REVIEW

Liposome–nanoparticle hybrids for multimodal diagnostic and therapeutic applications

Wafa' T Al-Jamal & Kostas Kostarelos†

†Author for correspondence Nanomedicine Laboratory, Centre for Drug Delivery Research, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, UK Tel.: +44 207 753 5861; Fax: +44 207 753 5942; E-mail: kostas.kostarelos@pharmacy.ac.uk

Liposomes have a decade-long clinical presence as nanoscale delivery systems of encapsulated anthracycline molecules. However, their use as delivery systems of nanoparticles is still in the preclinical development stages. Liposome–nanoparticle hybrid constructs present great opportunities in terms of nanoscale delivery system engineering for combinatory therapeutic–imaging modalities. Moreover, many novel materials are being developed in nanotechnology laboratories that often require methodologies to enhance their compatibility with the biological milieu *in vitro* and *in vivo*. Liposomes are structurally suitable to make nanoparticles biocompatible and offer a clinically proven, versatile platform for the further enhancement of pharmacological efficacy. Small iron oxide nanoparticles, quantum dots, liposomes, silica and polystyrene nanoparticles have been incorporated into liposomes for a variety of different applications. In this review, all such liposome–nanoparticle hybrid systems are described, both in terms of their structural characteristics and the potential they offer as diagnostic and therapeutic multimodality agents.

Liposomes are the most clinically established nanometer-scale systems that are used to deliver cytotoxic and antifungal drugs, genes and vaccines, and are also being used as imaging agents [1]. Liposomes consist of a single or multiple concentric lipid bilayers (called lamellae) that encapsulate an aqueous compartment. Biocompatibility, biodegradability, reduced toxicity and capacity for size and surface manipulations comprise the outstanding profile that liposomes offer compared with other delivery systems. Hydrophilic polymers, such as polyethylene glycol (PEG), can be attached onto the liposome surface to sterically stabilize and increase liposome blood circulation residence time; targeting ligands (e.g., antibodies or peptides) can also be attached to increase liposome specificity toward target tissues [2]. Liposomes constitute the most established nanoscale delivery system already in clinical use for over a decade and provide a leading example of how nanomedicines can be developed and have a valuable clinical history. Some of the commercially available liposome-based products in clinical use currently are listed in Table 1 and many more are still undergoing clinical trials [2–7].

Most clinically used liposomes encapsulate a hydrophilic therapeutic agent (e.g., anthracyclines) in their aqueous compartment, which is surrounded by a lipid bilayer that may contain polymers or targeting ligands on its surface. The pharmacokinetics of such systems have been studied thoroughly [8]. Drug encapsulation into liposome carriers can alter a drug's *in vivo* pharmacokinetic and pharmacodynamic profile, leading to an increase in the therapeutic index, reduction in tissue toxicity and other side effects [9–12], an increase in drug stability [13,14] or the emergence of a sustained-release profile formulation [7,15,16]. New-generation systems include liposomes responsive to external or environmental stimuli (e.g., pH, temperature or enzymes) that trigger drug release at specific and controlled sites [17].

The versatility of the liposomal structure lies in its capacity to cargo drug molecules and biological macromolecules that are hydrophilic (therefore entrapped in the liposome inner aqueous core) or hydrophobic (therefore incorporated within the lipid bilayer). In recent years, with the advent of nanotechnology, there has been a dramatic increase in the development of novel particulate systems that are of nanoscale dimensions. Nanometer-sized particles, such as superparamagnetic iron oxides (SPIOs) and semiconducting nanocrystals (quantum dots [QDs]), possess novel magnetic and optical properties that can be used as imaging probes. However, their hydrophobicity or poor colloidal stability at physiological conditions frequently renders them inappropriate for clinical use. Our proposal is to take advantage of the much more developed and sophisticated liposome technology as a platform for the delivery of novel nanoparticles. Encapsulation of these nanoparticles within liposomes can lead to enhanced

Keywords: double liposomes, liposome, polystyrene nanospheres, quantum dot, silica, superparamagnetic iron oxides, vesosomes

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Table 1. Commercially available liposomal preparations.

Category	Trade name	Manufacturer	Liposome system details	Therapeutic target
Cytotoxic	Doxil®/Caelyx®	Ortho Biotech (Doxil) Schering Plough (Caelyx)	80–100-nm sterically stabilized liposomes (HSPC:chol:DSPE-PEG2000) suspension encapsulating doxorubicin	Kaposi sarcoma, ovarian cancer
	DaunoXome®	Gilead	Small rigid (DSPC:chol) liposomes encapsulating daunorubicin	Kaposi sarcoma
	Myocet®	Zeneus Pharma	Liposomal formulation (EPC:chol) encapsulating doxorubicin citrate complex	Combinational therapy with cyclophosphamide for advanced stage or metastatic breast cancer
	DepoCyt®	Enzon Pharmaceuticals	Cytarabine liposome injection (DOPC:chol:DPPG multivesicular liposome)	Lymphomatous meningitis (cancer of the lymph system that has spread to the brain)
Fungicide	AmBisome®	Gilead	Small negatively charged liposomal suspension (HSPC:chol:DSPG) encapsulating amphotericin B	Systemic fungal infections (visceral leishmaniasis)
Vaccine	Epaxal®	Berna Biotech	Formalin-inactivated hepatitis A virus attached to phospholipid vesicles together with influenza virus hemagglutinin	Hepatitis A virus infections
	AVAXIM®	Sanofi Pasteur MSD	Liposome suspension contains inactivated hepatitis A virus	Hepatitis A virus infections
	Bio-Hep-B ®	Biotechnology General	HBs antigen vaccine	Hepatitis B virus infections
	Inflexal®V Berna	Berna Biotech AG	Purified influenza hemagglutinin glycoprotein and neuraminidase inserted into the liposomal membrane (lecithin)	Influenza prophylaxis
	FluMist®	MedImmune Vaccines	Nasal liposomal preparation contains weakened live influenza viruses	Influenza prophylaxis
	Newcastle disease vaccine (vet)	Schering-Plough Animal Health Corporation	Novasome is nonphospholipid liposomes containing killed Newcastle disease virus	Newcastle disease (a highly infectious viral disease of domestic and wild birds)
	Avian Rheovirus vaccine (vet)	Schering-Plough Animal Health Corporation	Nonphospholipid vesicle containing killed avian rheovirus	Passive protection of chickens against rheovirus infections
Others	Visudyne®	Novartis	Liposomal suspension encapsulating verteporfin drug	Photodynamic therapy for macular degeneration (ophthalmic preparation)

**The dates of clinical approval for some of the products listed: Doxil®/Caelyx®: 1995 (USA), 1996 (Europe); DaunoXome®: 1996 (USA & Europe); Myocet®:: 2005 (Europe & Canada); DepoCyt®: 1999 (USA); AmBisome®: 1990 (Europe), 1997 (USA); Epaxal®: 1994 (Switzerland); Inflexal®V Berna: 1997 (USA); FluMist®: 2003 (USA); Avian Rheovirus vaccine: 2006 (USA); Visudyne®: 2000 (USA).*
Chol: Cholesterol; DOPC: Dioleoyl phosphatidylcholine; DPPG: Dipalmitoylphosphatidylglycerol; DSPE: Distearoylphosphatidylethanolamine; EPC: Egg phosphatidylcholine; HBs: Hepatitis B surface; HSPC: Hydrogenated soy phosphatidylcholine; PEG: Polyethylene glycol.

nanoparticle hydrophilicity, stability in plasma and an overall improvement in their biocompatibility. Furthermore, by taking advantage of the capability offered by liposomes to carry hydrophilic and hydrophobic moieties, combinatory therapy/imaging modalities can be achieved by incorporating therapeutics and diagnostic agents in a single liposome-delivery system.

In this review, we highlight various types of nanometer-sized particles that have been encapsulated within phospholipid bilayers and their applications in the biomedical field, particularly in designing novel biosensor devices. Following is a more detailed description of most such types of liposome–nanoparticle hybrids.

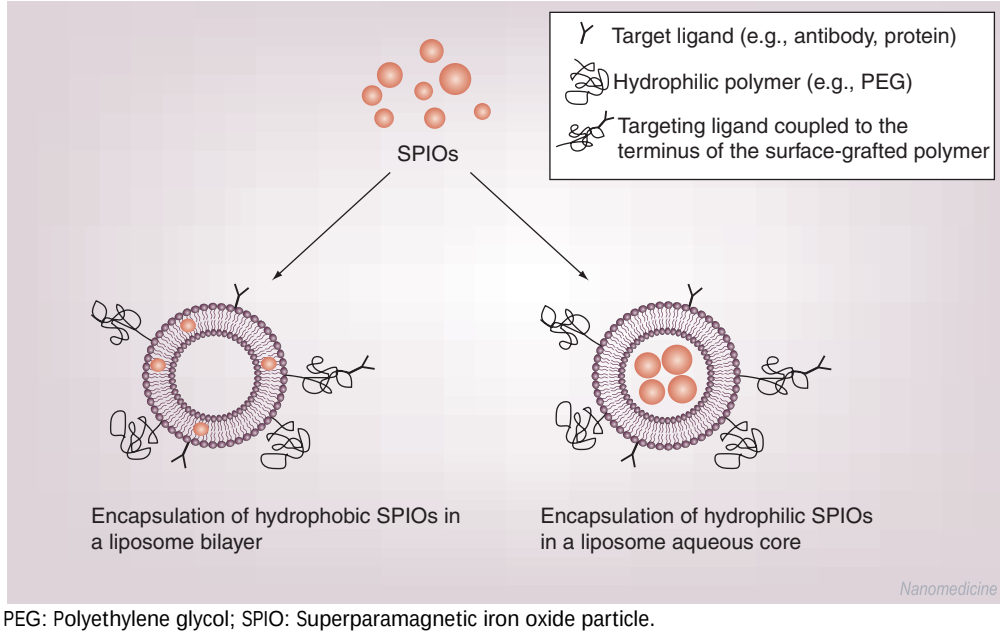
Liposome–SPIO particle hybrids

The unique properties of magnetic particles, such as gadolinium, magnetite and maghemite (γ-Fe_2O_3), when placed in magnetic fields, have attracted great attention with regards to their potential for magnetic resonance imaging (MRI). Their applications in the biomedical field, including noninvasive imaging, drug targeting, gene therapy, tissue engineering and, more recently, as heat mediators for cancer treatments, have been investigated. They have been studied thoroughly with attempts to improve the resulting MRI resolution and reduce toxicity. Most commonly, they have been embedded in stabilizing polymers, such as dextran or PEG to improve their biocompatibility and reduce their aggregation in physiological environments.

SPIO nanoparticles have been encapsulated within the phospholipid vesicles (Figure 1) using techniques, such as reverse phase evaporation [18–21], extrusion [22], freeze-thawing [21,23] or sonication [24,25]. Liposomes containing nanometer-sized superparamagnetic particles have been prepared to improve SPIO biocompatibility as the lipid bilayer is biocompatible with the biological membrane and enhances SPIO utilization by the cells [22]. Lipid bilayer coating of the magnetic nanoparticles preserved the magnetic characteristics of SPIOs, reduced SPIO aggregation in the blood stream, reduced the cytotoxicity of the free iron oxide particles [22] and, most importantly, provided a more effective MRI contrast agent. Encapsulation of SPIOs within liposomes (known as magnetoliposomes) provides a promising delivery system with combinatory capacity, whereby therapeutic agents can also be incorporated either in the aqueous core or in the lipid bilayer. Drug-loaded magnetoliposomes provide a specific targeting and therapeutic delivery system by applying magnetic forces. They achieve a high drug concentration at the specific site without associated toxicity to neighboring tissues [19,23,26]. Also, the magnetoliposome's surface can be modified chemically by specific targeting ligands [19,20,27–30] or PEG-grafted lipids [22,31] to reduce the nonspecific adsorption and avoid macrophage uptake. Functionalized PEG ligands have also been incorporated to improve the blood circulation time and target recognition [21,32].

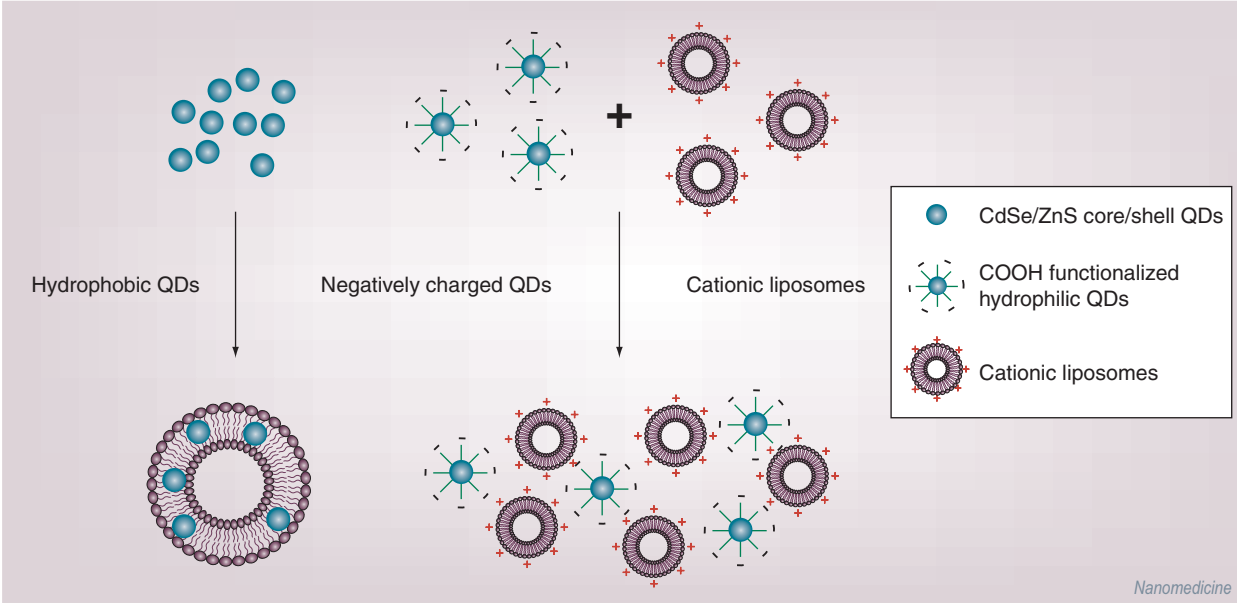
Hyperthermia is a promising tool for cancer therapy as it has fewer side effects than chemotherapy or radiotherapy. Hyperthermia involves raising the temperature of the tissue to 42–44°C, which causes physical damage. Many procedures have been followed to induce hyperthermia but they all cause damage to the normal tissues as well as to the tumor [33].

Figure 1. Diagram showing the incorporation of superparamagnetic iron oxide nanoparticles within a liposome bilayer (left) or a liposome aqueous core (right).



PEG: Polyethylene glycol; SPIO: Superparamagnetic iron oxide particle.

Figure 2. Incorporation of hydrophobic quantum dot nanoparticles within a liposome bilayer (left) and electrostatic complexation of negatively charged hydrophilic quantum dots with commercially available cationic liposomes (right).



QD: Quantum dot.

Magnetoliposomes can act as heat mediators within the tumor when an alternating magnetic field is applied, resulting in antitumor activity without heating of the surrounding healthy tissues [25,27–29,34]. Magnetoliposomes induce selective hyperthermia if they accumulate only within the tumor. Such accumulation has been achieved by direct intratumoral injection [24,25,33,34] or by antibody conjugation for inaccessible tumors [27,28,30], which makes them more convenient for intravenous administration. Interestingly, cationic magnetoliposomes (CMLs) have shown higher accumulation and affinity for the cell membrane after intratumoral injection than neutral liposomes [29,35]. Thus, CMLs induced efficient and selective intracellular hyperthermia in different tumor models without raising the whole body temperature, resulting in tumor necrosis and complete tumor regression after multiple exposures to an alternating magnetic field [25,29,34]. Furthermore, CMLs have potential applications in gene delivery as their positive charge can condense negatively charged DNA, which will facilitate cell association and transfection [36,37].

In addition to their antitumor activity, magnetoliposomes can be developed as triggerable systems since magnetically induced increases in local temperature can induce drug release from thermosensitive liposomes [18,38,39] or, more interestingly, may activate an encapsulated prodrug molecule prior to its release [40]. More complex fullerene–liposome hybrid nanoparticles and magneto-fullerene–liposomes have been constructed where fullerenes incorporated into the phosphatidylcholine liposome bilayer, which, on near-infrared (NIR) laser pulse, can trigger drug release from such nanocarriers [41]. Magnetoliposomes, directed by a magnetic field to the tumor site, can be used for targeting, imaging, therapeutic hyperthermia, gene transfection, prodrug activation and controlled drug release [42], all of which provide multiple options for cancer therapy.

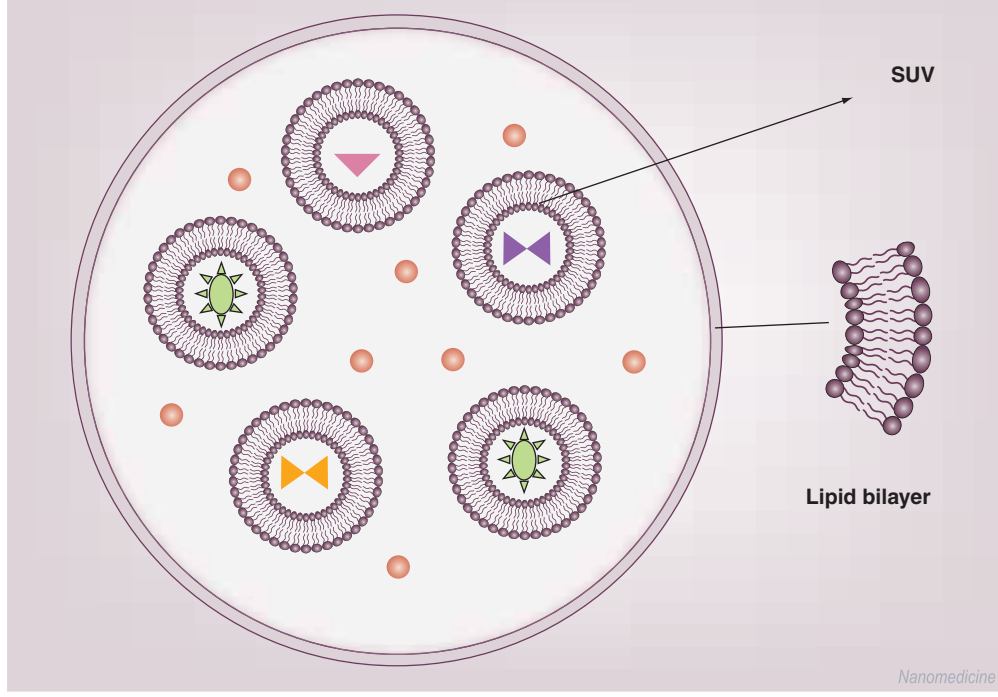
Liposome–QD hybrids

Semiconductor nanocrystals, known as QDs, are fluorescent nanoparticles of 1–10 nm in diameter [43–45] that offer distinct spectrofluorometric advantages over traditional fluorescent organic molecules. QDs exhibit fluorescence characteristics that are 10–20-times brighter than conventional dyes, greater photostability, broad excitation wavelength range, size-tuneable spectrum and narrow and symmetric emission spectrum, ranging from 400 to 2000 nm,

depending on their size and chemical composition. Owing to these photophysical characteristics they are being explored as potential imaging agents primarily in fluorescence-based diagnostic applications [45–47]. Recent reports have shown that QDs can also be linked to SPIO nanoparticles to develop a dual modality contrast agent for cell tracking *in vivo* via MRI and optical imaging [48,49]. Samia and colleagues and others have reported using QDs in photodynamic therapy (PDT) [50–52], since QD emission wavelength can excite a photosensitiser and QDs alone also have the potential to produce the reactive singlet oxygen, which can be used as a cytotoxic agent against tumor cells [50].

QDs are prepared originally in organic solvents [53], therefore their hydrophobic shells compromise their water solubility and consequently their compatibility with the biological milieu. In addition, their hydrophobic surface results in an unfavorable toxicity profile, introducing serious limitations in potential biomedical and clinical applications of QDs. Many strategies are being developed to overcome this limitation. The most succsessful approach has been to functionalize QDs with polar moieties and ligands with specific receptor recognition signals (e.g., peptides and monoclonal antibodies or their fragments) [44,54–56]. However, this surface modification leads to decreases in QD fluorescence intensity and photostability [57–59]. Preformed liposomes of cationic surface character have been electrostatically complexed with functionalized QDs (Figure 2) to enhance the cellular binding and internalization of QDs for cell labeling and tracking purposes [60–62]. However, these studies simply mixed commercially available, liposome-based transfection agents with QDs in order to translocate enough QD particles intracellularly to achieve efficient levels of mammalian cell fluorescent labeling. More recently, Feng and colleagues have reported the encapsulation of organic CdSe QDs within PEG-conjugated phosphatidylcholine liposomes (Figure 2). Gopalakrishnan and colleagues have reported the incorporation of hydrophobic QDs within fusogenic liposome bilayers that were able to translocate and stain the plasma membrane of cell cultures upon fusion [63]. Incorporation of the organic QDs within

Figure 3. Multicompartment liposome encapsulating SUVs within an outer bilayer membrane where different therapeutic molecules (cocktail therapies) can be loaded into SUVs.



SUV: Small unilamellar vesicle.

phospholipid bilayers renders QDs compatible with the aqueous environment and allows fluorescent labeling of the lipid bilayers for *in vivo* and *in vitro* imaging.

In an alternative approach, ultrasmall, uncapped QDs have been prepared using unilamellar phosphatidylcholine vesicles and electroporation. Schelly and colleagues have synthesised PbS [64] and AgBr subnanometer crystals [65] by encapsulating the metal ions within unilamellar liposomes and dispersing anionic ions in the surrounding aqueous phase. The application of high voltage induced the formation of reversible pores within the membrane, followed by adsorption of QD monomers on the external surface of the liposomes. With time, self-aggregation took place, resulting in crystals less than 10 Å in diameter.

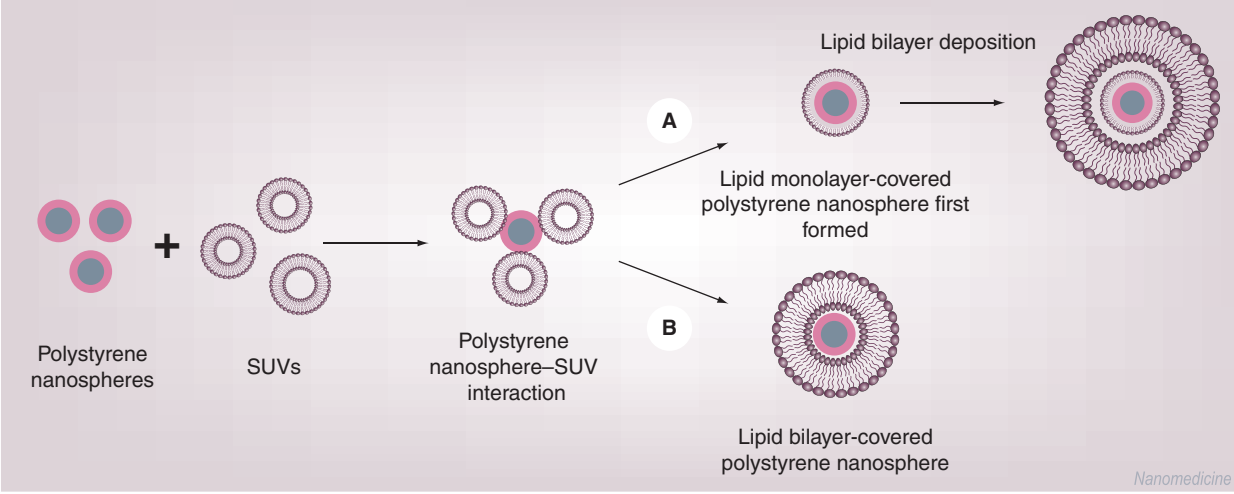
Liposomes-in-liposomes

Doxil® (Ortho Biotech Products, NJ, USA), Caelyx® (Schering-Plough, NJ, USA) and Myocet® (Zeneus Pharma, Oxford, UK) are nanometer-sized liposome systems (encapsulating doxorubicin in their aqueous core) that have been used in cancer clinics for over a decade. Administration of combination chemotherapy treatment regimens using a single delivery system is thought to significantly enhance therapeutic efficacy [66–68]. Therefore, the engineering of new types of multicompartment liposomes is needed to allow adoption of such novel modalities in a single liposome carrier system [68] (Figure 3).

Multivesicular systems were first described in 1982 [69], prepared by the double emulsification technique. Cytarabine-containing multivesicular liposomes in the micrometer scale (average diameter 6–30 µm) have been introduced to the market for cancer therapy (DepoCyt®, Enzon Pharmaceuticals, NJ, USA) (Table 1). These are large clusters of smaller lipid bilayers seemingly 'glued' to each other by triolein-rich hydrophobic regions. Multivesicular structures consisting of lipid bilayers encapsulating intact liposomes of smaller mean diameters (multicompartment liposome [MCL]) have been further developed by various laboratories [70], however, the minimum average diameter described for any MCL system is in the range of a few micrometers [7,71]. This inhibits the use of multivesicular systems for systemic indications, such as cancer, whereby blood circulation of the delivery systems is required. Micrometer-sized multivesicular systems have been reported recently as oral (for hormones, proteins and vaccine) [13,14,72] and local (intratumoral and intramuscular) [15,16] drug-delivery systems, providing a sustained drug-release profile, inner liposome protection and a higher drug-encapsulation efficiency.

An alternative MCL system, called vesosome, has been developed by Zasadzinski and coworkers using the self-assembly properties between streptavidin-coated cochleate cylinders [14,71–75] or ethanol interdigitated phospholipid bilayer sheets [73] and biotin-coated smaller liposomes. Such

Figure 4. Mechanism of interaction between polystyrene nanospheres and SUVs.



(A) A lipid monolayer-covered nanosphere can be formed if the lipid and nanosphere are similarly charged. If a neutral lipid is used, a lipid bilayer can be adsorbed on the lipid monolayer-covered nanosphere by further lipid deposition. **(B)** A lipid bilayer is formed on the surface of an oppositely charged nanosphere.
SUV: Small unilamellar vesicle.

micrometer-sized vesosome systems were reported recently as vaccine-delivery systems following topical (skin) immunization [76]. We have constructed a novel MCL system of nanometer dimensions (200 nm average diameter) recently that can potentially be a tool for systemic administration of combinatory therapeutic/imaging modalities [77].

Liposome–polystyrene nanosphere hybrids

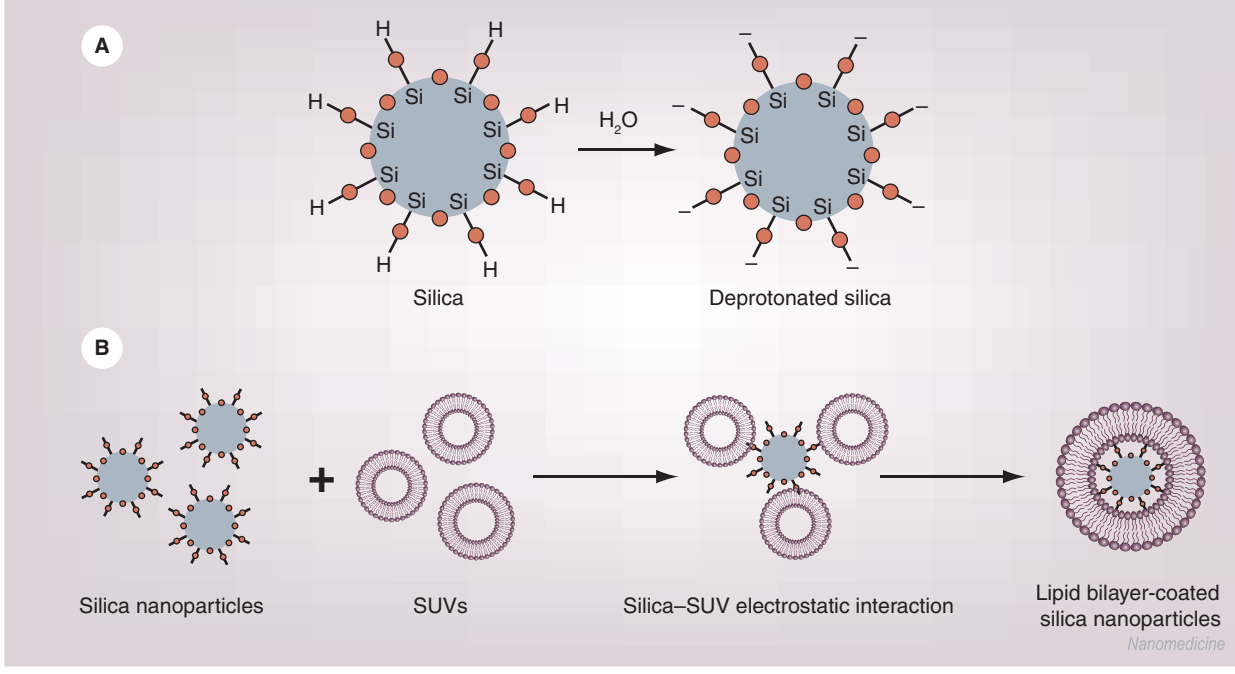
Adsorption of a lipid bilayer around the surface of polystyrene nanospheres [78–82] leads to the formation of hybrid systems consisting of nanoparticles within liposomes. The mechanism of interaction between liposome vesicles and the solid polystyrene particles is not yet well understood. Different lipids and types of nanoparticles have been studied and lipid deposition at the solid surface has been shown to depend on the lipid concentration, the surface charge and the hydrophilic/hydrophobic nature of the solid nanoparticles (Figure 4) [78,79,82].

Positively charged synthetic dioctadecyldimethylammonium bromide (DODAB) amphiphiles formed bilayers and had a high adsorption affinity for the negatively charged polystyrene nanosphere surface owing to electrostatic attraction [78]. However, negatively charged liposomes formed a lipid monolayer on the surface of sulphated polystyrene nanospheres with their polar heads directed towards the aqueous phase [79]. Unstable adsorption of neutral liposomes occurred on charged polystyrene nanosphere surfaces and depends on the hydrophobic attractions between the phospholipid bilayer(s) and the monolayer-covered nanospheres [82]. The self-assembled lipid on solid particles combines the intrinsic properties of both the solid core and the surface bilayer, which can be used as a model of the cell membrane. As such, it can act as a host for transmembrane proteins or receptors [83] and be of benefit in designing biosensors for optical or electrical detection [84,85].

Liposome–silica nanoparticle hybrids

Formation of a solid particle-supported bilayer has also been described by deposition of small unilmellar vesicles (SUVs), composed of either synthetic amphiphiles or natural phospholipids onto hydrophilic silica nanospheres [86–88]. Deposition is followed by vesicle distortion and rupture to form a continuous fluid bilayer membrane (Figure 5) [82,86–88]. The bilayer adsorption is as a result of electrostatic attractions between the silica surface and the vesicle polar groups. The adsorption stability of neutral phospholipid bilayer on a silica surface depends on the H-bridges formed between the deprotonated silanol groups (Si-OH) and the phosphate groups ($O=P^-$) of the phospholipids. Therefore,

Figure 5. (A) The chemical composition of a silica nanoparticle and (B) the mechanism of interaction between silica nanoparticles and SUVs.



SUV: Small unilamellar vesicle.

Table 2. Different liposome–nanoparticle hybrid systems

Nanoparticle composition	Vesicle lipid type	Method of nanoparticle encapsulation	Final product size	Advantages of liposome encapsulation	Application	Ref.
Superparamagnetic iron oxide particles						
Magnetite (Fe_3O_4)	TMAG:DLPC:DOPE (1:2:2 molar ratio)	SPIOs were encapsulated within a cationic liposome by lipid film hydration and sonication, followed by magnetoliposomes complexation to DNA	Nanoscale*	Magnetic separation of transfected cell	Gene delivery	[37]
Magnetite	TMAG:DLPC:DOPE (1:2:2 molar ratio)	Lipid film hydration and sonication	Nanoscale*	Cationic magnetoliposome provides selective intracellular hyperthermia and immune response induction	Cancer therapy	[24,25,34]
Magnetite	Lecithin:chol	Lipid film hydration and sonication	Nanoscale*	Drug targeting upon applying magnetic field	Targeted drug delivery	[26]
Magnetite	PC:PE	Lipid film hydration and sonication	Nanoscale*	Tumor-specific antibody-conjugated magnetoliposome	Local hyperthermia treatment of human renal cell carcinoma	[27]
Dextran–magnetite	Soy PC:chol:PS	REV and sonication	Nanoscale*	Monocyte/neutrophil-mediated active delivery of drug to inflammatory sites	Active drug delivery for cerebrovascular diseases	[19]
Dextran–magnetite	DPPC	REV	1 µm	Drug release from thermosensitive liposomes in response to magnetically induced hyperthermia	Can be used in cancer treatment providing magnetic targeting and drug release in response to local hyperthermia	[18]
Maghemite (γFe_2O_3)	EPC, EPC:DSPE-PEG2000 (95:5mol %)	Lipid film hydration and extrusion	200 nm	Highly efficient MR contrast agent by avoiding particles aggregation and blood dilution	MRI	[22]
Quantum dots						
Hydrophobic CdSe QD	DMPC:DOTAP:DPPE-PEG2000 (47.5:25:0.5 mol %)	Lipid film hydration and sonication	20 –100 nm	QD water solubilization and cell membrane labeling	Cell imaging	[63]
Hydrophobic CdSe QD	PC:PE:chol:PEG	Lipid film hydration followed by sonication and extrusion	20 nm	QD water solubilization	Could be used as fluorescent labels for biological applications	[91]

**The mean size of these systems was not mentioned, but it can be assumed that this will be in the range of 100–200 nm, depending on the method of liposome preparation.*

DHP: Dihexadecylphosphate; DLPC: Dilauroylphosphatidylcholine; DODAC: N-N-*dioleoyl*-N,N-*dimethylammonium chloride; DOPE: Dioleoylphosphatidylethanolamine; DOPS: Dioleoylphosphatidylserine; DOTAP: 1, 2-dioleoyl-3-trimethylammonium propane; DPPC: Dipalmitoylphosphatidylcholine; DPPG: Dipalmitoylphosphatidylglycerol; DSPE: Distearoylphosphatidylethanolamine; EDTA: Ethylenediaminetetraacetic acid; EPC: Egg phosphatidylcholine; MR: Magnetic resonance; MRI: Magnetic resonance imaging; NA: Not available; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PEG: Poly(ethylene glycol); PS: Phosphatidylserine; QD: Quantum dot; REV: Reverse-phase evaporation; SA: Stearylamine; SPIO: Superparamagnatic iron oxide particle; SUV: Small unilamellar vesicle; TMAG: N-α-(trimethylammonio-acetyl)-didodecyl-D-glutamate chloride.*

Table 2. Different liposome–nanoparticle hybrid systems (cont.).

Nanoparticle composition	Vesicle lipid type	Method of nanoparticle encapsulation	Final product size	Advantages of liposome encapsulation	Application	Ref.
Quantum dots						
PEG-coated QD (CdSe/ZnS) DHLA-capped QD	Lipofectamine 2000 (cationic liposomes)	QDs–liposome electrostatic complexation	NA	High intracellular QD delivery	Intracellular trafficking	[60,61]
Hydrophobic QD (CdSe/ZnS)	PC:PEG–PE	Lipid film hydration and sonication	20–100 nm	QD individualization, water solubilization and increase colloidal stability	*In vivo* and *in vitro* imaging	[47]
QD cationic ions	DOPC	Lipid film hydration and extrusion	100–200 nm	Preparation of Angstrom-sized QDs	N/A	[64,65]
Phospholipid vesicles						
DPPC:Soya PC:chol:SA (7:7:5:4) (200 nm)	DMPC:DMPG (10:1 molar ratio)	Glass bead method and REV, followed by sonication and extrusion	1–10 µm	Protect the antigen against pepsin and prolonged the antigen release	Oral vaccine	[72]
DOTAP:PC:DOPE (2:1:0.5)	DPPC:chol (97.5:2.5 molar ratio)	Ethanol interdigitation	3–5 µm	Fusogenic vesosomes deliver the antigen systemically via topical application	Topical immunization	[76]
Large vesicle aggregates of unilamellar DLPC liposomes (vesosomes)	DOPS cochleates (a cylinder formed upon the interaction of Ca^{+2} ions with anionic SUVs)	Cylinder-aggregate formation using biotin–streptavidin interaction, followed by unrolling of PS bilayer around the vesicle aggregates from the cochleate cylinder by adding EDTA	0.5–5 µm	Formation of multicompartment liposomes encapsulating vesicle aggregates within a bilayer	Could be used as a drug delivery system for combinatory therapy providing synergistic effect for therapeutic and diagnostic purposes	[75]
200-nm liposomes composed of DSPC:chol (2:1) or DSPC:chol:SA (2:1:1). 100 nm liposomes of DOPS	DOPS cochleate	Unrolling of PS bilayer around the colloidal nanoparticles from cochleate cylinder by adding EDTA	0.3–2 µm	Formation of multicompartment liposomes composed of anionic or neutral liposomes encapsulated within an anionic bilayer		[92]
50 nm or 200 nm vesicles of DSPC: chol (2:1) or DPPC: chol: SA (50:25:25 mol %).	DPPC, DSPC, DPPG, DPPC: chol (97.5: 2.5 molar ratio), DPPC-DPPE-PEG2000	Ethanol interdigitation	0.3–2 µm	Multicompartment liposomes		[73]

**The mean size of these systems was not mentioned, but it can be assumed that this will be in the range of 100–200 nm, depending on the method of liposome preparation.*

DHP: Dihexadecylphosphate; DLPC: Dilauroylphosphatidylcholine; DODAC: N-N-*dioleoyl*-N,N-*dimethylammonium chloride; DOPE: Dioleoylphosphatidylethanolamine; DOPS: Dioleoylphosphatidylserine; DOTAP: 1, 2-dioleoyl-3-trimethylammonium propane; DPPC: Dipalmitoylphosphatidylcholine; DPPG: Dipalmitoylphosphatidylglycerol; DSPE: Distearoylphosphatidylethanolamine; EDTA: Ethylenediaminetetraacetic acid; EPC: Egg phosphatidylcholine; MR: Magnetic resonance; MRI: Magnetic resonance imaging; NA: Not available; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PEG: Poly(ethylene glycol); PS: Phosphatidylserine; QD: Quantum dot; REV: Reverse-phase evaporation; SA: Stearylamine; SPIO: Superparamagnatic iron oxide particle; SUV: Small unilamellar vesicle; TMAG: N-α-(trimethylammonio-acetyl)-didodecyl-D-glutamate chloride.*

Table 2. Different liposome–nanoparticle hybrid systems (cont.).

Nanoparticle composition	Vesicle lipid type	Method of nanoparticle encapsulation	Final product size	Advantages of liposome encapsulation	Application	Ref.
Phospholipid vesicles						
Coatsome EL series®, which are 200-nm liposomes composed of DMPC:chol:SA:DMPG (52:40:8:0) (54:40:0:6)	HSPC, DMPC, EPC	Glass bead method	0.25–10 µm	Preparation of double liposomes, which retard the drug release		[71]
Liposome vesicles composed of HSPC, HSPC:SA, HSPC:PS	HSPC, HSPC:SA, HSPC:PS	Glass filter method	2–10 µm	Better hypoglycemic effect owing to insulin release retardation and inner liposome protection against enzymatic degradation	Oral administration of peptide drugs	[13,70]
Silicon-based nanoparticles						
Organic-capped silicon	EYPC, 16-DOXYL, 5-DOXYL	Lipid film hydration followed by sonication and extrusion	60 nm, 150 nm, 300 nm	Water solubilization of photoluminescent hydrophobic silicon	Can be useful in the development of aqueous-based sensors and providing model systems to study nanoparticle/ cell interaction	[93]
Hydrophilic silica	DOTAP, DOPC, DOPS, DOPC:DOPS PC, DHP, DODAB	Adsorption of SUVs on the silica nanoparticles surface, followed by SUV rupture, forming a lipid monolayer or bilayer(s)	100–150 nm	Preparation of supported lipid bilayer on silica nanoparticles combine the intrinsic properties of silica and the bilayer	Can be used in designing biosensors	[88,89]
Polystyrene nanospheres						
Sulphate and amidine polystyrene nanorosphere	DODAB, DODAC and DHP	Adsorption of SUV on the polystyrene nanoparticles surface, followed by SUV rupture forming a lipid monolayer or bilayer(s)	100–200 nm	Production of monodispersed and smooth bilayer nanosphere	Defloculating or stabilizing of oppositely charged latex	[78]
Amidine nanorosphere	PC		170–200 nm	Incorporation of cholera toxin receptors into bilayer-covered nanospheres	Development of cholera toxin biosensors	[81,83]

**The mean size of these systems was not mentioned, but it can be assumed that this will be in the range of 100–200 nm, depending on the method of liposome preparation.*

DHP: Dihexadecylphosphate; DLPC: Dilauroylphosphatidylcholine; DODAC: N-N-*dioleoyl*-N,N-*dimethylammonium chloride; DOPE: Dioleoylphosphatidylethanolamine; DOPS: Dioleoylphosphatidylserine; DOTAP: 1, 2-dioleoyl-3-trimethylammonium propane; DPPC: Dipalmitoylphosphatidylcholine; DPPG: Dipalmitoylphosphatidylglycerol; DSPE: Distearoylphosphatidylethanolamine; EDTA: Ethylenediaminetetraacetic acid; EPC: Egg phosphatidylcholine; MR: Magnetic resonance; MRI: Magnetic resonance imaging; NA: Not available; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PEG: Poly(ethylene glycol); PS: Phosphatidylserine; QD: Quantum dot; REV: Reverse-phase evaporation; SA: Stearylamine; SPIO: Superparamagnatic iron oxide particle; SUV: Small unilamellar vesicle; TMAG: N-α-(trimethylammonio-acetyl)-didodecyl-D-glutamate chloride.*

adsorption of the lipid bilayer on the solid silica nanoparticle depends on the buffer used, the amphiphile's polar headgroup and the physical state of the bilayer [86,87].

Liposome vesicles composed of the synthetic DODAB amphiphile demonstrated a high affinity for silica surface. By contrast, negatively charged liposomes composed of dihexadecylphosphate (DHP) amphiphile did not form a continuous layer on the hydrophilic silica, presumably owing to electrostatic repulsive forces. However, neutral phospholipid bilayer deposition can be driven by stabilizing the H-bridges between the interacting particles [86,87]. These liposome–silica nanoparticle hybrid systems can be used in designing biosensors whereby the physical (e.g., semiconductors) characteristics of silica can be matched with the biocompatibility and pharmaceutical and pharmacodynamicproperties of liposomes [89,90].

Future perspective

Several liposomal products are licensed for clinical use in cancer therapy and for vaccination. Encapsulation of amphotericin B and anthracyline cytotoxic drugs into liposome carriers significantly increased the drug therapeutic index and reduced associated cytotoxicity. Most importantly, they have established clinically nanoscale liposome delivery systems. One future direction in liposome research will be in combination with other nanoparticulate systems for the construction of multiple modality systems. In the diagnostic field, bilayer-coated nanoparticles have already been described for the design of multifunctional biosensors. These types of biosensors can increase the detection sensitivity and save time and effort, as protein receptors can be anchored into lipid membranes to detect the presence of several antigens and antibodies present in biological fluids. Combinatory imaging–therapeutic applications will include liposome–SPIO hybrid nanoparticles that have been shown to be nontoxic and highly stable, offering multimodal capacities. Within the next few years, we expect the development of liposomal SPIO products encapsulating different drug molecules for cancer therapy. Another hybrid system at earlier development stages is based on QD–liposome hybrid nanoparticles. We speculate that incorporation of QDs with the liposomal structure can dramatically improve QDs biocompatibility under physiological conditions and can help bring QD technologies to the clinic faster by reducing QD dose and their associated toxicity. Furthermore, liposomes that are combined with NIR nanoparticles, which can be imaged within the NIR light 'transparency window' deeper within human tissue, can have a tremendous impact in imaging and therapeutic combined modality agents.

Executive summary

- Liposomes are the most clinically established nanomedicines today, with a decade-long clinical history as nanoscale delivery systems of anticancer drug molecules.
- Intensive research in nanotechnology has led to the development of many types of nanoparticles not compatible with the biological milieu.
- Liposome–nanoparticle hybrid constructs improve the biocompatibility of novel nanoparticles.
- Liposome–nanoparticle hybrids are structurally diverse nanosystems offering a wide variety of opportunities for engineering to achieve specific biological functions (e.g., tissue targeting and triggerable release).
- Liposome–nanoparticle hybrids constitute candidates for clinically viable and easily translational combinatory therapeutic–diagnostic modalities.

Conclusion

In this review, we have shown that liposomes, apart from being clinically used delivery systems of anticancer agents and vaccines, can also be considered as carriers for different types of nanoparticles. The variety of novel, usually biologically incompatible, nanoparticles developed as a result of advances in nanotechnology represents a rich source of materials that liposomes can transform into clinically relevant diagnostic or therapeutic agents. Different strategies to achieve encapsulation of solid or semi-solid nanoparticles within liposomes have been reported. All such strategies offer improvements in the nanoparticle aqueous solubilization and offer a viable platform (the liposome surface) for further bioconjugation. Moreover, liposome–nanoparticle hybrids can increase blood circulation times on systemic administration and thus improve accumulation within sites of leaky vasculature (tumor or inflammation) and offer opportunities for the development of combinatory imaging and therapeutic modalities at those sites.

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Bibliography

1. Lasic DD, Papahadjopoulos D: *Medical Applications of Liposomes.* Elsevier Science, Amsterdam, The Netherlands (1998).
2. Torchilin VP: Recent advances with liposomes as pharmaceutical carriers. *Nat. Rev. Drug Discov.* 4, 145–160 (2005).
•• One of the most comprehensive liposome reviews that describes the new types of liposomes and their applications.
3. Lasic DD: Novel applications of liposomes. *Trends Biotechnol.* 16, 307–321 (1998).
4. Lasic DD: Recent development in medical applications of liposomes: sterically stabilized liposomes in cancer therapy and gene delivery *in vivo*. *J. Control. Release* 48, 203–222 (1997).
5. Mischler R, Metcalfe IC: Inflexal V a trivalent virosome subunit influenza vaccine: production. *Vaccine* 20(Suppl. 5), B17–B23 (2002).
6. Voinea M, Simionescu M: Designing of 'intelligent' liposomes for efficient delivery of drugs. *J. Cell Mol. Med.* 6, 465–474 (2002).
7. Mantripragada S: A lipid based depot (DepoFoam technology) for sustained release drug delivery. *Prog. Lipid Res.* 41, 392–406 (2002).
8. Allen TM, Hansen CB, Demenezes DEL: Pharmacokinetics of long-circulating liposomes. *Adv. Drug Deliv. Rev.* 16, 267–284 (1995).
9. Ewer MS, Martin FJ, Henderson C *et al.*: Cardiac safety of liposomal anthracyclines. *Semin. Oncol.* 31, 161–181 (2004).
10. Orditura M, Quaglia F, Morgillo F *et al.*: Pegylated liposomal doxorubicin: pharmacologic and clinical evidence of potent antitumor activity with reduced anthracycline-induced cardiotoxicity (review). *Oncol. Rep.* 12, 549–556 (2004).
11. Minodier P, Retornaz K, Horelt A, Garnier JM: Liposomal amphotericin B in the treatment of visceral leishmaniasis in immunocompetent patients. *Fundam. Clin. Pharmacol.* 17, 183–188 (2003).
12. Burger KN, Staffhorst RW, de Vijlder HC *et al.*: Nanocapsules: lipid-coated aggregates of cisplatin with high cytotoxicity. *Nat. Med.* 8, 81–84 (2002).
• Interesting paper achieved high encapsulation efficiency of cisplatin into liposomes with high cytotoxicity *in vitro* compared with free cisplatin.
13. Katayama K, Kato Y, Onishi H, Nagai T, Machida Y: Double liposomes: hypoglycemic effects of liposomal insulin on normal rats. *Drug Dev. Ind. Pharm.* 29, 725–731 (2003).
14. Yamabe K, Kato Y, Onishi H, Machida Y: Potentiality of double liposomes containing salmon calcitonin as an oral dosage form. *J. Control. Release* 89, 429–436 (2003).
15. Zhong H, Deng Y, Wang X, Yang B: Multivesicular liposome formulation for the sustained delivery of breviscapine. *Int. J. Pharm.* 301, 15–24 (2005).
16. Xiao C, Qi X, Maitani Y, Nagai T: Sustained release of cisplatin from multivesicular liposomes: potentiation of antitumor efficacy against S180 murine carcinoma. *J. Pharm. Sci.* 93, 1718–1724 (2004).
17. Andresen TL, Jensen SS, Jorgensen K: Advanced strategies in liposomal cancer therapy: problems and prospects of active and tumor specific drug release. *Prog. Lipid Res.* 44, 68–97 (2005).
18. Viroonchatapan E, Ueno M, Sato H *et al.*: Preparation and characterization of dextran magnetite-incorporated thermosensitive liposomes: an on-line flow system for quantifying magnetic responsiveness. *Pharm. Res.* 12, 1176–1183 (1995).
19. Jain S, Mishra V, Singh P *et al.*: RGD-anchored magnetic liposomes for monocytes/neutrophils-mediated brain targeting. *Int. J. Pharm.* 261, 43–55 (2003).
20. Elmi MM, Sarbolouki MN: A simple method for preparation of immuno-magnetic liposomes. *Int. J. Pharm.* 215, 45–50 (2001).
21. Domingo JC, Mercadal M, Petriz J, de Madariaga MA: Preparation of PEG-grafted immunomagnetoliposomes entrapping citrate stabilized magnetite particles and their application in $CD34^+$ cell sorting. *J. Microencapsul.* 18, 41–54 (2001).
22. Martina MS, Fortin JP, Menager C *et al.*: Generation of superparamagnetic liposomes revealed as highly efficient MRI contrast agents for *in vivo* imaging. *J. Am. Chem. Soc.* 127, 10676–10685 (2005).
23. Chen H, Langer R: Magnetically-responsive polymerized liposomes as potential oral delivery vehicles. *Pharm. Res.* 14, 537–540 (1997).
24. Yanase M, Shinkai M, Honda H *et al.*: Antitumor immunity induction by intracellular hyperthermia using magnetite cationic liposomes. *Jpn. J. Cancer Res.* 89, 775–782 (1998).
25. Yanase M, Shinkai M, Honda H *et al.*: Intracellular hyperthermia for cancer using magnetite cationic liposomes: an *in vivo* study. *Jpn. J. Cancer Res.* 89, 463–469 (1998).
26. Kuznetsov AA, Filippov VI, Alyautdin RN, Torshina NL, Kuznetsov OA: Application of magnetic liposomes for magnetically guided transport of muscle relaxants and anti-cancer photodynamic drugs. *J. Magn. Magn. Mater.* 225, 95–100 (2001).
27. Shinkai M, Le B, Honda H *et al.*: Targeting hyperthermia for renal cell carcinoma using human MN antigen-specific magnetoliposomes. *Jpn. J. Cancer Res.* 92, 1138–1145 (2001).
28. Le B, Shinkai M, Kitade T *et al.*: Preparation of tumor-specific magnetoliposomes and their application for hyperthermia. *J. Chem. Eng. Jpn.* 34, 66–72 (2001).
29. Ito A, Shinkai M, Honda H, Kobayashi T: Medical application of functionalized magnetic nanoparticles. *J. Biosci. Bioeng.* 100, 1–11 (2005).
30. Ito A, Kuga Y, Honda H *et al.*: Magnetite nanoparticle-loaded anti-HER2 immunoliposomes for combination of antibody therapy with hyperthermia. *Cancer Lett.* 212, 167–175 (2004).
31. Bulte JWM, de Cuyper M, Despres D, Frank JA: Preparation, relaxometry, and biokinetics of PEGylated magnetoliposomes as MR contrast agent. *J. Magn. Magn. Mater.* 194, 204–209 (1999).
32. Hodenius M, De CM, Desender L *et al.*: Biotinylated Stealth magnetoliposomes. *Chem. Phys. Lipids* 120, 75–85 (2002).
33. Hamaguchi S, Tohnai I, Ito A *et al.*: Selective hyperthermia using magnetoliposomes to target cervical lymph node metastasis in a rabbit tongue tumor model. *Cancer Sci.* 94, 834–839 (2003).
34. Kawai N, Ito A, Nakahara Y *et al.*: Anticancer effect of hyperthermia on prostate cancer mediated by magnetite cationic liposomes and immune-response induction in transplanted syngeneic rats. *Prostate* 64, 373–381 (2005).
• Describes the anticancer effect of hyperthermia mediated by cationic magnetoliposomes in rats.
35. Shinkai M, Yanase M, Honda H *et al.*: Intracellular hyperthermia for cancer using magnetite cationic liposomes: *in vitro* study. *Jpn. J. Cancer Res.* 87, 1179–1183 (1996).
36. Hirao K, Sugita T, Kubo T *et al.*: Targeted gene delivery to human osteosarcoma cells with magnetic cationic liposomes under a magnetic field. *Int. J. Oncol.* 22, 1065–1071 (2003).
37. Nagatani N, Shinkai M, Honda H, Kobayashi T: Development of a new transformation method using magnetite cationic liposomes and magnetic selection of transformed cells. *Biotechnol. Tech.* 12, 525–528 (1998).

38. Viroonchatapan E, Sato H, Ueno M *et al.*: Microdialysis assessment of 5-fluorouracil release from thermosensitive magnetoliposomes induced by an electromagnetic field in tumor-bearing mice. *J. Drug Target.* 5, 379–390 (1998).
39. Babincova M: Targeted and controlled release of drugs using magnetoliposomes. *Ceska. Slov. Farm.* 48, 27–29 (1999).
40. Babincova M, Leszczynska D, Sourivong P, Babinec P, Leszczynski J: Principles of magnetodynamic chemotherapy. *Med. Hypotheses* 62, 375–377 (2004).
41. Babincova M, Sourivong P, Leszczynska D, Babinec P: Fullerenosomes: design of a novel nanomaterial for laser controlled topical drug release. *Phys. Medica.* 19, 213–216 (2003).
42. Babincova M, Sourivong P, Chorvat D, Babinec P: Laser triggered drug release from magnetoliposomes. *J. Magn. Magn. Mater.* 194, 163–166 (1999).
43. Alivisatos AP: Semiconductor clusters, nanocrystals, and quantum dots. *Science* 271, 933–937 (1996).
44. Akerman ME, Chan WC, Laakkonen P, Bhatia SN, Ruoslahti E: Nanocrystal targeting *in vivo*. *Proc. Natl Acad. Sci. USA* 99, 12617–12621 (2002).
45. Michalet X, Pinaud FF, Bentolila LA *et al.*: Quantum dots for live cells, *in vivo* imaging, and diagnostics. *Science* 307, 538–544 (2005).
46. Parak WJ, Pellegrino T, Plank C: Labelling of cells with quantum dots. *Nanotechnology* 16, R9–R25 (2005).
47. Dubertret B, Skourides P, Norris DJ *et al.: In vivo* imaging of quantum dots encapsulated in phospholipid micelles. *Science* 298, 1759–1762 (2002).

•• One of the first papers to describe quantum dots in phospholipid micelles as biocompatible fluorescent probe for *in vivo* imaging

48. Vuu K, Xie J, McDonald MA *et al.*: Gadolinium–rhodamine nanoparticles for cell labeling and tracking via magnetic resonance and optical imaging. *Bioconjug. Chem.* 16, 995–999 (2005).
49. Mulder WJ, Koole R, Brandwijk RJ *et al.*: Quantum dots with a paramagnetic coating as a bimodal molecular imaging probe. *Nano Lett.* 6, 1–6 (2006).
50. Samia AC, Chen X, Burda C: Semiconductor quantum dots for photodynamic therapy. *J. Am. Chem Soc.* 125, 15736–15737 (2003).
51. Hsieh JM, Ho ML, Wu PW *et al.*: Iridium-complex modified CdSe/ZnS quantum dots; a conceptual design for bi-functionality toward imaging and photosensitization. *Chem. Commun. (Camb.)* 14, 615–617 (2006).
52. Samia AC, Dayal S, Burda C: Quantum dot-based energy transfer: perspectives and potential for applications in photodynamic therapy. *Photochem. Photobiol.* 82, 617–625 (2006).
53. Chan WC, Maxwell DJ, Gao X *et al.*: Luminescent quantum dots for multiplexed biological detection and imaging. *Curr. Opin. Biotechnol.* 13, 40–46 (2006).
54. Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS: Noninvasive imaging of quantum dots in mice. *Bioconjug. Chem.* 15, 79–86 (2004).
55. Gao X, Cui Y, Levenson RM, Chung LW, Nie S: *In vivo* cancer targeting and imaging with semiconductor quantum dots. *Nat. Biotechnol.* 22, 969–976 (2004).
56. Wu X, Liu H, Liu J *et al.*: Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. *Nat. Biotechnol.* 21, 41–46 (2003).
57. Kalyuzhny G, Murray RW: Ligand effects on optical properties of CdSe nanocrystals. *J. Phys. Chem. B* 109, 7012–7021 (2005).
58. Kuno M, Lee JK, Dabbousi BO, Mikulec FV, Bawendi MG: The band edge luminescence of surface modified CdSe nanocrystallites: probing the luminescing state. *J. Chem. Phys.* 106, 9869–9882 (1997).
59. Mekis I, Talapin DV, Kornowski A, Haase M, Weller H: One-pot synthesis of highly luminescent CdSe/CdS core–shell nanocrystals via organometallic and "greener" chemical approaches. *J. Phys. Chem. B* 107, 7454–7462 (2003).
60. Derfus AM, Chan WC, Bhatia SN: Intracellular delivery of quantum dots for live cell labeling and organelle tracking. *Adv. Mater.* 16, 961–966 (2004).
61. Voura EB, Jaiswal JK, Mattoussi H, Simon SM: Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy. *Nat. Med.* 10, 993–998 (2004).

• Interesting paper that describes using quantum dots as a potential tool to track metastasis cancer *in vivo*.

62. Hsieh SC, Wang FF, Lin CS *et al.*: The inhibition of osteogenesis with human bone marrow mesenchymal stem cells by CdSe/ZnS quantum dot labels. *Biomaterials* 27, 1656–1664 (2006).
63. Gopalakrishnan G, Danelon C, Izewska P *et al.*: Multifunctional lipid/quantum dot hybrid nanocontainers for controlled targeting of live cells. *Angew. Chem. Int. Ed. Engl.* 45, 5478–5483 (2006).
64. Wu S, Zeng H, Schelly ZA: Preperation of ultrasmall, uncapped PbS quantum dots via electroporation of vesicles. *Langmuir* 21, 686–691 (2005).
65. Correa NM, Zhang H, Schelly ZA: Preparation of AgBr quantum dots via electroporation of vesicles. *J. Am. Chem. Soc.* 122, 6432–6434 (2000).
66. Mayer LD, Harasym TO, Tardi PG *et al.*: Ratiometric dosing of anticancer drug combinations: controlling drug ratios after systemic administration regulates therapeutic activity in tumor-bearing mice. *Mol. Cancer Ther.* 5, 1854–1863 (2006).
67. Ramsay EC, Dos Santos N, Dragowska WH, Laskin JJ, Bally MB: The formulation of lipid-based nanotechnologies for the delivery of fixed dose anticancer drug combinations. *Curr. Drug Deliv.* 2, 341–351 (2005).
68. Kisak ET, Coldren B, Evans CA, Boyer C, Zasadzinski JA: The vesosome – a multicompartment drug delivery vehicle. *Curr. Med. Chem.* 11, 199–219 (2004).
69. Kim S, Turker MS, Chi EY, Sela S, Martin GM: Preparation of multivesicular liposomes. *Biochim. Biophys. Acta* 728, 339–348 (1983).
70. Katayama K, Kato Y, Onishi H, Nagai T, Machida Y: Preparation of novel double liposomes using the glass-filter method. *Int. J. Pharm.* 248, 93–99 (2002).
71. Yamabe K, Kato Y, Onishi H, Machida Y: *In vitro* characteristics of liposomes and double liposomes prepared using a novel glass beads method. *J. Control. Release* 90, 71–79 (2003).
72. Ogue S, Takahashi Y, Onishi H, Machida Y: Preparation of double liposomes and their efficiency as an oral vaccine carrier. *Biol. Pharm. Bull.* 29, 1223–1228 (2006).
73. Kisak ET, Coldren B, Zasadzinski JA: Nanocompartments enclosing vesicles, colloids, and macromolecules via interdigitated lipid bilayers. *Langmuir* 18, 284–288 (2002).

•• Report encapsulating a wide variety of colloidal nanoparticles into a lipid bilayer via ethanol intergdigitation.

74. Lasic DD: Colloid chemistry. Liposomes within liposomes. *Nature* 387, 26–27 (1997).
75. Walker SA, Kennedy MT, Zasadzinski JA: Encapsulation of bilayer vesicles by self-assembly. *Nature* 387, 61–64 (1997).

76. Mishra V, Mahor S, Rawat A *et al.*: Development of novel fusogenic vesosomes for transcutaneous immunization. *Vaccine* 24, 5559–5570 (2006).
77. Al-Jamal W, Kostareols K: Construction of nanoscale multicompartment liposomes for combinatory drug delivery. *Int. J. Pharm.* DOI 10.1016/j.ijpharm.2006.11.020 (2007) (Epub ahead of prints).
78. Carmona-Ribeiro AM, Midmore BR: Synthetic bilayer adsorption onto polystyrene microspheres. *Langmuir* 8, 801–806 (1991).
79. Carmona-Ribeiro AM, Lessa MD: Interactions between bilayer membranes and latex. *Colloids Surf. A* 153, 355–361 (1999).
80. Carmona-Ribeiro AM: Interactions between cationic liposomes and drugs or biomolecules. *An. Acad. Bras. Cienc.* 72, 39–43 (2000).
81. Carmona-Ribeiro AM: Bilayer vesicles and liposomes as interface agents. *Chem. Soc. Rev.* 30, 241–247 (2001).
82. Carmona-Ribeiro AM, Herrington TM: Phospholipid adsorption onto polystyrene microspheres. *J. Colloid Interface Sci.* 156, 19–23 (1993).
83. Sicchierolli SMa, Carmona-Ribeiro AM: Incorporation of the cholera toxin receptor in phospholipid-covered polystyrene microspheres. *Colloids Surf. B* 5, 57–61 (1995).
84. Sackmann E: Supported membranes: scientific and practical applications. *Science* 271, 43–48 (1996).
85. Stelzle M, Weissmuller G, Sackmann E: On the application of supported bilayers as receptive layers for biosensors with electrical detection. *J. Phys. Chem.* 97, 2974–2981 (1993).
86. Rapuano R, Carmona-Ribeiro AM: Physical adsorption of bilayer membranes on silica. *J. Colloid Interface Sci.* 193, 104–111 (1997).
87. Rapuano R, Carmona-Ribeiro AM: Supported bilayers on silica. *J. Colloid Interface Sci.* 226, 299–307 (2000).
88. Mornet S, Lambert O, Duguet E, Brisson A: The formation of supported lipid bilayers on silica nanoparticles revealed by cryoelectron microscopy. *Nano Lett.* 5, 281–285 (2005).
89. Moura SP, Carmona-Ribeiro AM: Biomimetic particles for isolation and reconstitution of receptor function. *Cell Biochem. Biophys.* 44, 446–452 (2006).
90. Puu G, Artursson E, Gustafson I, Lundstrom M, Jass J: Distribution and stability of membrane proteins in lipid membranes on solid supports. *Biosens. Bioelectron.* 15, 31–41 (2000).
91. Feng L, Kong X, Chao K *et al.*: Efficient phase transfer of hydrophobic CdSe quantum dots: from nonpolar organic solvent to biocompatible water buffer. *Mater. Chem. Phys.* 93, 310–313 (2005).
92. Evans CC, Zasadzinski J: Encapsulating vesicles and colloids from cochleate cylinders. *Langmuir* 19, 3109–3113 (2003).
93. Jang H, Pell LE, Korgel BA, English DS: Photoluminescence quenching of silicon nanoparticles in phospholipid vesicle bilayers. *J. Photochem. Photobiol. A.* 158, 111–117 (2003).