ISSN 0006-2979, Biochemistry (Moscow), 2010, Vol. 75, No. 7, pp. 811-824. © Pleiades Publishing, Ltd., 2010.
Published in Russian in Biokhimiya, 2010, Vol. 75, No. 7, pp. 920-935.

REVIEW

# "Smart" Liposomal Nanocontainers in Biology and Medicine

**Y. S. Tarahovsky**

*Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia, fax: (4967) 330-553; E-mail: tarahov@rambler.ru*

Received November 23, 2009

**Abstract**—The perspectives of using liposomes for delivery of drugs to desired parts of the human body have been intensively investigated for more than 30 years. During this time many inventions have been suggested and different kinds of liposomal devices developed, and a number of them have reached the stages of preclinical or clinical trials. The latest techniques can be used to develop biocompatible nano-sized liposomal containers having some abilities of artificial intellect, such as the presence of sensory and responsive units. However, only a few have been clinically approved. Further improvements in this area depend on our knowledge of the interactions of drugs with the lipid bilayer of liposomes. Further studies on liposomal transport through the human body, their targeting of cells requiring therapeutic treatment, and finally, the development of techniques for controlled drug delivery to desired acceptors on cell surfaces or in cytoplasm are still required.

**DOI**: 10.1134/S0006297910070023

*Key words*: liposomes, intellectual nanocontainers, drugs targeting, nanomedicine

Liposomes, like other particles of micro- and nanosize, can be used for development of containers for storage and targeting of drugs [1-4]. Depending on their solubility in water, the drugs can be contained in the internal aqueous space of liposomes or in the hydrophobic region of the lipid bilayer. Isolation inside liposomes prevents biodegradation or undesirable toxic effects of drugs. Control in space and time of delivery of drugs developed with liposomes revolutionizes modern therapy. The targeting of presumably toxic compounds precisely to the area of therapeutic treatment prevents undesirable side effects of the drugs. Controllable drug release allows their monitoring in the body according to the clinical course and fluctuations of physiological requirements that can vary with the patient's schedule or according to circadian biorhythms [5].

*Abbreviations*: CHEMS, cholesteryl hemisuccinate; DOPE, dioleoyl phosphatidylethanolamine; DPPC, dipalmitoyl phosphatidylcholine; DSPC, distearoyl phosphatidylcholine; EPR, enhanced permeability and retention; FR, folate receptor; HePC, hexadecyl phosphocholine; IR, infrared light; OA, oleic acid; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEG, polyethyleneglycol; PS, phosphatidylserine; RES, reticuloendothelial system; RFC, reduced folate carrier; UV, ultraviolet light.

## TARGETING OF SMART LIPOSOMAL CONTAINERS

We can distinguish active and passive drug targeting of liposomal containers. For passive targeting the delivery depends on the pharmacokinetics of the particles in the body. For active targeting the containers are supplied with means for monitoring their distribution in the body and can initiate drug release by an external signal, or particles could have devices for independent reaching of the target and drug release at a proper time and in a proper place (space–time self targeting). For this purpose the containers should be able to circulate in the bloodstream for a sufficiently long time to reach the target. But not all liposomes are able to remain in the blood for a long time.

Macrophages of the reticuloendothelial system (RES) can remove foreign bodies from the blood in a few minutes after their administration and thus prevent prolonged circulation of liposomes [6]. Kupffer cells, the liver macrophages, are the most active in removing particles. The Kupffer cells cannot identify liposomes, but they can recognize them through specific opsonins, blood proteins attached to the surface of liposomes during the process called opsonization [7].

A number of approaches have been developed to prevent opsonization. Specially designed camouflage

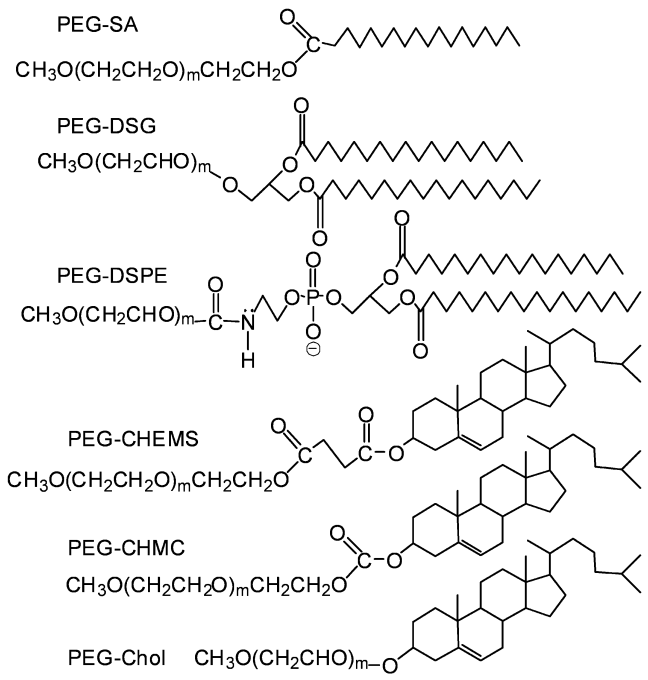


**Fig. 1.** Structure of PEG conjugates with different lipids. PEG-SA, conjugate of PEG with stearic acid; PEG-DSG, conjugate with distearoylglycerol; PEG-DSPE, conjugate with distearoyl phosphatidylethanolamine; PEG-CHEMS, conjugate with cholesteryl hemisuccinate; PEG-CHMC, conjugate with cholesteryl chloroformate; PEG-Chol, conjugate with cholesterol.

coverings help particles to escape RES [8]. The most effective and widely used is polyethyleneglycol (PEG), which can be attached to the polar heads of some lipids (Fig. 1). Some other polymeric compounds like polyvinylpyrrolidone, poly(ε-caprolactone), poly(β-hydroxybutyrate), polystyrene, serum albumin, gelatin (type B), polysaccharides, polyacrylamides, polyvinyl alcohols, and others [9-18] can also prevent opsonization. Particles covered with polymers preventing opsonization are called sterically stabilized. The time of their circulation in blood can be prolonged up to many hours and even days [19-22]. Modification of liposomal surfaces with PEG gave rise to manufacturing of sterically stabilized liposomes named Stealth® (ALZA Corporation, USA) [23]. The term "stealth liposomes" is now widely used. The "stealth" approach was also used for development of DOXIL® (Centocor Ortho Biotech Products, USA; Schering-Plough Corporation, Israel) liposomes loaded with the anticarcinogenic compound doxorubicin [24] and DaunoXome® (NeXstar Pharmaceuticals, Inc., USA) liposomes loaded with daunorubicin [25]. The prolonged circulation of liposomes in the blood stream and slow drug release considerably decrease the side effects of chemotherapy.

## PHARMACOKINETICS AND PASSIVE TARGETING OF LIPOSOMES

Some nanocontainers can be spontaneously accumulated in the area of a pathological disorder. It is remarkable that areas of inflammation or oncogenesis can attract nanoparticles if they have not been removed earlier by RES macrophages. Conditions retarding RES acquisition can favor particle accumulation in the pathologically transformed sites. For example, it was experimentally demonstrated that the longer particles circulate in the blood stream, the higher their accumulation in solid tumors [26-28].

One of the most accepted explanations of this phenomenon suggests that the size of pores between endothelial cell covered capillaries is sufficiently small to prevent particle release from the blood stream into the surrounding tissue. However, tumor capillaries are defective and their epithelium is permeable for nanoparticles. The process of particle capture by tissues as a result of increased permeability of capillaries is called enhanced permeability and retention (EPR) [29-31]. The EPR effect facilitates delivery of particles into tumors [26-28, 32, 33].

The size of particles is very important for EPR. Particles of 80-150 nm are usually more effectively delivered into tumors compared to particles of larger or smaller size [26, 34-38]. It is possible that effectiveness of EPR depends not only on the size of particles, but also on the structure of tumor tissue, which could vary for different diseases. Tumors with higher vascularization and loosely arranged endothelial cells with larger intercellular clearance can accumulate particles of larger size compared to tumors with tight endothelium where the intercellular clearance is smaller [39].

## ACTIVE TARGETING AND CONTROLLABLE DRUG RELEASE

Liposomal containers can contain specific surface markers for their recognition by cells of targeted tissues. They can also be supplied with "smart" molecular devices to release their load in response to changes in pH, temperature, light exposure, magnetic field, osmotic strength, and other factors.

**pH-Sensitive liposomes.** Normally the pH of fluids of human or other animal bodies can considerably vary in different tissues and in different compartments of the cell cytoplasm. The typical pH of blood is slightly basic (pH 7.4), but in areas of disorders, particularly inside solid tumors, the pH values can decrease to 6.5-7.2 [40, 41]. The observed decrease in pH can be explained by accumulation of lactic acid and products of ATP hydrolysis resulting from high level of malignant cell metabolism under conditions of hypoxia. An additional pH decrease appears when particles are endocytosed into

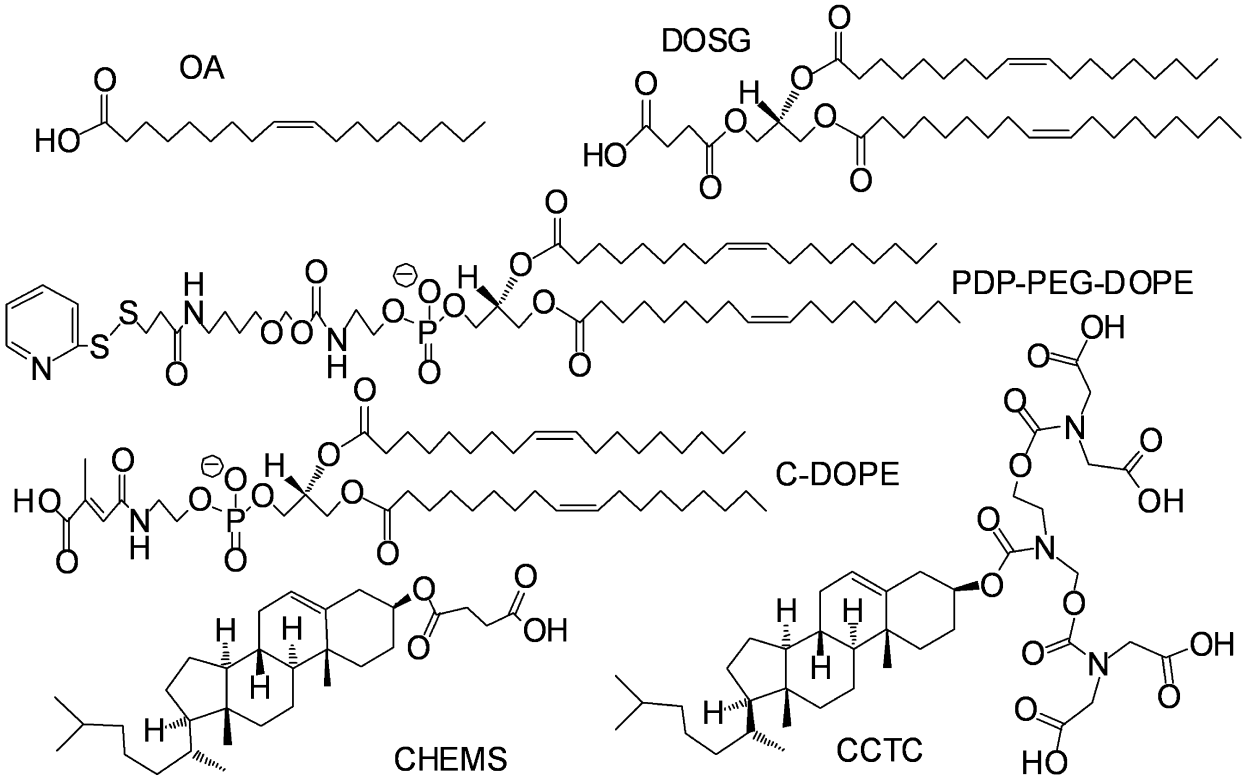


**Fig. 2.** pH-Sensitive lipids used for liposomes preparation. OA, oleic acid [47, 49]; DOSG, dioleoyl succinyl glycerol [46, 51, 54]; PDP-PEG-DOPE, copolymer composed of pyridyl dithiopropionate (PDP), PEG, and DOPE [50]; C-DOPE, N-citraconyl DOPE; CHEMS, cholesteryl hemisuccinate [48, 53]; CCTC, ((2-(cholesteryloxycarbonyl-(2-(bis-carboxymethyl-carbamoyloxy)-ethyl)-amino)-ethoxycarbonyl)-carboxymethyl-amino)-acetic acid [52].

endosomal and lysosomal compartments of the cell where the pH can be as low as 5.0-6.5. These changes can be utilized as a signal for drug release by pH-sensitive nanoparticles.

Liposomes sensitive to changes in pH were suggested as a device to improve drug delivery to tumors. A mixture of oleic acid (OA) and dioleoyl phosphatidylethanolamine (DOPE) was used in the first pH-sensitive liposomes [42-45]. The bilayer permeability of this lipid mixture was increased at pH 6.5, which can result in undesirable drug release outside of the cytoplasm, or during the early stages of endocytosis. In contrast, mixtures of DOPE/CHEMS can release drugs at pH 5.5, which corresponds to later phases of endocytosis when nanoparticles are delivered into lysosomes. Conjugation of lipids to different molecular moieties (Fig. 2) allows "tuning" liposomes to release a drug at optimal range of pH [42, 46-55]. Some lipids can also be used for the tuning for necessary range of pH. For example, cholesterol can additionally stabilize liposomes. The response to pH changes can be facilitated by fusion peptides [56], pH sensitive polymers, attached to the surface of liposomes [57-59], or pH sensitive lipids [48, 53].

**Thermosensitive liposomes.** The ability of liposomes to release drugs in response to temperature changes was first demonstrated more than 30 years ago [60]. The mechanism of their functioning was based on the destabilization of the bilayer at the main transition temperature of lipid ($T_m$), known also as the temperature of lipid melting [61]. For this purpose the authors used a mixture of DPPC ($T_m$ = 41.4°C) and DSPC ($T_m$ = 54.9°C) that reveal drug release at temperature >43°C. Lysolipids, for example 1-palmitoyl-2-lysophosphatidylcholine (lysoPC), can decrease the temperature to the clinically more reasonable (39.5-41.5°C) and increase the rate of release of the drug (Fig. 3). Thus, it was demonstrated that doxorubicin can be completely released from liposomes in 20 sec [62]. Using these results the Celsion company developed the liposomal product ThermoDox® (Celsion Corporation, USA) that in 2008 successfully passed stage I clinical tests on patients with liver cancer and in 2009 on patients with breast cancer.

Recently the thermosensitive phospholipid DPPGOG (Fig. 3) was produced [63]. In a mixture with unsaturated phosphatidylcholines the authors developed thermosensitive liposomes of 175-nm size that are able to

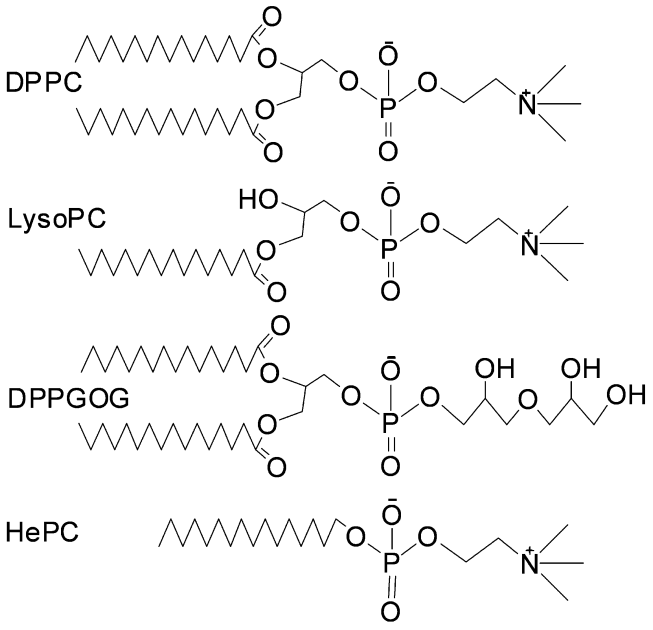


**Fig. 3.** Some phospholipids used for preparation of thermosensitive liposomes. See details in text.

release doxorubicin at 41-42°C, while at body temperature the liposomes were very stable and their half period of drug release was 5-10 h. Alkyl phosphocholines, a new class of compounds, were suggested recently to produce thermosensitive liposomes for drug delivery in anticancer therapy [64]. Among them hexadecyl phosphocholine (HePC in Fig. 3), known in medicine as miltefosine, is the most remarkable compound. This lipid is effective against parasitic diseases like leishmaniasis [65], it is clinically approved for some cancer diseases [66], and it is promising in therapy of HIV infection [67]. Liposomes containing HePC and loaded with doxorubicin revealed pronounced antitumor activity [68, 69]. It was found recently that liposomes with HePC are thermally sensitive. Thus, the antitumor effect of HePC could be facilitated by doxorubicin release from liposomes on local heating of the tumor [64].

**Light-sensitive liposomes.** Biocompatible light-sensitive materials were suggested more than 20 years ago [70]. These molecular devices are used for designing liposomes able to release their load in response to exposure to light [71, 72]. The depth of light penetration into the human body depends on wavelength. The ultraviolet (UV) and blue part of the visible spectrum penetrates into the human body no more than 1-2 mm and allows controlling some processes on the surface and inside the skin. A considerable increase in penetration begins from orange light (>590 nm). Wavelengths >700 nm (red and near infrared (IR) light) penetrate to approximately 1 cm and can be used for controlling light sensitive devices inside the body and also for visualization of some internal processes [73, 74].

A disruption of barrier properties of the lipid bilayer of liposomes is necessary to release loaded water-soluble drugs in tissues or the cell cytoplasm. The disruption can be initiated by photochemical reactions of oxidation by singlet oxygen. Singlet oxygen can be produced by natural or artificial photosensitizers designed to adsorb red and infrared light, which is especially promising for medical applications. For example, the porphyrins and related compounds are very effective [75-77]. Other classes of compounds include, for example, chlorins [78], bacteriochlorophyll *a* [79], and phthalocyanine dendrimers [80, 81].

Singlet oxygen is highly reactive and can damage cells by oxidizing various compounds [82-84]. However, the lifetime of singlet oxygen is very short (<0.1 μsec). During this time singlet oxygen can migrate by a distance of 10-20 nm and thus is able to oxidize molecules located in the immediate vicinity of the photosensitizer [85]. This allows safe use of photosensitizers for light-dependent compound release from liposomes and development of techniques for photochemical drug and genetic material internalization into the cytoplasm [75, 76].

The produced reactive oxygen species can oxidize both endogenous lipids of the cell membranes and externally added molecules having increased oxidability, for example derivates of natural lipids like bis-SorbPC$_{17,17}$ (Fig. 4a, I). This lipid can increase stealth liposome permeability 200-fold even on short (a few minutes) exposure to UV light [86]. There is a report of 28,000-fold increase in permeability after adjustment of lipid composition and exposure to 254-nm UV light. It is supposed that bilayer defects originate from cross-linking between molecules of bis-SorbPC$_{17,17}$ produced by UV radiation in presence of oxygen. This results in production of hard and compact "shrunken domains" and formation of packing defects of lipids resulting in transient increase in the permeability of the bilayer [87].

Singlet oxygen oxidizes lipids near double bonds. For example, in plasmenylcholine the breaking of a double bond of vinyl plasmalogen leads to disintegration of liposomes and formation of micelles [79] (Fig. 4b). Infrared light of 800 nm rapidly releases contents of liposomes modified by bacteriochlorophyll *a* as photosensitizer. Thus, the rate of calcein release from photosensitive liposomes was two orders of magnitude higher than that of regular liposomes composed from egg lecithin.

Light-sensitive liposomes can contain synthetic lipids with chromophores responsible for light harvesting and light-dependent conformational transitions of molecules. In response to light exposure derivatives of azobenzene, cinnamoyl, and spirobenzopyran moieties can change their polarity and corresponding hydrophobicity as a result of *cis-trans* isomerization, or changes in number of charged groups [72, 88, 89] (Fig. 4a, II-IV).

The *cis-trans* isomerization of azobenzene can change the packing and phase state of lipids. For exam-

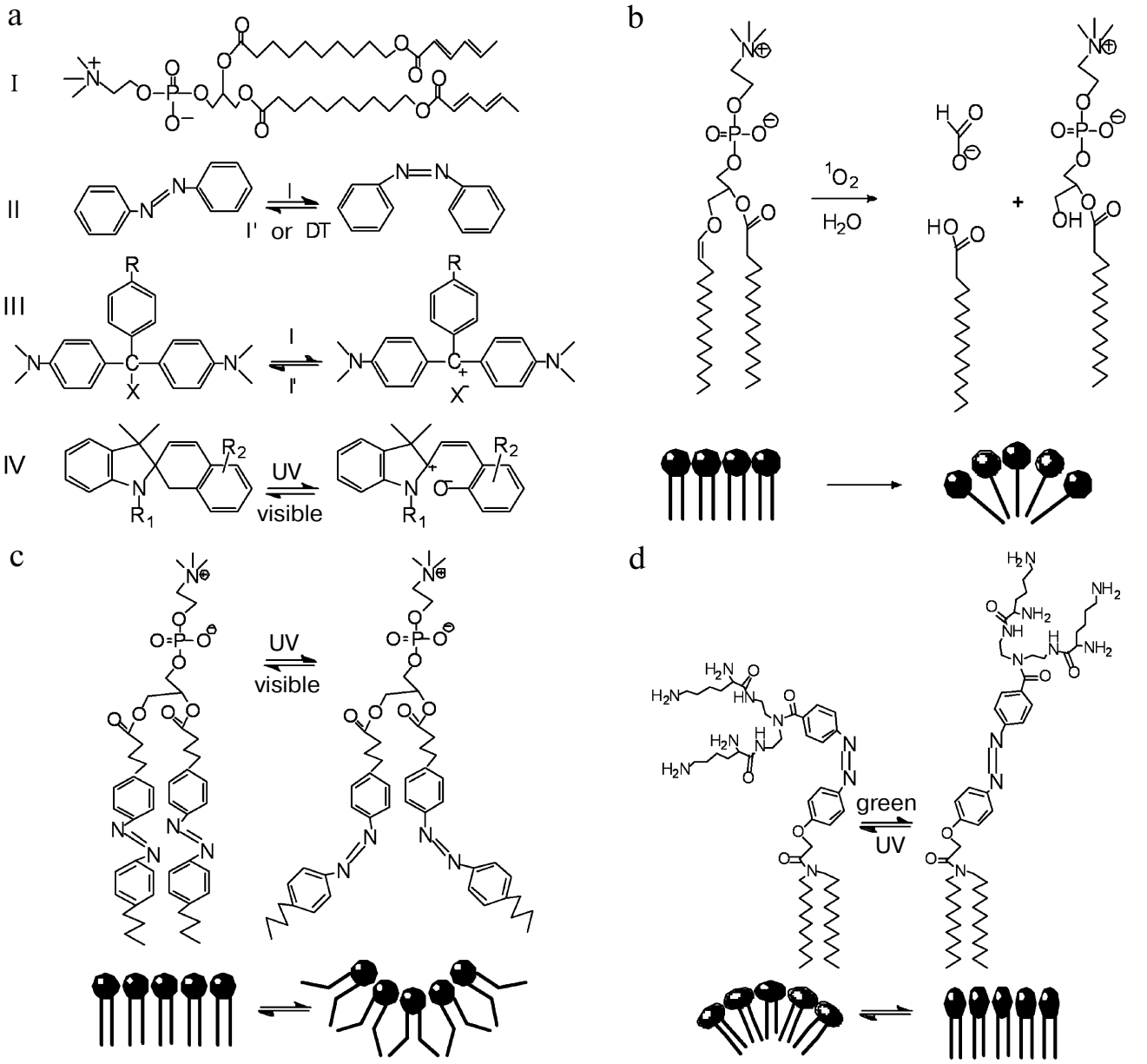


**Fig. 4.** a) Molecules used for preparation of photosensitive liposomes. Photoreactive phosphatidylcholine bis-SorbPC$_{17,17}$ (1,2-bis[10-(2′,4′-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphocholine) (I). *Cis-trans* isomerization of azobenzene (II). Change in charge of cinnamoyl (III) or spirobenzopyran (IV) groups when exposed to light of different wavelengths [72]. b) Changes in lipid packing initiated by detaching of one hydrophobic hydrocarbon chain in plasmenylcholine oxidized by singlet oxygen produced by ultraviolet radiation. c) Conformational transformations and resulting molecular packing changes in the azobenzene-derivative phosphatidylcholine Bis-Azo-PC. d) Isomerization in azobenzene-derivative lipid KAON12 under exposure to green (546 nm) or ultraviolet (365 nm) light [95].

ple, UV treatment of liposomes containing phospholipid Bis-Azo-PC can initiate transition from a planar structure present in bilayer vesicles to inverted micellar structures (Fig. 4c). The conformational transition is reversible and can be controlled by light of different wavelengths. This allows regulation of the permeability and release of internal content from liposomes containing 6% of Bis-Azo-PC [90-93]. The changes in permeability were reversible because the amount of light sensitive lipid in liposomes was rather small. Thus, the local and temporal changes in lipid packing in liposomes can induce reversible changes in the bilayer permeability. It is remarkable that in darkness the liposomes were very stable and impermeable for the loaded compounds.

Azobenzene derivatives of cholesterol can also be utilized for regulation of liposomes permeability. Cholesterol derivatives with different polarity and charge can change the sensitivity of liposomes to light exposure

and the rate of drug release at different wavelengths and temperatures [94].

The isomerization of lipid can also induce changes in shape of liposomes. Reversible changes in shape could be observed on gigantic liposomes containing lipid KAON12 when they were exposed to light of different wavelengths [95]. Light can initiate isomerization of the azobenzene chromophore moiety (Fig. 4d). Isomerization of the lipid can initiate changes in lipid bilayer curvature. The shape changes can be observed under a light microscope as invaginations or protrusions on the surface of gigantic liposomes. The changes are reversible and controlled by light of different wavelengths. The phenomenon could be applicable for manipulation of liposomes with light [96].

**Echoliposomes.** Submicron gas bubbles stabilized with lipid and sensitive to ultrasound treatment are known as echogenic liposomes or as echoliposomes [97-99]. They can be used for delivery of drugs incorporated inside the bubbles or as contrasting agents for ultrasound visualization and treatment of pathological processes. Then, a focused ultrasound beam can be applied for acoustic targeting of drugs loaded inside the liposomes or for selective disintegration of desired tissues (acoustic scalpel).

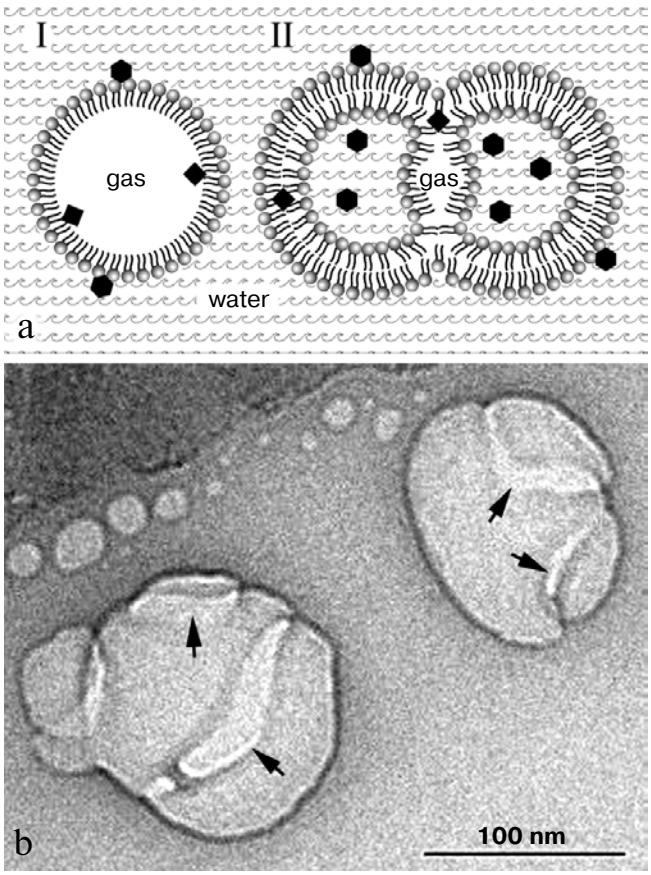


**Fig. 5.** a) Schematic representation of two types of echoliposomes. In the first type gas occupies the internal space of the liposomes and is surrounded with a lipid monolayer. Drugs (black polyhedrons) can be located on the external or on the internal surface of the lipid, but never inside the bubbles where the gas is located (I). Multisectional liposome filled with drug solution. Some drugs could be located on the surface or in the hydrophobic region of the bilayer. Gas occupies only a few sections of liposome (II). b) Cryo-transmission electron microscopy of echoliposomes. The air bubbles inside of liposomes are indicated by arrows. Bar is 100 nm.

Ultrasound is designated as sound waves with frequency higher than 20 kHz. The frequency range from tens of kHz to a few MHz is applicable for therapy. Theoretically, cavitation of liquids could be promoted by the presence of appropriate centers of nucleation. Gaseous bubbles are the most appropriate centers of nucleation. In model tissues, if the initial centers of nucleation were not present, spontaneous nucleation does not depend on the frequency of waves in the range of 1-15 MHz and produced at sound pressure above 4 MPa [100]. But actual threshold values in tissues are higher than in liquids [101]. This is the reason why centers of artificial nucleation are necessary to produce cavitation in tissues. The first ultrasound contrasting compound Albunex® was produced by Molecular Biosystems (USA) and medically approved in 1994. Its improved version Opsiton™ appeared in 1997. Later two more compounds, Definity® and Imagent®, were approved for application in the USA in 2001 and 2002, respectively. There are also medically approved Canadian and European products [102].

The ultrasound contrasting agencies are presented by microbubbles of gas stabilized by surface active compounds, surfactants including long chain fatty acids, alcohols, esters, and various lipids. Because of low dielectric permeability, gases behave similarly to hydrophobic compounds. Lipids therefore produce a monolayer surrounded the gas bubbles with their polar heads turned outside in the water and with the hydrophobic moieties exposed to the gas (Fig. 5a). Acoustically active ultrasound contrast agents containing air bubbles of submicron size are sometimes called echogenic liposomes or echoliposomes [97]. Unfortunately, the survival of liposomes containing air is rather short because the air is soluble in water and can be readily removed from the liposomes. Gases with low solubility or insoluble in water can be utilized to increase the stability of bubbles. The gases must be chemically inert, metabolically inactive, and nontoxic. Perfluoro-organic compounds having 3-6 carbon atoms length, like perfluoropropane or perfluorohexane, are often used to fill bubbles.

Electron microscopy of echogenic liposomes revealed single bubbles filled with gases or rather complex liposomal structures containing compartments filled with both air and water (Fig. 5b). This kind of liposomes can be called multisectional. Compartments filled with water can contain also water-soluble drugs that can be released when the liposomes are exposed to ultrasound (Fig. 5a, II).

A number of different aspects of the use of echoliposomes in medicine should be mentioned [97, 99, 103, 104]. They can be used as contrasting agencies for ultrasound visualization and identification of pathological processes, as sources of local increase in temperature, concentration of free radicals, or compounds able to increase cell membranes permeability for drugs. Echoliposomes are also applicable for ultrasound visualization and treatment of pathological processes including ulcers and inflammations [105-108], tumor angiogenesis [109-112], or ischemia [113-116].

Water-soluble compounds contained in the internal volume of echoliposomes can be released by ultrasound [117]. In addition, echoliposomes can be directed to various targets [118-121]. For example, microbubbles conjugated to monoclonal antibodies against endoglin (CD 105) or VEGFR-2 receptor of tumor vasculature [122] have been developed. Targeting of echogenic liposomes to marrow cells implanted in muscles and subsequent treatment by ultrasound induced angiogenesis and improved implant viability [123].

Numerous studies have been devoted to the application of echogenic liposomes in gene delivery. The adjustment of ultrasound treatment protocol allows manifold increase in cell transfection [118, 124]. The ability of ultrasound to deeply penetrate into tissues facilitates gene expression in transfected myocardium [111] or hepatocytes [125].

As demonstrated recently, the permeability of the blood–brain barrier can be temporary and reversibly increased by a short impulse of focused ultrasound directed from the surface of the head into the brain [126]. Targeting of echogenic liposomes to endothelial cells of brain vessels and subsequent ultrasound treatment helps to treat desired regions of the brain [127]. Thus, large molecules like horseradish peroxidase, a protein of 40 kDa, can pass through the blood brain barrier [128].

The ultrasound-initiated cavitation of bubbles might facilitate arterial thrombus disaggregation and lysis. In the mid 1990s first model experiments demonstrated the possibility of thrombus destruction by ultrasound treatment [129]. In this case the ultrasound of not very high frequency, a few MHz or even tens of kHz, which is able to penetrate deeply into tissues and is therefore most useful [114, 130]. A source of focused ultrasound located on the surface of the body, for example on the surface of the head, could be used to disaggregate thrombus of brain vessels [103]. For further improvement of the thrombolysis the stability of echo contrast devices and their high concentration near the thrombus is very important. The bubbles should be filled with highly stable, inert, and water-insoluble gases with large molecular weight and increased boiling temperature, for example with perfluorocarbons [114, 115].

**Magnetoliposomes.** The therapeutic application of magnetic micro- and nano-particles is constantly growing [131, 132]. Possibilities of controlling particle delivery to a desired area, visualization of the delivery process with NMR, and drug release initiated by heating of particles in a magnetic field are their important advantages.

There are some limitations to the use of magnetic field for drug delivery. Based on clinical studies in 1987, the Food and Drug Administration of the USA (FDA) classified magnets with field strength of less than 2 T as devices having insignificant risk [132, 133]. In 1996 field of 4 T was determined to be insignificant. In 2003 the threshold was increased to 8 T, although 30% decrease in blood flow *in vitro* and changes in erythrocyte behavior were revealed [134]. Clinical tests with field strength of 8 T have not detected any pathological changes *in vivo* [135]. The field of 10 T was designated as harmless [136] or initiating only reversible changes after short (10 sec) application [137], though changes in blood flow were experimentally revealed. Modern permanent magnets are able to monitor particles up to 10-15 cm inside the body [132]. Moreover, manipulation of the shape of the magnetic field allows locating particles in a region for therapeutic treatment [138, 139].

The selection of appropriate material for production of magnetic particles is also a very important subject. Ferromagnetic materials, iron oxide (magnetite) for example, are magnetized strongly in the direction of an external magnetic field and tend to retain their magnetization after the external magnetic field is removed [131, 140]. This leads to aggregation of particles and increases the risk of blood vessel thrombosis. But sufficiently small particles, less than 150-200 nm, undergo drastic thermal fluctuations and loose magnetization and tendency to aggregate immediately after removing the external magnetic field. This phenomenon is called superparamagnetism [141].

Large-scale studies of pharmacokinetics and toxicity of superparamagnetic particles of magnetite did not reveal any acute or subacute toxic effect even when the concentration of magnetite particles was increased to 150 mg/kg body weight, which considerable exceeds therapeutic doses. Magnetite did not influence blood pressure, cardiac rhythm, or respiratory rate and is regarded as a material that is well tolerated by the cardiovascular system [142]. The injection of magnetite into the bloodstream is permitted by the US Food and Drug Administration [143].

To prepare magnetoliposomes the superparamagnetic particles should be enclosed into lipid vesicles. The first magnetoliposomes and the term itself were suggested by Margolis and colleagues in 1983 [144]. Later two basic types of liposomes were studied. The first classical type [145] is magnetic spheres occupying the whole space inside of bilayer vesicles (Fig. 6a). In this type of liposomes the ratio of magnetic material to lipid is very high. When large quantities of paramagnetic material are endocytosed into the cytoplasm, the lipids can minimize the toxic effect [146]. The liposomes do not have a space for drugs. Only compounds dissolved in the lipid bilayer or

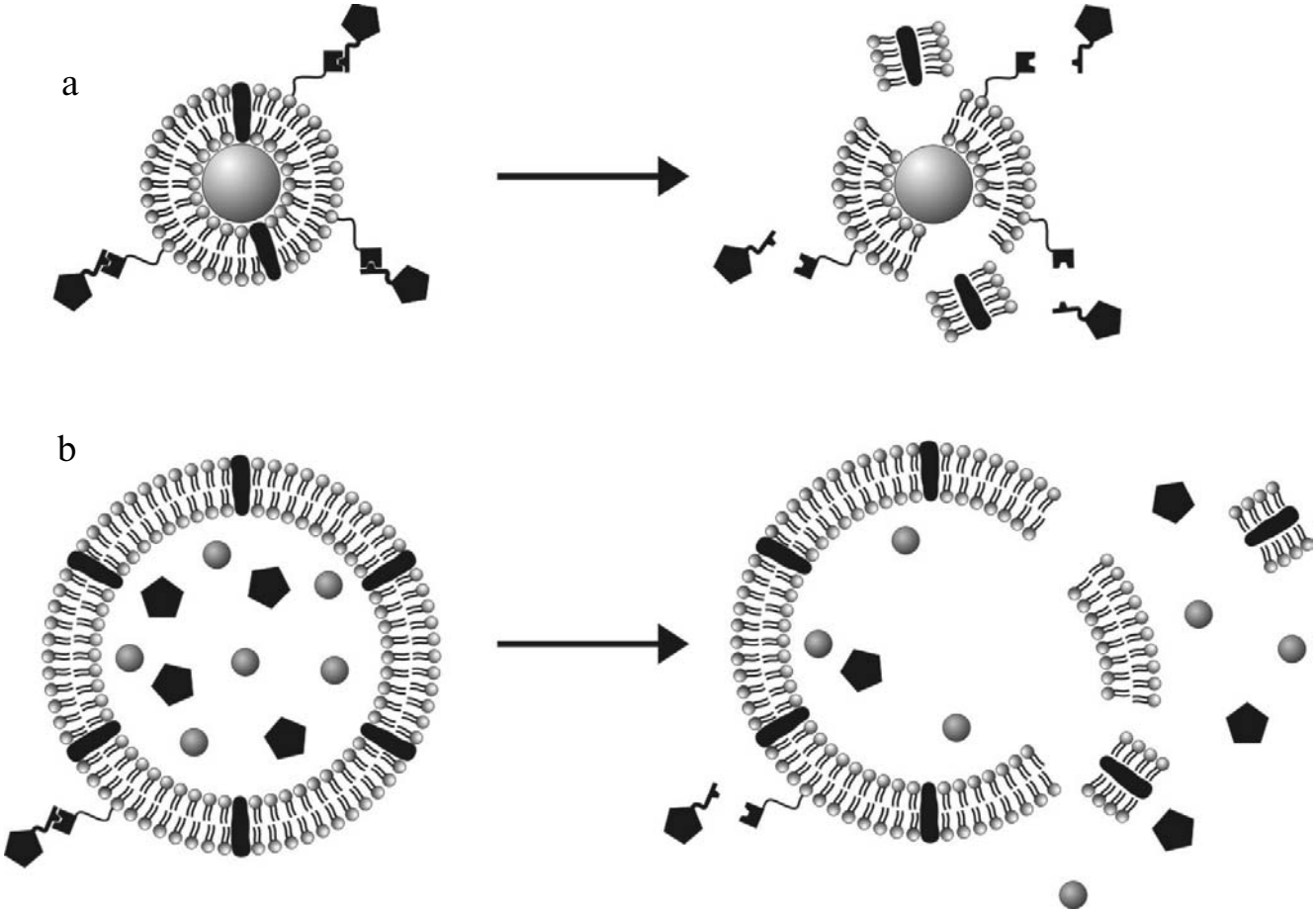


**Fig. 6.** a) Classical magnetoliposomes are filled with large magnetic particles (gray spheres) and do not have an internal space for drugs. In this case drugs (black polyhedrons) can be attached to ligands on the surface of the liposomes or located inside the lipid bilayer. When exposed to external signal (changes in temperature, pH, light, etc.) drugs can be released. b) Large liposomes filled with super-small magnetic particles and thus able to contain drugs not only on the surface, but also in the internal space of the liposome or in the hydrophobic region of the bilayer. Various factors resulting to disintegration of the bilayer or interaction with ligands can initiate drug release.

attached to the surface of the bilayer can be loaded using this type of liposomes.

The second type of magnetoliposomes [147] is vesicles of 100-500 nm containing a suspension of very small superparamagnetic particles (1-10 nm) in the internal space filled with water (Fig. 6b). The advantage of this type of liposomes is the possibility to introduce water-soluble compounds inside the internal space of the liposomes, although their magnetizability is not high because the magnetic moment of small particles is decreased.

Heating of magnetic particles in an alternating magnetic field allows release of compounds from the internal space of the second type of liposomes. For example, calcein could be released from DPPC magnetoliposomes heated above the lipid melting point ($T_m$ = 42°C) [148]. Later this approach was applied for drug release *in vivo* [149, 150]. Thermosensitive sterically stabilized liposomes composed of DPPC/DPPE/PEG2000 mixture revealed a phase transition at 41°C [151]. Within 10 min they where able to release up to 85% of internal doxorubicin when heated to 42°C, which is advantageous for drug delivery to tumor cells.

Magnetoliposomes could also be used for tissue hyperthermia. The superparamagnetic particles could be heated up to sufficiently high temperatures for killing cells and thus destroy tumors. The effectiveness of the hyperthermia depends on the size and composition of magnetic particles. In experiments with cobalt ferrite and maghemite good results were obtained with particles of about 14 nm [152, 153]. Heating to 41-46°C is regarded as moderate hyperthermia and can initiate immune response. Thus, this approach can be regarded as a nonspecific immunization against cancer [154, 155]. Cell death, tumor necrosis, and removal (thermoablation) could be achieved on heating to 46-56°C [156]. In this case it is very important not to damage the surrounding tissues. Liposomes are very convenient to solve this problem because the targeting can be considerably improved

by addition of specific ligands to the surface of liposomes [157].

**Folate-modified liposomes.** Use of specific receptors not only improves liposomal targeting, but it also extends the diversity of targets for drug delivery. Folate receptors (FR) known also as folate-binding proteins are the most often utilized targets [158]. The most useful α-FR and β-FR are membrane proteins attached to glycosyl phosphatidylinositol [159]. It should be mentioned also that there is a difference between FR and the well-known reduced folate carrier (RFC). RFC is involved in transmembrane folate transport. This protein is expressed in the all kind of cells, while FR is not normally required for cell survival and the expression of FR is very restricted. Moreover, the affinity of FR to folate is more than $10^3$-fold higher than RFC, which allows specifically targeting FR without side effects on RFC.

The increased expression of FR on the surface of tumor cells was found in the early 1990s [160-162]. Some tumors reveal elevated expression of α-FR. The list of diseases is rather long including ovarian, lung, breast, kidney, brain, and colon cell carcinomas. Diseases related to macrophage dysfunction and overexpression of β-FR include rheumatoid arthritis, psoriasis, atherosclerosis, diabetes, arthritis, glomerulonephritis, and most inflammatory diseases [163]. Thus α-FR receptor is regarded as a marker of cancerogenic transformations [10, 164, 165], while β-FR is a marker of myeloid leukemia and chronic inflammations [166]. Therapeutic agents specifically targeted to folate receptors are now under development [164, 167-169].

The affinity of folic acid to FR is very high. The dissociation constant of α-FR to folic acid is $K_d = 10^{-10}$ M [170, 171], while for β-FR it is $K_d = 10^{-9}$ M [172]. The dissociation constant is rather low even in conjugates of folate with delivered cargo. For example, the conjugate of folate with ribonuclease reveals $K_d = 24$ nM [173]. The effectiveness of conjugates of folate with some toxins is outstanding. Thus, folate–momordin kills 50% of tumor cells at conjugate concentration of $IC_{50} \approx 10^{-9}$ M [174]. The conjugate of folate with pseudomonad toxin was even more effective: $IC_{50} \approx 10^{-11}$ M [175].

The interaction of folate with corresponding receptors can activate endocytosis and deliver compounds into the cytoplasm [176]. It was demonstrated that when folate was attached directly to lipid molecules, liposomes could not effectively bind to the surface receptors of cells [177]. However, when folate was attached to liposome through the PEG chain (PEG 3350 Da, length 250 Å) the entry of the liposomes into cells was very effective [178]. The conjugates of folate–polyethyleneglycol–distearoyl phosphatidylethanolamine (folate-PEG-DSPE), or folate–polyethyleneglycol–cholesterol (folate-PEG-Chol) could also be used for this purpose [177, 179].

Folate-modified liposomes have been applied to delivery of various drugs including doxorubicin [178, 180, 181], daunorubicin [182, 183], cytosine arabinofuranoside [184], paclitaxel [185], radioactive boron [186, 187], photosensitizing agents [188], and antisense oligonucleotides [189]. Experiments on delivery of doxorubicin-loaded liposomes revealed that folate-modified liposomes enter tumor cells 45-fold more effectively than unmodified liposomes. The therapeutic efficiency of folate-modified liposomes was 85-fold higher than that of unmodified liposomes [178]. Moreover, doxorubicin delivery by folate-modified liposomes helps to bypass cell resistance to this drug [190].

The limitations of folate-modified liposomes are related to a greater rate of their removal from blood by the RES system compared to the normal sterically stabilized liposomes. This phenomenon can be explained by the increased affinity of macrophages to these liposomes originating from the existence of some β-FR expression by normal macrophages [191]. The abovementioned difficulties limit the application of folate-modified liposomes in medicine in spite of their successful use in experiments on animals [158].

**Immunoliposomes.** Immunoliposomes produced by attachment of antibodies to the surface of liposomes is the best way for their targeting [192, 193]. The perspectives for application of antibodies for targeting of liposomes have been studied since the 1970s [194, 195]. Considerable progress in this area has been achieved owing to application of sterically stabilized liposomes covered with polymers like PEG. Liposomes with antibodies attached to the distant ends of PEG chains are the most effective. This type of liposomes was suggested in the end of the 1990s [196-198] and is now the most often used.

The specific targeting of liposomes is based on monoclonal antibodies. Attachment of antibodies to liposomes results in considerable decrease in the effectiveness of their interaction with antigens. Thus, monoclonal antibodies trastuzumab (herceptin) express up to 95% of activity to antigen HER2/neu. But with immunoliposomes their activity drops to 2.0-4.5% [199]. The antibody–antigen binding constant decreased 15-fold when antibodies were attached to the surface of liposomes. This probably originates from steric restrictions of liposome interactions with the cell surface [200]. However, when recalculated for a whole liposome containing many antibodies simultaneously interacting with a cell, the decrease is not very large and the effectiveness of liposomes interaction with cell surface can be rather high.

Attachment of intact IgG antibodies to the surface of liposomes can shorten their presence in the blood because of recognition by macrophages. Attachment to liposomes of Fab and scFv fragments of antibodies is a good solution because the fragments retain effective binding to antigens and can prolong the presence of the liposomes in the blood [192, 201, 202]. It was demonstrated that immunoliposomes are able to effectively penetrate inside cells by

means of receptor-mediated endocytosis. About 90% of immunoliposomes could be endocytosed, while similar liposomes without antibodies were endocytosed less than 5% [202]. Thus, a considerably higher effectiveness of immunoliposomes can be explained by delivery of drugs directly into the cytoplasm.

The application of immunoliposomes for delivery of contrast compounds for visualization of pathological processes with magnetic resonance imaging (MRI) or radioscintigraphy has also been studied [201-208]. Contrast materials for MRI, for example Gd, are attached to hydrophobic chelators and incorporated into liposomes [209]. This approach is called anchoring. Polychelating amphiphilic polymer (PAP) can be attached to lipid and incorporated in the bilayer. In this case the contrast is improved because numerous atoms of Gd are attached to one anchor [208, 210]. Similar approaches are applicable also for targeting of immunoliposomes loaded with isotope $^{111}$In [205, 211] used for gamma scintigraphic imaging of different tumors. Immunoliposomes loaded with $\alpha$-particles emitting isotopes $^{212}$Bi, $^{225}$Ac, $^{223}$Ra can be used for high-precision radiotherapy [199, 212, 213]. Immunoliposomes may find wide application in medicine, though only early stages of clinical trials are being conducted at present [214-216].

The lipid bilayer is characterized by complex behavior and a variety of properties that living organisms have been exploiting for hundreds of millions of years. Now it is a time to use the results of the natural "experiment" for designing "smart" artificial devices. At present liposomes composed of lipid bilayer and accordingly liposomal drug delivery technologies are a fast developing area of investigation. Liposomes are regarded not only as a convenient and capacious tool for storage and delivery of medical compounds, but also a useful platform for attachment of various molecular devices providing their controlled transportation and targeting in the human body. This allows combining our knowledge in lipid behavior with the most staggering achievements in chemistry of intelligent polymers. Moreover, the liposomal platform can be also equipped with diagnostics and visualization devices based on the latest achievements in magnetic resonance, infrared, and ultrasound spectroscopy. The development of nanoparticles equipped with abilities not only to control the drug delivery processes, but also to evaluate the results of their action is the subject of forthcoming studies.

## REFERENCES


1. Dass, C. R. (2008) *Meth. Mol. Biol.*, **437**, 177-182.
2. Huwyler, J., Drewe, J., and Krahenbuhl, S. (2008) *Int. J. Nanomed.*, **3**, 21-29.
3. Schiffelers, R. M., and Storm, G. (2008) *Int. J. Pharm.*, **364**, 258-264.
4. Torchilin, V. P. (2006) *Annu. Rev. Biomed. Eng.*, **8**, 343-375.
5. Hermida, R. C., Fernandez, J. R., Ayala, D. E., Mojon, A., Alonso, I., and Smolensky, M. (2001) *Chronobiol. Int.*, **18**, 475-489.
6. Gref, R., Minamitake, Y., Peracchia, M. T., Trubetskoy, V., Torchilin, V., and Langer, R. (1994) *Science*, **263**, 1600-1603.
7. Frank, M. M., and Fries, L. F. (1991) *Immunol. Today*, **12**, 322-326.
8. Kaul, G., and Amiji, M. (2002) *Pharm. Res.*, **19**, 1061-1067.
9. Dumortier, G., Grossiord, J. L., Agnely, F., and Chaumeil, J. C. (2006) *Pharm. Res.*, **23**, 2709-2728.
10. Gabizon, A., Shmeeda, H., Horowitz, A. T., and Zalipsky, S. (2004) *Adv. Drug Deliv. Rev.*, **56**, 1177-1192.
11. Illum, L., Davis, S. S., Muller, R. H., Mak, E., and West, P. (1987) *Life Sci.*, **40**, 367-374.
12. Nishiyama, N., and Kataoka, K. (2003) *Adv. Exp. Med. Biol.*, **519**, 155-177.
13. Parveen, S., and Sahoo, S. K. (2006) *Clin. Pharmacokinet.*, **45**, 965-988.
14. Redhead, H. M., Davis, S. S., and Illum, L. (2001) *J. Control Release*, **70**, 353-363.
15. Sharpe, M., Easthope, S. E., Keating, G. M., and Lamb, H. M. (2002) *Drugs*, **62**, 2089-2126.
16. Torchilin, V. P. (2002) *Adv. Drug Deliv. Rev.*, **54**, 235-252.
17. Zhang, W., Liu, J., Li, S., Chen, M., and Liu, H. (2008) *J. Microencapsul.*, **25**, 203-209.
18. Owens, D. E., III, and Peppas, N. A. (2006) *Int. J. Pharm.*, **307**, 93-102.
19. Awasthi, V. D., Garcia, D., Goins, B. A., and Phillips, W. T. (2003) *Int. J. Pharm.*, **253**, 121-132.
20. Prencipe, G., Tabakman, S. M., Welsher, K., Liu, Z., Goodwin, A. P., Zhang, L., Henry, J., and Dai, H. (2009) *J. Am. Chem. Soc.*, **131**, 4783-4787.
21. Shi, B., Fang, C., and Pei, Y. (2006) *J. Pharm. Sci.*, **95**, 1873-1887.
22. Allen, T. M., Mumbengegwi, D. R., and Charrois, G. J. (2005) *Clin. Cancer Res.*, **11**, 3567-3573.
23. Allen, T. M. (1994) *Trends Pharmacol. Sci.*, **15**, 215-220.
24. Lasic, D. D. (1996) *Nature*, **380**, 561-562.
25. Gill, P. S., Espina, B. M., Muggia, F., Cabriales, S., Tulpule, A., Esplin, J. A., Liebman, H. A., Forssen, E., Ross, M. E., and Levine, A. M. (1995) *J. Clin. Oncol.*, **13**, 996-1003.
26. Charrois, G. J., and Allen, T. M. (2003) *Biochim. Biophys. Acta*, **1609**, 102-108.
27. Nagayasu, A., Uchiyama, K., and Kiwada, H. (1999) *Adv. Drug Deliv. Rev.*, **40**, 75-87.
28. Ishida, O., Maruyama, K., Sasaki, K., and Iwatsuru, M. (1999) *Int. J. Pharm.*, **190**, 49-56.
29. Lukyanov, A. N., Hartner, W. C., and Torchilin, V. P. (2004) *J. Control Release*, **94**, 187-193.
30. Li, S. D., and Huang, L. (2008) *Mol. Pharm.*, **5**, 496-504.
31. Brannon-Peppas, L., and Blanchette, J. O. (2004) *Adv. Drug Deliv. Rev.*, **56**, 1649-1659.
32. Mayer, L. D., Nayar, R., Thies, R. L., Boman, N. L., Cullis, P. R., and Bally, M. B. (1993) *Cancer Chemother. Pharmacol.*, **33**, 17-24.
33. Zou, Y., Ling, Y. H., Reddy, S., Priebe, W., and Perez-Soler, R. (1995) *Int. J. Cancer*, **61**, 666-671.

34. Allen, T. M., Hansen, C., Martin, F., Redemann, C., and Yau-Young, A. (1991) *Biochim. Biophys. Acta*, **1066**, 29-36.
35. Klibanov, A. L., Maruyama, K., Beckerleg, A. M., Torchilin, V. P., and Huang, L. (1991) *Biochim. Biophys. Acta*, **1062**, 142-148.
36. Litzinger, D. C., and Huang, L. (1992) *Biochim. Biophys. Acta*, **1113**, 201-227.
37. Litzinger, D. C., Buiting, A. M., van Rooijen, N., and Huang, L. (1994) *Biochim. Biophys. Acta*, **1190**, 99-107.
38. Maruyama, K., Yuda, T., Okamoto, A., Kojima, S., Suginaka, A., and Iwatsuru, M. (1992) *Biochim. Biophys. Acta*, **1128**, 44-49.
39. Allen, T. M., Cheng, W. W., Hare, J. I., and Laginha, K. M. (2006) *Anticancer Agents Med. Chem.*, **6**, 513-523.
40. Ojugo, A. S., McSheehy, P. M., McIntyre, D. J., McCoy, C., Stubbs, M., Leach, M. O., Judson, I. R., and Griffiths, J. R. (1999) *NMR Biomed.*, **12**, 495-504.
41. Stubbs, M., McSheehy, P. M., Griffiths, J. R., and Bashford, C. L. (2000) *Mol. Med. Today*, **6**, 15-19.
42. Bergstrand, N., Arfvidsson, M. C., Kim, J. M., Thompson, D. H., and Edwards, K. (2003) *Biophys. Chem.*, **104**, 361-379.
43. Connor, J., and Huang, L. (1986) *Cancer Res.*, **46**, 3431-3435.
44. Duzgunes, N., Straubinger, R. M., Baldwin, P. A., Friend, D. S., and Papahadjopoulos, D. (1985) *Biochemistry*, **24**, 3091-3098.
45. Straubinger, R. M., Duzgunes, N., and Papahadjopoulos, D. (1985) *FEBS Lett.*, **179**, 148-154.
46. Collins, D., Litzinger, D. C., and Huang, L. (1990) *Biochim. Biophys. Acta*, **1025**, 234-242.
47. De Oliveira, M. C., Rosilio, V., Lesieur, P., Bourgaux, C., Couvreur, P., Ollivon, M., and Dubernet, C. (2000) *Biophys. Chem.*, **87**, 127-137.
48. Drummond, D. C., and Daleke, D. L. (1995) *Chem. Phys. Lipids*, **75**, 27-41.
49. Hong, M. S., Lim, S. J., Oh, Y. K., and Kim, C. K. (2002) *J. Pharm. Pharmacol.*, **54**, 51-58.
50. Kim, M. J., Lee, H. J., Lee, I. A., Kim, I. Y., Lim, S. K., Cho, H. A., and Kim, J. S. (2008) *Arch. Pharm. Res.*, **31**, 539-546.
51. Liu, D., and Huang, L. (1990) *Biochim. Biophys. Acta*, **1022**, 348-354.
52. Mignet, N., Richard, C., Seguin, J., Largeau, C., Bessodes, M., and Scherman, D. (2008) *Int. J. Pharm.*, **361**, 194-201.
53. Reddy, J. A., and Low, P. S. (2000) *J. Control Release*, **64**, 27-37.
54. Tari, A. M., Fuller, N., Boni, L. T., Collins, D., Rand, P., and Huang, L. (1994) *Biochim. Biophys. Acta*, **1192**, 253-262.
55. Drummond, D. C., Zignani, M., and Leroux, J. (2000) *Prog. Lipid Res.*, **39**, 409-460.
56. Turk, M. J., Reddy, J. A., Chmielewski, J. A., and Low, P. S. (2002) *Biochim. Biophys. Acta*, **1559**, 56-68.
57. Leroux, J., Roux, E., Le Garrec, D., Hong, K., and Drummond, D. C. (2001) *J. Control Release*, **72**, 71-84.
58. Mizoue, T., Horibe, T., Maruyama, K., Takizawa, T., Iwatsuru, M., Kono, K., Yanagie, H., and Moriyasu, F. (2002) *Int. J. Pharm.*, **237**, 129-137.
59. Roux, E., Francis, M., Winnik, F. M., and Leroux, J. C. (2002) *Int. J. Pharm.*, **242**, 25-36.
60. Yatvin, M. B., Weinstein, J. N., Dennis, W. H., and Blumenthal, R. (1978) *Science*, **202**, 1290-1293.
61. Mabrey, S., and Sturtevant, J. M. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 3862-3866.
62. Mills, J. K., and Needham, D. (2005) *Biochim. Biophys. Acta*, **1716**, 77-96.
63. Lindner, L. H., Eichhorn, M. E., Eibl, H., Teichert, N., Schmitt-Sody, M., Issels, R. D., and Dellian, M. (2004) *Clin. Cancer Res.*, **10**, 2168-2178.
64. Lindner, L. H., Hossann, M., Vogeser, M., Teichert, N., Wachholz, K., Eibl, H., Hiddemann, W., and Issels, R. D. (2008) *J. Control Release*, **125**, 112-120.
65. Sundar, S., Jha, T. K., Thakur, C. P., Engel, J., Sindermann, H., Fischer, C., Junge, K., Bryceson, A., and Berman, J. (2002) *N. Engl. J. Med.*, **347**, 1739-1746.
66. Leonard, R., Hardy, J., van Tienhoven, G., Houston, S., Simmonds, P., David, M., and Mansi, J. (2001) *J. Clin. Oncol.*, **19**, 4150-4159.
67. Collini, P., Premchand, N., Lockwood, D., and Greig, J. (2009) *Ann. Trop. Med. Parasitol.*, **103**, 455-459.
68. Papagiannaros, A., Hatziantoniou, S., Lelong-Rebel, I. H., Papaioannou, G. T., Dimas, K., and Demetzos, C. (2006) *In vivo*, **20**, 129-135.
69. Papagiannaros, A., Hatziantoniou, S., Dimas, K., Papaioannou, G. T., and Demetzos, C. (2006) *Biomed. Pharmacother.*, **60**, 36-42.
70. Lohmann, D., and Petrak, K. (1989) *Crit Rev. Ther. Drug Carrier Syst.*, **5**, 263-320.
71. Alvarez-Lorenzo, C., and Concheiro, A. (2008) *Mini. Rev. Med. Chem.*, **8**, 1065-1074.
72. Alvarez-Lorenzo, C., Bromberg, L., and Concheiro, A. (2009) *Photochem. Photobiol.*, **85**, 848-860.
73. Mochizuki-Oda, N., Kataoka, Y., Cui, Y., Yamada, H., Heya, M., and Awazu, K. (2002) *Neurosci. Lett.*, **323**, 207-210.
74. Li, Z., Zhang, M., Zhang, X., Dai, S., Yu, X., and Wang, Y. (2009) *Eur. J. Appl. Physiol.*, **107**, 281-287.
75. Hogset, A., Prasmickaite, L., Selbo, P. K., Hellum, M., Engesaeter, B. O., Bonsted, A., and Berg, K. (2004) *Adv. Drug Deliv. Rev.*, **56**, 95-115.
76. Prasmickaite, L., Hogset, A., Engesaeter, B. B., Bonsted, A., and Berg, K. (2004) *Expert. Opin. Biol. Ther.*, **4**, 1403-1412.
77. Shieh, M. J., Peng, C. L., Lou, P. J., Chiu, C. H., Tsai, T. Y., Hsu, C. Y., Yeh, C. Y., and Lai, P. S. (2008) *J. Control Release*, **129**, 200-206.
78. Mojzisova, H., Bonneau, S., Maillard, P., Berg, K., and Brault, D. (2009) *Photochem. Photobiol. Sci.*, **8**, 778-787.
79. Thompson, D. H., Gerasimov, O. V., Wheeler, J. J., Rui, Y., and Anderson, V. C. (1996) *Biochim. Biophys. Acta*, **1279**, 25-34.
80. Nishiyama, N., Arnida, Jang, W. D., Date, K., Miyata, K., and Kataoka, K. (2006) *J. Drug Target*, **14**, 413-424.
81. Nishiyama, N., Morimoto, Y., Jang, W. D., and Kataoka, K. (2009) *Adv. Drug Deliv. Rev.*, **61**, 327-338.
82. Henderson, B. W., and Dougherty, T. J. (1992) *Photochem. Photobiol.*, **55**, 145-157.
83. Moan, J., and Juzenas, P. (2006) *J. Environ. Pathol. Toxicol. Oncol.*, **25**, 29-50.
84. Tarr, M., and Valenzeno, D. P. (2003) *Photochem. Photobiol. Sci.*, **2**, 355-361.
85. Moan, J., and Berg, K. (1991) *Photochem. Photobiol.*, **53**, 549-553.
86. Bondurant, B., Mueller, A., and O'Brien, D. F. (2001) *Biochim. Biophys. Acta*, **1511**, 113-122.

87. Spratt, T., Bondurant, B., and O'Brien, D. F. (2003) *Biochim. Biophys. Acta*, **1611**, 35-43.
88. Zhao, Y. (2007) *Chem. Rec.*, **7**, 286-294.
89. Zhao, Y. (2009) *J. Mater. Chem.*, **19**, 4887-4895.
90. Bisby, R. H., Mead, C., and Morgan, C. G. (2000) *Biochem. Biophys. Res. Commun.*, **276**, 169-173.
91. Yagai, S., Karatsu, T., and Kitamura, A. (2005) *Chemistry*, **11**, 4054-4063.
92. Bisby, R. H., Mead, C., and Morgan, C. G. (2000) *Photochem. Photobiol.*, **72**, 57-61.
93. Bisby, R. H., Mead, C., Mitchell, A. C., and Morgan, C. G. (1999) *Biochem. Biophys. Res. Commun.*, **262**, 406-410.
94. Liu, X. M., Yang, B., Wang, Y. L., and Wang, J. Y. (2005) *Biochim. Biophys. Acta*, **1720**, 28-34.
95. Ishii, K., Hamada, T., Hatakeyama, M., Sugimoto, R., Nagasaki, T., and Takagi, M. (2009) *Chembiochem.*, **10**, 251-256.
96. Hamada, T., Sato, Y. T., Yoshikawa, K., and Nagasaki, T. (2005) *Langmuir*, **21**, 7626-7628.
97. Huang, S. L. (2008) *Adv. Drug Deliv. Rev.*, **60**, 1167-1176.
98. O'Brien, W. D., Jr. (2007) *Prog. Biophys. Mol. Biol.*, **93**, 212-255.
99. Unger, E. C., Zutshi, R., Matsunaga, T. O., and Ramaswami, R. (2006) in *Nanoparticulates as Drug Carriers* (Torchilin, V., ed.) Imperial College Press, London, pp. 225-254.
100. Church, C. C. (2002) *Ultrasound Med. Biol.*, **28**, 1349-1364.
101. Yang, X., and Church, C. C. (2005) *J. Acoust. Soc. Am.*, **118**, 3595-3606.
102. Miller, A. P., and Nanda, N. C. (2004) *Ultrasound Med. Biol.*, **30**, 425-434.
103. Dijkmans, P. A., Juffermans, L. J., Musters, R. J., van Wamel, A., ten Cate, F. J., van Gilst, W., Visser, C. A., de Jong, N., and Kamp, O. (2004) *Eur. J. Echocardiogr.*, **5**, 245-256.
104. Unger, E. C., Porter, T., Culp, W., Labell, R., Matsunaga, T., and Zutshi, R. (2004) *Adv. Drug Deliv. Rev.*, **56**, 1291-1314.
105. Lindner, J. R., Song, J., Xu, F., Klibanov, A. L., Singbartl, K., Ley, K., and Kaul, S. (2000) *Circulation*, **102**, 2745-2750.
106. Lindner, J. R., Dayton, P. A., Coggins, M. P., Ley, K., Song, J., Ferrara, K., and Kaul, S. (2000) *Circulation*, **102**, 531-538.
107. Lindner, J. R., Song, J., Christiansen, J., Klibanov, A. L., Xu, F., and Ley, K. (2001) *Circulation*, **104**, 2107-2112.
108. Naito, Y., Ito, M., Watanabe, T., and Suzuki, H. (2005) *Digestion*, **72**, 164-180.
109. Willmann, J. K., Cheng, Z., Davis, C., Lutz, A. M., Schipper, M. L., Nielsen, C. H., and Gambhir, S. S. (2008) *Radiology*, **249**, 212-219.
110. Willmann, J. K., Paulmurugan, R., Chen, K., Gheysens, O., Rodriguez-Porcel, M., Lutz, A. M., Chen, I. Y., Chen, X., and Gambhir, S. S. (2008) *Radiology*, **246**, 508-518.
111. Korpanty, G., Chen, S., Shohet, R. V., Ding, J., Yang, B., Frenkel, P. A., and Grayburn, P. A. (2005) *Gene Ther.*, **12**, 1305-1312.
112. Weller, G. E., Wong, M. K., Modzelewski, R. A., Lu, E., Klibanov, A. L., Wagner, W. R., and Villanueva, F. S. (2005) *Cancer Res.*, **65**, 533-539.
113. Andonian, S., Coulthard, T., Smith, A. D., Singhal, P. S., and Lee, B. R. (2009) *J. Endourol.*, **23**, 373-378.
114. Fatar, M., Stroick, M., Griebe, M., Alonso, A., Kreisel, S., Kern, R., Hennerici, M., and Meairs, S. (2008) *Ultrasound Med. Biol.*, **34**, 1414-1420.
115. Jing, X. X., Wang, Z. G., Ran, H. T., Li, L., Wu, X., Li, X. D., Peng, X. Q., Yang, C. J., Li, X. S., and Zhang, Q. X. (2008) *Clin. Imaging*, **32**, 178-182.
116. Title, L. M., Iles, S. E., Gardner, M. J., Penney, C. J., Clements, J. C., and Horacek, B. M. (2003) *J. Electrocardiol.*, **36**, 17-26.
117. Kee, P. H., Abruzzo, T. A., Smith, D. A., Kopechek, J. A., Wang, B., Huang, S. L., MacDonald, R. C., Holland, C. K., and McPherson, D. D. (2008) *J. Liposome Res.*, **18**, 263-277.
118. Hernot, S., and Klibanov, A. L. (2008) *Adv. Drug Deliv. Rev.*, **60**, 1153-1166.
119. Bull, J. L. (2007) *Expert. Opin. Drug Deliv.*, **4**, 475-493.
120. Kheirolomoom, A., Dayton, P. A., Lum, A. F., Little, E., Paoli, E. E., Zheng, H., and Ferrara, K. W. (2007) *J. Control Release*, **118**, 275-284.
121. Liu, Y., Miyoshi, H., and Nakamura, M. (2006) *J. Control Release*, **114**, 89-99.
122. Korpanty, G., Carbon, J. G., Grayburn, P. A., Fleming, J. B., and Brekken, R. A. (2007) *Clin. Cancer Res.*, **13**, 323-330.
123. Imada, T., Tatsumi, T., Mori, Y., Nishiue, T., Yoshida, M., Masaki, H., Okigaki, M., Kojima, H., Nozawa, Y., Nishiwaki, Y., Nitta, N., Iwasaka, T., and Matsubara, H. (2005) *Arterioscler. Thromb. Vasc. Biol.*, **25**, 2128-2134.
124. Meijering, B. D., Henning, R. H., van Gilst, W. H., Gavrilovic, I., van Wamel, A., and Deelman, L. E. (2007) *J. Drug Target*, **15**, 664-671.
125. Shen, Z. P., Brayman, A. A., Chen, L., and Miao, C. H. (2008) *Gene Ther.*, **15**, 1147-1155.
126. Hynynen, K., McDannold, N., Vykhodtseva, N., and Jolesz, F. A. (2003) *Acta Neurochir. Suppl.*, **86**, 555-558.
127. Meairs, S., and Alonso, A. (2007) *Prog. Biophys. Mol. Biol.*, **93**, 354-362.
128. Hynynen, K., McDannold, N., Vykhodtseva, N., and Jolesz, F. A. (2001) *Radiology*, **220**, 640-646.
129. Tachibana, K., and Tachibana, S. (1995) *Circulation*, **92**, 1148-1150.
130. Shaw, G. J., Meunier, J. M., Huang, S. L., Lindsell, C. J., McPherson, D. D., and Holland, C. K. (2009) *Thromb. Res.*, **124**, 306-310.
131. Polyak, B., and Friedman, G. (2009) *Expert. Opin. Drug Deliv.*, **6**, 53-70.
132. Arruebo, M., Fernandez-Pacheco, R., Ibarra, M. R., and Santamaria, J. (2007) *Nanotoday*, **2**, 22-32.
133. Schenck, J. F. (2005) *Prog. Biophys. Mol. Biol.*, **87**, 185-204.
134. Haik, Y., Pai, V., and Chen, C. J. (2001) *J. Magn. Magn. Mater.*, **225**, 180-186.
135. Chakeres, D. W., and de Vocht, F. (2005) *Prog. Biophys. Mol. Biol.*, **87**, 255-265.
136. Seiyama, A., Seki, J., Iwamoto, M., and Yanagida, T. (2005) *Dyn. Med.*, **4**, 5.
137. Chan, P., Eng, L. F., Lee, Y. L., and Lin, V. W. (1999) *J. Neurosci. Res.*, **55**, 238-244.
138. Dobson, J. (2009) *Drug. Dev. Res.*, **67**, 55-60.
139. Iacob, G., Rotariu, O., Strachan, N. J., and Hafeli, U. O. (2004) *Biorheology*, **41**, 599-612.

140. Olsvik, O., Popovic, T., Skjerve, E., Cudjoe, K. S., Hornes, E., Ugelstad, J., and Uhlen, M. (1994) *Clin. Microbiol. Rev.*, **7**, 43-54.
141. Pankhurst, Q. A., Connolly, J., Jones, S. K., and Dobson, J. (2003) *J. Phys. D.: Appl. Phys.*, **36**, R167-R181.
142. Lubbe, A. S., Alexiou, C., and Bergemann, C. (2001) *J. Surg. Res.*, **95**, 200-206.
143. Kaminski, M. D., and Rosengart, A. J. (2005) *J. Magn. Magn. Mater.*, **293**, 389-403.
144. Margolis, L. B., Namiot, V. A., and Kljukin, L. M. (1983) *Biochim. Biophys. Acta*, **735**, 193-195.
145. De Cuyper, M., and Joniau, M. (1988) *Eur. Biophys. J.*, **15**, 311-319.
146. Soenen, S. J., Baert, J., and de Cuyper, M. (2007) *Chembiochem.*, **8**, 2067-2077.
147. Martina, M. S., Fortin, J. P., Menager, C., Clement, O., Barratt, G., Grabielle-Madelmont, C., Gazeau, F., Cabuil, V., and Lesieur, S. (2005) *J. Am. Chem. Soc.*, **127**, 10676-10685.
148. Viroonchatapan, E., Ueno, M., Sato, H., Adachi, I., Nagae, H., Tazawa, K., and Horikoshi, I. (1995) *Pharm. Res.*, **12**, 1176-1183.
149. Viroonchatapan, E., Sato, H., Ueno, M., Adachi, I., Murata, J., Saiki, I., Tazawa, K., and Horikoshi, I. (1998) *J. Drug Target*, **5**, 379-390.
150. Viroonchatapan, E., Sato, H., Ueno, M., Adachi, I., Tazawa, K., and Horikoshi, I. (1996) *Life Sci.*, **58**, 2251-2261.
151. Chiu, G. N., Abraham, S. A., Ickenstein, L. M., Ng, R., Karlsson, G., Edwards, K., Wasan, E. K., and Bally, M. B. (2005) *J. Control Release*, **104**, 271-288.
152. Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Vander, E. L., and Muller, R. N. (2008) *Chem. Rev.*, **108**, 2064-2110.
153. Fortin, J. P., Wilhelm, C., Servais, J., Menager, C., Bacri, J. C., and Gazeau, F. (2007) *J. Am. Chem. Soc.*, **129**, 2628-2635.
154. Ito, A., Shinkai, M., Honda, H., and Kobayashi, T. (2005) *J. Biosci. Bioeng.*, **100**, 1-11.
155. Kawai, N., Ito, A., Nakahara, Y., Futakuchi, M., Shirai, T., Honda, H., Kobayashi, T., and Kohri, K. (2005) *Prostate*, **64**, 373-381.
156. Gupta, A. K., and Gupta, M. (2005) *Biomaterials*, **26**, 3995-4021.
157. Shinkai, M., Le, B., Honda, H., Yoshikawa, K., Shimizu, K., Saga, S., Wakabayashi, T., Yoshida, J., and Kobayashi, T. (2001) *Jpn. J. Cancer Res.*, **92**, 1138-1145.
158. Zhao, X., Li, H., and Lee, R. J. (2008) *Expert. Opin. Drug Deliv.*, **5**, 309-319.
159. Elnakat, H., and Ratnam, M. (2004) *Adv. Drug Deliv. Rev.*, **56**, 1067-1084.
160. Campbell, I. G., Jones, T. A., Foulkes, W. D., and Trowsdale, J. (1991) *Cancer Res.*, **51**, 5329-5338.
161. Coney, L. R., Tomassetti, A., Carayannopoulos, L., Frasca, V., Kamen, B. A., Colnaghi, M. I., and Zurawski, V. R., Jr. (1991) *Cancer Res.*, **51**, 6125-6132.
162. Weitman, S. D., Lark, R. H., Coney, L. R., Fort, D. W., Frasca, V., Zurawski, V. R., Jr., and Kamen, B. A. (1992) *Cancer Res.*, **52**, 3396-3401.
163. Low, P. S., Henne, W. A., and Doorneweerd, D. D. (2008) *Acc. Chem. Res.*, **41**, 120-129.
164. Gibbs, D. D., Theti, D. S., Wood, N., Green, M., Raynaud, F., Valenti, M., Forster, M. D., Mitchell, F., Bavetsias, V., Henderson, E., and Jackman, A. L. (2005) *Cancer Res.*, **65**, 11721-11728.
165. Lu, Y., and Low, P. S. (2002) *Adv. Drug Deliv. Rev.*, **54**, 675-693.
166. Salazar, M. D., and Ratnam, M. (2007) *Cancer Metastasis Rev.*, **26**, 141-152.
167. Jackman, A. L., Theti, D. S., and Gibbs, D. D. (2004) *Adv. Drug Deliv. Rev.*, **56**, 1111-1125.
168. Nagayoshi, R., Nakamura, M., Ijiri, K., Yoshida, H., Komiya, S., and Matsuyama, T. (2003) *Clin. Exp. Rheumatol.*, **21**, 719-725.
169. Theti, D. S., Bavetsias, V., Skelton, L. A., Titley, J., Gibbs, D., Jansen, G., and Jackman, A. L. (2003) *Cancer Res.*, **63**, 3612-3618.
170. Low, P. S., and Antony, A. C. (2004) *Adv. Drug Deliv. Rev.*, **56**, 1055-1058.
171. Kamen, B. A., and Caston, J. D. (1986) *Biochem. Pharmacol.*, **35**, 2323-2329.
172. Da Costa, M., and Rothenberg, S. P. (1996) *Biochim. Biophys. Acta*, **1292**, 23-30.
173. Leamon, C. P., and Low, P. S. (1993) *Biochem. J.*, **291** (Pt. 3), 855-860.
174. Leamon, C. P., and Low, P. S. (1992) *J. Biol. Chem.*, **267**, 24966-24971.
175. Leamon, C. P., Pastan, I., and Low, P. S. (1993) *J. Biol. Chem.*, **268**, 24847-24854.
176. Turek, J. J., Leamon, C. P., and Low, P. S. (1993) *J. Cell Sci.*, **106** (Pt. 1), 423-430.
177. Lee, R. J., and Low, P. S. (1994) *J. Biol. Chem.*, **269**, 3198-3204.
178. Lee, R. J., and Low, P. S. (1995) *Biochim. Biophys. Acta*, **1233**, 134-144.
179. Zhao, X. B., Muthusamy, N., Byrd, J. C., and Lee, R. J. (2007) *J. Pharm. Sci.*, **96**, 2424-2435.
180. Pan, X. Q., Wang, H., and Lee, R. J. (2003) *Pharm. Res.*, **20**, 417-422.
181. Lu, Y., Wu, J., Wu, J., Gonit, M., Yang, X., Lee, A., Xiang, G., Li, H., Liu, S., Marcucci, G., Ratnam, M., and Lee, R. J. (2007) *Mol. Pharm.*, **4**, 707-712.
182. Ni, S., Stephenson, S. M., and Lee, R. J. (2002) *Anticancer Res.*, **22**, 2131-2135.
183. Pan, X. Q., and Lee, R. J. (2005) *Anticancer Res.*, **25**, 343-346.
184. Sudimack, J. J., Guo, W., Tjarks, W., and Lee, R. J. (2002) *Biochim. Biophys. Acta*, **1564**, 31-37.
185. Wu, J., Liu, Q., and Lee, R. J. (2006) *Int. J. Pharm.*, **316**, 148-153.
186. Stephenson, S. M., Yang, W., Stevens, P. J., Tjarks, W., Barth, R. F., and Lee, R. J. (2003) *Anticancer Res.*, **23**, 3341-3345.
187. Sudimack, J. J., Adams, D., Rotaru, J., Shukla, S., Yan, J., Sekido, M., Barth, R. F., Tjarks, W., and Lee, R. J. (2002) *Pharm. Res.*, **19**, 1502-1508.
188. Stevens, P. J., Sekido, M., and Lee, R. J. (2004) *Anticancer Res.*, **24**, 161-165.
189. Chiu, S. J., Marcucci, G., and Lee, R. J. (2006) *Anticancer Res.*, **26**, 1049-1056.
190. Goren, D., Horowitz, A. T., Tzemach, D., Tarshish, M., Zalipsky, S., and Gabizon, A. (2000) *Clin. Cancer Res.*, **6**, 1949-1957.
191. Gabizon, A., Horowitz, A. T., Goren, D., Tzemach, D., Shmeeda, H., and Zalipsky, S. (2003) *Clin. Cancer Res.*, **9**, 6551-6559.

192. Sofou, S., and Sgouros, G. (2008) *Expert. Opin. Drug Deliv.*, **5**, 189-204.
193. Torchilin, V. (2008) *Expert. Opin. Drug Deliv.*, **5**, 1003-1025.
194. Gregoriadis, G., and Neerunjun, E. D. (1975) *Biochem. Biophys. Res. Commun.*, **65**, 537-544.
195. Leserman, L. D., Weinstein, J. N., Blumenthal, R., Sharrow, S. O., and Terry, W. D. (1979) *J. Immunol.*, **122**, 585-591.
196. Hansen, C. B., Kao, G. Y., Moase, E. H., Zalipsky, S., and Allen, T. M. (1995) *Biochim. Biophys. Acta*, **1239**, 133-144.
197. Allen, T. M., Brandeis, E., Hansen, C. B., Kao, G. Y., and Zalipsky, S. (1995) *Biochim. Biophys. Acta*, **1237**, 99-108.
198. Sapra, P., Tyagi, P., and Allen, T. M. (2005) *Curr. Drug Deliv.*, **2**, 369-381.
199. Sofou, S., Kappel, B. J., Jaggi, J. S., McDevitt, M. R., Scheinberg, D. A., and Sgouros, G. (2007) *Bioconj. Chem.*, **18**, 2061-2067.
200. Klibanov, A. L., Muzykantov, V. R., Ivanov, N. N., and Torchilin, V. P. (1985) *Anal. Biochem.*, **150**, 251-257.
201. Kirpotin, D. B., Drummond, D. C., Shao, Y., Shalaby, M. R., Hong, K., Nielsen, U. B., Marks, J. D., Benz, C. C., and Park, J. W. (2006) *Cancer Res.*, **66**, 6732-6740.
202. Mamot, C., Drummond, D. C., Noble, C. O., Kallab, V., Guo, Z., Hong, K., Kirpotin, D. B., and Park, J. W. (2005) *Cancer Res.*, **65**, 11631-11638.
203. Elbayoumi, T. A., and Torchilin, V. P. (2006) *Pharm. Eng.*, **26**, 96-104.
204. Elbayoumi, T. A., Pabba, S., Roby, A., and Torchilin, V. P. (2007) *J. Liposome Res.*, **17**, 1-14.
205. Elbayoumi, T. A., and Torchilin, V. P. (2009) *Mol. Pharm.*, **6**, 246-254.
206. Emfietzoglou, D., Kostarelos, K., Papakostas, A., Yang, W. H., Ballangrud, A., Song, H., and Sgouros, G. (2005) *J. Nucl. Med.*, **46**, 89-97.
207. Erdogan, S., Roby, A., and Torchilin, V. P. (2006) *Mol. Pharm.*, **3**, 525-530.
208. Erdogan, S., Roby, A., Sawant, R., Hurley, J., and Torchilin, V. P. (2006) *J. Liposome Res.*, **16**, 45-55.
209. Kabalka, G. W., Davis, M. A., Moss, T. H., Buonocore, E., Hubner, K., Holmberg, E., Maruyama, K., and Huang, L. (1991) *Magn Reson. Med.*, **19**, 406-415.
210. Torchilin, V. P. (2000) *Curr. Pharm. Biotechnol.*, **1**, 183-215.
211. Elbayoumi, T. A., and Torchilin, V. P. (2009) *Clin. Cancer Res.*, **15**, 1973-1980.
212. Jonasdottir, T. J., Fisher, D. R., Borrebaek, J., Bruland, O. S., and Larsen, R. H. (2006) *Anticancer Res.*, **26**, 2841-2848.
213. Sofou, S., Thomas, J. L., Lin, H. Y., McDevitt, M. R., Scheinberg, D. A., and Sgouros, G. (2004) *J. Nucl. Med.*, **45**, 253-260.
214. Matsumura, Y., Gotoh, M., Muro, K., Yamada, Y., Shirao, K., Shimada, Y., Okuwa, M., Matsumoto, S., Miyata, Y., Ohkura, H., Chin, K., Baba, S., Yamao, T., Kannami, A., Takamatsu, Y., Ito, K., and Takahashi, K. (2004) *Ann. Oncol.*, **15**, 517-525.
215. Matsumura, Y. (2003) *Adv. Exp. Med. Biol.*, **519**, 179-193.
216. Barrajon-Catalan, E., Menendez-Gutierrez, M. P., Falco, A., Carrato, A., Saceda, M., and Micol, V. (2009) *Cancer Lett.*, **290**, 192-203.