) is regarded as a gold standard for improved safety, bioavailability and blood persistence, resulted from the reduced interactions between CPP-NPs and opsonins by the hydrophilic shell of PEG [89-92].

Crosslinkedmultilamellar liposomes

A strategy to improve liposome-based anticancer drugs should involve the development of a stable liposomal formulation with improved drug release from the carrier in a controlled and sustained manner, thereby enhancing bioavailability. Based on this idea, Joo*et al*., have developed a new liposomal formulation involving the creation of a robust multilamellar structure of the liposome by covalently crosslinking inter-lipid bilayers. The main goal of these authors was to generate a liposomal formulation with improved bioavailability of liposomal drugs and enhanced vesicle stability. The multilamellar vesicles were formed through covalently crosslinking functionalized headgroups of adjacent lipid bilayers. As a nanocarrier platform for chemotherapy drug delivery applications, this study demonstrates that these crosslinkedmultilamellar liposomes (CMLs) can lower systemic toxicity and enhance therapeutic efficacy. Effectively, this study demonstrated that the enhanced delivery of CML-DOX to tumor cell *in vitro* and *in vivo* improved anticancer activity and led to better tumor reduction and inhibition of tumor progression, when compared with the antitumor activity of non-CML with the same lipid composition [93].

Special types of liposomes

Niosomes

Niosomes are similar, in terms of structure and physical properties, to liposomes [94]. Niosomes are non-ionic surfactant vesicles made up from single chain surfactant molecules often in combination with cholesterol. They alleviate the disadvantages associated with liposomes, like chemical instability and variable purity of phospholipids [95]. Additionally, the research interest in niosomal formulations is recently widening because surfactants are easily derivative and give a higher versatility to the vesicular structure and moreover they have lower costs than phospholipids [31]. They have longer shelf life, stability and ability to deliver drug at target site in a controlled or sustained manner which enhances bioavailability [94,96,97]. Nonionic surfactants used due to their ability to enhance solubility are used to increase bioavailability of poor water soluble drugs. Nonionic surfactants increase both permeability and fluidity of biological membranes and drugs show enhanced bioavailability by transdermal route via niosomes. Furthermore, nonionic surfactants are preferred due to less irritation power which decreases in order of cationic>anionic>amphoteric>nonionic [98]. They are inhibitors of p-glycoprotein, hence increasing bioavailability of some anticancer drugs, HIV drugs and other class of drugs [99-101].

Kong *et al*., have developed a novel drug nanocarrier HA niosome, which combine transdermal delivery and tumor targeting. These authors have concluded that incorporating HA significantly promoted the endocytosed amount of nanocarrier by tumor cell. HA-niosome is not only efficient and secure for transdermal permeation, but it also offers a useful and promising carrier for tumor therapy through percutaneous administration [102]. Tavano*et al*., have developed magneto-niosomes, in which both the magnetic material and antitumoral drug have been incorporated into the niosome aqueous compartment. Vesicles have been prepared by Tween® 60 and Pluronic® L64 surfactants and DOX was used as a model drug. Magneto-niosome formulations were stable for long periods and exhibited a controlled drug release. It has been concluded that DOX loading and release behavior of magneto-niosomes could probably promote them as effective functional materials for magnetically controlled cancer therapy [103]. Paolino and collaborators have made innovative bola-surfactant niosomes as topical delivery systems of 5-FU (5-Fluorouracil) for the treatment of skin cancer. 5-FU-loaded bola-niosomes showed an improvement of the cytotoxic effect with respect to the free drug. Bola-niosomes also provided an increase of the drug penetration of 8- and 4-folds with respect to a drug aqueous solution and to a mixture of empty bola-niosomes with a drug aqueous solution [104].

Transfersomes

Deformable liposomes (Transfersomes®) are the first generation of elastic vesicles introduced by Cev*et al*. and were reported to penetrate intact skin carrying therapeutic concentrations of drugs, but only when applied under non-occluded conditions [105]. These systems consist of phospholipids and an edge activator. An edge activator is often a single chain surfactant that destabilizes lipid bilayers of the vesicles and increases deformability of the bilayers [106]. Sodium cholate, Span® 80, Tween® 80 and dipotassiumglycyrrhizinate were employed as edge activators [107]·[108]. Ultra-deformable liposomes have shown potential as carriers for topical drug delivery systems because they can penetrate the intact skin, improving drug delivery of various drugs, with efficiency comparable with subcutaneous administration [107]-[110].

Maghraby*et al*., have evaluated the potential use of deformable and standard liposomes as skin drug delivery systems of 5-FU. These authors have observed that ultra-deformable formulation was superior to standard liposomes in the skin delivery of 5-FU and concluded that ultra-deformable vesicles are promising agents for skin delivery of drugs [111].

Recent work has shown that bleomycin can be encapsulated in ultra-deformable liposomes and it has been suggested that this preparation may be useful for topical chemotherapy of non-melanoma skin cancer [112]. Hiruta and collaborators have incorporated beta-sitosterol 3-β-D-glucoside (Sit-G) as absorption enhancer into ultra-deformable liposomes containing bleomycin to attenuate drug toxicity in human keratinocytes. The presence of Sit-G increased drug entrapment and improved *in vitro* stability. Furthermore, treatment with preparations incorporating Sit-G resulted in elevated epidermal and dermal concentrations of bleomycin. Ultra-deformable formulation containing Sit-G maintained flexibility for penetration through the skin, increased entrapment efficiency of bleomycin and stability *in vitro*, and significantly increased the distribution of bleomycin in epidermis and dermis compared with the formulations without Sit-G [113].

Ethosomes

Modern approaches for drug delivery via skin have resulted in the design of modified liposomes. Basically these approaches use the non-toxic and biodegradable, characteristics of phospholipids and for this reason they are able to prolong the half-life of a drug to attain a sustained-release effect. On the other hand, previous studies demonstrated that phospholipids can exhibit their enhancing effect on the skin in the presence of organic solvents such as ethanol, as in the case of ethosomes[114]. Ethosomesare non-invasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active substances. They are composed mainly of phospholipids, (phosphatidylcholine, phosphatidylserine, phosphatidic acid), high concentration of ethanol and water [115]. Ethanol is known as an efficient permeation enhancer [116]. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, it gives the vesicle the ability to penetrate the stratum corneum. Also,

because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improving drug distribution ability in stratum corneum lipids [117].

Paolinho*et al*., have developed paclitaxel-loaded ethosomes for potential topical treatment of squamous cell carcinoma, a malignant transformation of actinic keratosis. The paclitaxel-loaded ethosomes were proposed as topical drug delivery systems for treatment of this pathology due to their suitable physicochemical characteristics and enhanced skin permeation ability for deep dermal delivery. The obtained results showed that the proposed formulation enhanced the drug permeation through the skin and also increase its anti-proliferative activity compared to the free drug [118].

CONCLUSION

The research for novel drug delivery systems that could provide a controlled release, increase efficacy or reduce side effects of antitumor drugs is an important field that is at the forefront of the pharmaceutical technology. Particularly, the nanocarriers have been extensively studied in last years, demonstrating compliance with the referred objectives. The main advantages of nanocarriers can be a real asset to carry cytotoxic drugs, because, due to lack of specificity, these drugs exhibit various harmful effects typical from chemotherapy.

Liposomes represent one of the most popular nanocarriers for the delivery of anticancer drugs. From its discovery to date, liposomes have been intensively investigated in the context of its application as anticancer drug carriers. Liposomes are proven to be efficient drug delivery systems. In a general manner, this research has been carried out with the intention to increase the specificity to cancer cells. Additionally, the special types of liposomes, that is, niosomes, transfersomes and ethosomes, were also investigated as carriers to cytotoxic drugs, showing very promising results.

REFERENCES

1. Defining Cancer. National Cancer Institute at the National Institutes of Health National Cancer Institute at the National Institutes of Health; 2013. Available from: http://www.cancer.gov/cancertopics/cancerlibrary/what-is-cancer
2. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. World Health Organization/ International Agency for Research on Cancer; 2014. Available from: http://globocan.iarc. fr/Default.aspx
3. Brizel DM, Albers ME, Fisher SR, Scher RL, Richtsmeier WJ, Hars V, *et al*. Hyperfractionated irradiation with or without concurrent chemotherapy for locally advanced head and neck cancer. N Engl J Med 1998;338:1798-804.
4. Chaplin DJ, Hill SA, Bell KM, Tozer GM. Modification of tumor blood flow: current status and future directions. Semin Radiat Oncol 1998;8:151-63.
5. Needham D, Dewhirst MW. The development and testing of a new temperature-sensitive drug delivery system for the treatment of solid tumors. Adv Drug Deliv Rev 2001;53:285-305.
6. Jain RK. Barriers to drug delivery in solid tumors. Sci Am 1994;271:58-65.
7. Yuan F. Transvascular drug delivery in solid tumors. Semin Radiat Oncol 1998;8:164-75.
8. Kong G, Anyarambhatla G, Petros WP, Braun RD, Colvin OM, Needham D, *et al*. Efficacy of liposomes and hyperthermia in a human tumor xenograft model: importance of triggered drug release. Cancer Res 2000;60:6950-57.
9. Needham D. Materials engineering of lipid bilayers for drug carrier performance. MRS Bull 1999;24:32-41.
10. Aderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. Annu Rev Immunol 1999;17:593-623.
11. Yoo J-W, Doshi N, Mitragotri S. Adaptive micro and nanoparticles: temporal control over carrier properties to facilitate drug delivery. Adv Drug Deliv Rev 2011;63:1247-56.
12. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. Pharmacol Rev 2001;53:283-318.
13. Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Ipe BI, *et al*. Renal clearance of quantum dots. Nat Biotechnol 2007;25:1165-78.
14. Rejman J, Oberle V, Zuhorn IS, Hoekstra D. Size-dependent internalization of particles via the pathways of clathrin-and caveolae-mediated endocytosis. Biochem J 2004;377:159-69.
15. Laverman P, Boerman OC, Oyen WJG, Dams ETM, Storm G, Corstens FHM. Liposomes for scintigraphic detection of infection and inflammation. Adv Drug Deliv Rev 1999;37:225-35.
16. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 1986;46:6387-92.
17. Cho K, Wang X, Nie S, Chen ZG, Shin DM. Therapeutic nanoparticles for drug delivery in cancer. Clin Cancer Res 2008;14:1310-6.
18. Ferrari M. Cancer nanotechnology: opportunities and challenges. Nat Rev Cancer 2005;5:161-71.
19. Fang J, Nakamura H, Maeda H. The EPR effect: unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. Adv Drug Deliv Rev 2011;63:136-51.
20. Allen TM. Liposomes: opportunities in drug delivery. Drugs 1997;54:8-14.
21. Lasic DD. Novel applications of liposomes. Trends Biotechnol 1998;16:307-21.
22. P Torchilin V. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov 2005;4:145-60.
23. Sandip BT, Udupa N, Rao BSS, Devi PU. Thermosensitive liposomes and localised hyperthermia-an effective bimodality approach for tumour management. Indian J Pharmacol 2000;32:214-20.
24. Verma RK, Garg S. Drug delivery technologies and future directions. Pharm Technol On-Line 2001;25:1-14.
25. Edwards KA, Baeumner AJ. Liposomes in analyses. Talanta 2006;68:1421-31.
26. Park J. Liposome-based drug delivery in breast cancer treatment. Breast Cancer Res 2002;4:95-9.
27. Drummond DC, Noble CO, Hayes ME, Park JW, Kirpotin DB. Pharmacokinetics and *in vivo* drug release rates in liposomal nanocarrier development. J Pharm Sci 2008;97:4696-740.
28. Moutinho C, Matos C, Balcão V. Development of innovative nanotechnology-based drug delivery systems for cancer therapy. Revista FCS-UFP 2007;4:94-104.
29. Oberoi HS, Nukolova NV, Kabanov AV, Bronich TK. Nanocarriers for delivery of platinum anticancer drugs. Adv Drug Deliv Rev 2013;65:1667-85.
30. Vemuri S, Rhodes CT. Preparation and characterization of liposomes as therapeutic delivery systems: a review. Pharm Acta Helv 1995;70:95-111.
31. Marianecci C, Di Marzio L, Rinaldi F, Celia C, Paolino D, Alhaique F, *et al*. Niosomes from 80s to present: The state of the art. Adv Colloid Interface Sci 2014;205:187-206.
32. Sharma A, Sharma US. Liposomes in drug delivery: Progress and limitations. Int J Pharm 1997;154:123-40.
33. Yarosh DB. Liposomes in investigative dermatology. Photodermatol Photoimmunol Photomed 2001;17:203-12.
34. Zuidam NJ, Gouw HKME, Barenholz Y, Crommelin DJA. Physical (in) stability of liposomes upon chemical hydrolysis: the role of lysophospholipids and fatty acids. Biochim Biophys Acta 1995;1240:101-10.
35. Hwang KJ. Liposome pharmacokinetics. In: Ostro MJ, ed. *Liposome from Biophysics to Therapeutics*. New York: Marcel Dekker Inc; 1987. p. 109–56.
36. Senior JH. Fate and behavior of liposomes *in vivo*: a review of controlling factors. Crit Rev Ther Drug Carrier Syst 1987;3:123-93.
37. Maruyama K, Ishida O, Takizawa T, Moribe K. Possibility of active targeting to tumor tissues with liposomes. Adv Drug Deliv Rev 1999;40:89-102.

38. Wu NZ, Da D, Rudoll TL, Needham D, Whorton AR, Dewhirst MW. Increased microvascular permeability contributes to preferential accumulation of Stealth liposomes in tumor tissue. Cancer Res 1993;53:3765-70.
39. Gabizon AA. Stealth liposomes and tumor targeting: one step further in the quest for the magic bullet. Clin Cancer Res 2001;7:223-5.
40. O'Shaughnessy JA. Pegylated Liposomal Doxorubicin in the Treatment of Breast Cancer. Clin Breast Cancer 2003;4:318-28.
41. Gabizon AA. Pegylated liposomal doxorubicin: metamorphosis of an old drug into a new form of chemotherapy. Cancer Invest 2001;19:424-36.
42. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. Sci 2004;303:1818-22.
43. Laginha KM, Verwoert S, Charrois GJ, Allen TM. Determination of doxorubicin levels in whole tumor and tumor nuclei in murine breast cancer tumors. Clin Cancer Res 2005;11:6944-9.
44. Al-Jamal WT, Al-Ahmady ZS, Kostarelos K. Pharmacokinetics and tissue distribution of temperature-sensitive liposomal doxorubicin in tumor-bearing mice triggered with mild hyperthermia. Biomaterials 2012;33:4608-17.
45. Van Lummel M, van Blitterswijk WJ, Vink SR, Veldman RJ, van der Valk MA, Schipper D, *et al*. Enriching lipid nanovesicles with short-chain glucosylceramide improves doxorubicin delivery and efficacy in solid tumors. FASEB J 2011;25:280-9.
46. Barenholz Y. Doxil® — The first FDA-approved nano-drug: lessons learned. J Cont Rel 2012;160:117-34.
47. Dass CR, Walker TL, Burton MA, Decruz EE. Enhanced anticancer therapy mediated by specialized liposomes. J Pharm Pharmacol 1997;49:972-5.
48. Derksen JTP, Morselt HWM, Scherphof GL. Uptake and processing of immunoglobulin-coated liposomes by subpopulations of rat liver macrophages. Biochim Biophys Acta 1988;971:127-36.
49. Maruyama K, Holmberg E, Kennel SJ, Klibanov A, Torchilin VP, Huang L. Characterization of *in vivo* immunoliposome targeting to pulmonary endothelium. J Pharm Sci 1990;79:978-84.
50. Torchilin VP, Klibanov AL, Huang L, O'Donnell S, Nossiff ND, Khaw BA. Targeted accumulation of polyethylene glycol-coated immunoliposomes in infarcted rabbit myocardium. FASEB J 1992;6:2716-9.
51. Torchilin VP. Immunoliposomes and PEGylated Immunoliposomes: possible use for targeted delivery of imaging agents. Immunomethods 1994;4:244-58.
52. Torchilin VP, Narula J, Halpern E, Khaw BA. Poly(ethylene glycol)-coated anti-cardiac myosin immunoliposomes: factors influencing targeted accumulation in the infarcted myocardium. Biochim Biophys Acta 1996;1279:75-83.
53. Torchilin V. Antibody-modified liposomes for cancer chemotherapy. Expert Opin Drug Deliv 2008;5:1003-25.
54. Koshkaryev A, Sawant R, Deshpande M, Torchilin V. Immunoconjugates and long circulating systems: origins, current state of the art and future directions. Adv Drug Deliv Rev 2013;65:24-35.
55. Duggan S, Keating G. Pegylated liposomal doxorubicin. Drugs 2011;71:2531-58.
56. Park JW, Hong K, Carter P, Asgari H, Guo LY, Keller GA, *et al*. Development of anti-p185HER2 immunoliposomes for cancer therapy. Proc Natl Acad Sci U S A 1995;92:1327-31.
57. Park JW, Hong K, Kirpotin DB, Colbern G, Shalaby R, Baselga J, *et al*. Anti-HER2 Immunoliposomes: enhanced efficacy attributable to targeted delivery. Clin Cancer Res 2002;8:1172-81.
58. Park JW, Kirpotin DB, Hong K, Shalaby R, Shao Y, Nielsen UB, *et al*. Tumor targeting using anti-her2 immunoliposomes. J Control Release 2001;74:95-113.
59. Shmeeda H, Tzemach D, Mak L, Gabizon A. Her2-targeted pegylated liposomal doxorubicin: retention of target-specific binding and cytotoxicity after *in vivo* passage. J Cont Rel 2009;136:155-60.
60. Cheng WWK, Allen TM. Targeted delivery of anti-CD19 liposomal doxorubicin in B-cell lymphoma: a comparison of whole monoclonal antibody, Fabfragments and single chain Fv. J Cont Rel 2008;126:50-8.
61. Allen TM, Mumbengegwi DR, Charrois GJR. Anti-CD19-targeted liposomal doxorubicin improves the therapeutic efficacy in murine b-cell lymphoma and ameliorates the toxicity of liposomes with varying drug release rates. Clin Cancer Res 2005;11:3567-73.
62. Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB, *et al*. Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. Cancer Res 2006;66:6732-40.
63. Sapra P, Allen TM. Improved outcome when b-cell lymphoma is treated with combinations of immunoliposomal anticancer drugs targeted to both the cd19 and cd20 epitopes. Clin Cancer Res 2004;10:2530-37.
64. Lopus M. Antibody-DM1 conjugates as cancer therapeutics. Cancer Lett 2011;307:113-18.
65. Hosokawa S, Tagawa T, Niki H, Hirakawa Y, Nohga K, Nagaike K. Efficacy of immunoliposomes on cancer models in a cell-surface-antigen-density-dependent manner. Br J Cancer 2003;89:1545–51.
66. Allen TM. Ligand-targeted therapeutics in anticancer therapy. Nat Rev Cancer 2002;2:750–63.
67. Needham D, Anyarambhatla G, Kong G, Dewhirst MW. A New Temperature-sensitive Liposome for Use with Mild Hyperthermia: Characterization and Testing in a Human Tumor Xenograft Model. Cancer Res 2000;60:1197-201.
68. Zhu L, Huo Z, Wang L, Tong X, Xiao Y, Ni K. Targeted delivery of methotrexate to skeletal muscular tissue by thermosensitive magnetoliposomes. Int J Pharm 2009;370:136-43.
69. Alexiou C, Arnold W, Klein RJ, Parak FG, Hulin P, Bergemann C, *et al*. Locoregional cancer treatment with magnetic drug targeting. Cancer Res 2000;60:6641-8.
70. Schroeder A, Honen R, Turjeman K, Gabizon A, Kost J, Barenholz Y. Ultrasound triggered release of cisplatin from liposomes in murine tumors. J Cont Rel 2009;137:63-68.
71. Koning G, Eggermont AM, Lindner L, Hagen TM. Hyperthermia and thermosensitive liposomes for improved delivery of chemotherapeutic drugs to solid tumors. Pharm Res 2010;27:1750-54.
72. Yatvin MB, Weinstein JN, Dennis WH, Blumenthal R. Design of liposomes for enhanced local release of drugs by hyperthermia. Sci 1978;202:1290-3.
73. Gaber MH, Wu NZ, Hong K, Huang SK, Dewhirst MW, Papahadjopoulos D. Thermosensitive liposomes: extravasation and release of contents in tumor microvascular networks. Int J Radiat Oncol Biol Phys 1996;36:1177-87.
74. Torchilin VP. Targeted pharmaceutical nanocarriers for cancer therapy and imaging. AAPS J 2007;9:E128-47.
75. Torchilin VP. Multifunctional nanocarriers. Adv Drug Deliv Rev 2006;58:1532-55.
76. Basel MT, Shrestha TB, Troyer DL, Bossmann SH. protease-sensitive, polymer-caged liposomes: a method for making highly targeted liposomes using triggered release. ACS Nano 2011;5:2162-75.
77. Hällbrink M, Florén A, Elmquist A, Pooga M, Bartfai T, Langel Ü. Cargo delivery kinetics of cell-penetrating peptides. Biochim Biophys Acta 2001;1515:101-09.
78. Frankel AD, Pabo CO. Cellular uptake of the tat protein from human immunodeficiency virus. Cell 1988;55:1189-93.
79. Torchilin VP, Rammohan R, Weissig V, Levchenko TS. TAT peptide on the surface of liposomes affords their efficient intracellular delivery even at low temperature and in the presence of metabolic inhibitors. Proc Natl Acad Sci U S A 2001;98:8786-91.
80. Tachibana R, Harashima H, Shono M, Azumano M, Niwa M, Futaki S, *et al*. Intracellular regulation of macromolecules using ph-sensitive liposomes and nuclear localization signal: qualitative and quantitative evaluation of intracellular trafficking. Biochem Biophys Res Commun 1998;251:538-44.
81. Jiang T, Zhang Z, Zhang Y, Lv H, Zhou J, Li C, *et al*. Dual-functional liposomes based on pH-responsive cell-penetrating peptide and hyaluronic acid for tumor-targeted anticancer drug delivery. Biomaterials 2012;33:9246-58.

82. Cheng CJ, Saltzman WM. Enhanced siRNA delivery into cells by exploiting the synergy between targeting ligands and cell-penetrating peptides. Biomaterials 2011;32:6194-203.
83. Lee JY, Bae KH, Kim JS, Nam YS, Park TG. Intracellular delivery of paclitaxel using oil-free, shell cross-linked HSA--multi-armed PEG nanocapsules. Biomaterials 2011;32:8635-44.
84. Liu J, Zhao Y, Guo Q, Wang Z, Wang H, Yang Y, *et al*. TAT-modified nanosilver for combating multidrug-resistant cancer. Biomaterials 2012;33:6155-61.
85. Koren E, Apte A, Sawant RR, Grunwald J, Torchilin VP. Cell-penetrating TAT peptide in drug delivery systems: proteolytic stability requirements. Drug Deliv 2011;18:377-84.
86. Eliyahu H, Servel N, Domb AJ, Barenholz Y. Lipoplex-induced hemagglutination: potential involvement in intravenous gene delivery. Gene Ther 2002;9:850-58.
87. Li S, Tseng WC, Stolz DB, Wu SP, Watkins SC, Huang L. Dynamic changes in the characteristics of cationic lipidic vectors after exposure to mouse serum: implications for intravenous lipofection. Gene Ther 1999;6:585-94.
88. Fischer D, Li Y, Ahlemeyer B, Krieglstein J, Kissel T. In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. Biomaterials 2003;24:1121-31.
89. Crawford J. Clinical uses of pegylated pharmaceuticals in oncology. Cancer Treat Rev 2002;28:7-11.
90. Lee ES, Gao Z, Kim D, Park K, Kwon IC, Bae YH. Super pH-sensitive multifunctional polymeric micelle for tumor pHe specific TAT exposure and multidrug resistance. J Con Rel 2008;129:228-36.
91. Koren E, Apte A, Jani A, Torchilin VP. Multifunctional PEGylated 2C5-immunoliposomes containing pH-sensitive bonds and TAT peptide for enhanced tumor cell internalization and cytotoxicity. J Con Re 2012;160:264-73.
92. Sethuraman VA, Bae YH. TAT peptide-based micelle system for potential active targeting of anti-cancer agents to acidic solid tumors. J Cont Rel 2007;118:216-24.
93. Joo K-I, Xiao L, Liu S, Liu Y, Lee C-L, Conti PS, *et al*. Crosslinked multilamellar liposomes for controlled delivery of anticancer drugs. Biomaterials 2013;34:3098-109.
94. Uchegbu IF, Florence AT. Non-ionic surfactant vesicles (niosomes): Physical and pharmaceutical chemistry. Adv Colloid Interf Sci 1995;58:1-55.
95. Farkas E, Schubert R, Zelkó R. Effect of β-sitosterol on the characteristics of vesicular gels containing chlorhexidine. Int J Pharm 2004;278:63-70.
96. Bajaj A, Desai M. Challenges and strategies in novel drug delivery technologies. Pharm Times 2006;38:12-6.
97. Wadhe K, Kalsait R, Umekar M. Alternate drug delivery system: recent advancement and future challenges. Arch Pharm Sci Res 2009;1:97–105.
98. Mahale NB, Thakkar PD, Mali RG, Walunj DR, Chaudhari SR. Niosomes: novel sustained release nonionic stable vesicular systems-An overview. Adv Colloid Interface Sci 2012;183–184:46-54.
99. Jiao J. Polyoxyethylated nonionic surfactants and their applications in topical ocular drug delivery. Adv Drug Deliv Rev 2008;60:1663-73.
100. Zografi G. Interfacial phenomena. In: Gennaro AR, ed. *Remington: the science and practice of pharmacy*. 17 ed. Pennsylvania: Mark Publishing; 1995. p. 241-51.
101. Kumar GP, Rajeshwarrao P. Nonionic surfactant vesicular systems for effective drug delivery—an overview. Acta Pharm Sin B 2011;1:208-19.
102. Kong M, Park H, Feng C, Hou L, Cheng X, Chen X. Construction of hyaluronic acid noisome as functional transdermal nanocarrier for tumor therapy. Carbohydr Polym 2013;94:634-41.
103. Tavano L, Vivacqua M, Carito V, Muzzalupo R, Caroleo MC, Nicoletta F. Doxorubicin loaded magneto-niosomes for targeted drug delivery. Colloids Surf B Biointerfac 2013;102:803-07.
104. Paolino D, Cosco D, Muzzalupo R, Trapasso E, Picci N, Fresta M. Innovative bola-surfactant niosomes as topical delivery systems of 5-fluorouracil for the treatment of skin cancer. Int J Pharm 2008;353:233-42.
105. Cevc G, Blume G. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochim Biophys Acta 1992;1104:226-32.
106. Honeywell-Nguyen PL, Bouwstra JA. Vesicles as a tool for transdermal and dermal delivery. Drug Discov Today Technol 2005;2:67-74.
107. Cevc G. Transfersomes, liposomes and other lipid suspensions on the skin: Permeation enhancement, vesicle penetration, and transdermal drug delivery. Crit Rev Ther Drug Carrier Syst 1996;13:257-388.
108. Trotta M, Peira E, Carlotti ME, Gallarate M. Deformable liposomes for dermal administration of methotrexate. Int J Pharm 2004;270:119-25.
109. El Maghraby GMM, Williams AC, Barry BW. Skin delivery of oestradiol from deformable and traditional liposomes: Mechanistic studies. J Pharm Pharmacol 1999;51:1123-34.
110. Cevc G, Schätzlein A, Richardsen H. Ultradeformable lipid vesicles can penetrate the skin and other semi-permeable barriers unfragmented. Evidence from double label CLSM experiments and direct size measurements. Biochim Biophys Acta 2002;1564:21-30.
111. El Maghraby GMM, Williams AC, Barry BW. Skin delivery of 5-fluorouracil from ultradeformable and standard liposomes *in-vitro*. J Pharm Pharmacol 2001;53:1069-77.
112. Lau KG, Chopra S, Maitani Y. Entrapment of bleomycin in ultra-deformable liposomes. STP Pharm Sci 2003;13:237-9.
113. Hiruta Y, Hattori Y, Kawano K, Obata Y, Maitani Y. Novel ultra-deformable vesicles entrapped with bleomycin and enhanced to penetrate rat skin. J Control Rel 2006;113:146-54.
114. Maheshwari RGS, Tekade RK, Sharma PA, Darwhekar G, Tyagi A, Patel RP, *et al*. Ethosomes and ultradeformable liposomes for transdermal delivery of clotrimazole: a comparative assessment. Saudi Pharm J 2012;20:161-70.
115. Merdan V, Alhaique F, Touitou E. Vesicular carriers for topical delivery. Acta Technol Legis Medicamenti 1998;12:1-6.
116. Berner B, Liu P. Alcohols; in Smith EW, Maibach HI (eds) CRC Press. Percutaneous Penetration Enhancers Boca Raton; 1995. p. 45-60.
117. Barry BW. Is transdermal drug delivery research still important today? Drug Discov Today 2001;6:967-71.
118. Paolino D, Celia C, Trapasso E, Cilurzo F, Fresta M. Paclitaxel-loaded ethosomes®: Potential treatment of squamous cell carcinoma, a malignant transformation of actinic keratoses. Eur J Pharm Biopharm 2012;81:102-12.