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Liposomes: From a Clinically Established Drug Delivery System to a Nanoparticle Platform for Theranostic Nanomedicine

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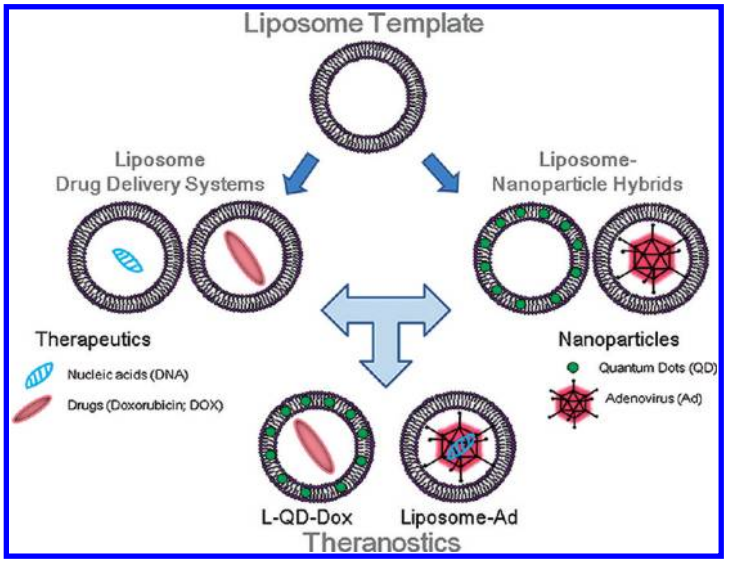

For decades, clinicians have used liposomes, self-assembled lipid vesicles, as nanoscale systems to deliver encapsulated anthracycline molecules for cancer treatment. The more recent proposition to combine liposomes with nanoparticles remains at the preclinical development stages; however, such hybrid constructs present great opportunities to engineer theranostic nanoscale delivery systems, which can combine simultaneous therapeutic and imaging functions. Many novel nanoparticles of varying chemical compositions are being developed in nanotechnology laboratories, but further chemical modification is often required to make these structures compatible with the biological milieu in vitro and in vivo. Such nanoparticles have shown promise as diagnostic and therapeutic tools and generally offer a large surface area that allows covalent and non-covalent surface functionalization with hydrophilic polymers, therapeutic moieties, and targeting ligands. In most cases, such surface manipulation diminishes the theranostic properties of nanoparticles and makes them less stable. From our perspective, liposomes offer structural features that can make nanoparticles biocompatible and present a clinically proven, versatile platform for further enhancement of the pharmacological and diagnostic efficacy of nanoparticles.

In this Account, we describe two examples of liposome–nanoparticle hybrids developed as theranostics: liposome–quantum dot hybrids loaded with a cytotoxic drug (doxorubicin) and artificially enveloped adenoviruses. We incorporated quantum dots into lipid bilayers, which rendered them dispersible in physiological conditions. This overall vesicular structure allowed them to be loaded with doxorubicin molecules. These structures exhibited cytotoxic activity and labeled cells both in vitro and in vivo. In an alternative design, lipid bilayers assembled around non-enveloped viral nanoparticles and altered their infection tropism in vitro and in vivo with no chemical or genetic capsid modifications. Overall, we have attempted to illustrate how alternative strategies to incorporate nanoparticles into liposomal nanostructures can overcome some of the shortcomings of nanoparticles. Such hybrid structures could offer diagnostic and therapeutic combinations suitable for biomedical and even clinical applications.

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Introduction

Liposomes are the most clinically established nanometer-scale systems that are used to deliver cytotoxic and antifungal drugs, genes, vaccines, as well as imaging agents.[1] Liposome vesicles consist of single or multiple concentric lipid bilayers (called lamellae) encapsulating an aqueous compartment. Biocompatibility, biodegradability, reduced toxicity, and capacity for size and surface manipulations comprise the outstanding profile that liposomes offer compared to other delivery systems.

The past decade has witnessed tremendous advances in the field of nanoparticle engineering technologies. Particles with a wide range of consistency, composition, and functionality have been developed. Nanoparticles such as iron oxide, quantum dots, and gold are recognized to offer extraordinary features for diagnostics as well as therapeutics. Imaging modalities, such as optical imaging, magnetic resonance imaging (MRI), and surface plasmon resonance (SPR), are rapid, noninvasive techniques that have been used in cancer therapy where early detection and disease prognosis have improved. Iron oxide nanoparticles, for example, are clinically approved MRI contrast agents and have also been proposed as a platform for development of targeted contrast agents, due to their large surface area that can be functionalized with different targeting ligands, their blood circulation can be modulated according to their physicochemical properties, and contrast agents and drugs can be included at predetermined ratios either in the interior or on the surface of the nanoparticles.

"Theranostic" is a term which defines ongoing efforts to develop more specific, individualized therapies for various diseases, combined with diagnostic capabilities into a single pharmaceutical agent. Merging diagnosis with therapeutic intervention is aiming to provide clinical protocols that are more specific to individuals and, therefore, more likely to offer improved prognoses and monitoring of disease. The field of theranostics is growing especially with the development of various delivery systems, such as liposomes, micelles, dendrimers, carbon nanotubes, in combination with imaging agents. The concept of simultaneous transport of therapeutic and diagnostic agents in a single delivery system is being intensively explored, either by upgrading theranostic nanoparticles to deliver drugs or by simply incorporating two moieties (therapeutic and diagnostic) in the same delivery system.[2,3] The present work is based only on the second concept, illustrating how therapeutic and imaging agents can be codelivered using liposomal carriers.

An overview of liposomes as delivery systems and as soft nanoscale platforms will be offered, highlighting on selective liposome–nanoparticle hybrids and their applications developed for the theranostic field. Furthermore, we will describe two examples of liposome–nanoparticle hybrid theranostics developed in our laboratory. The first study will report engineering artificial envelopes for non-enveloped, fluorescently labeled adenoviruses. In the second study, we will discuss doxorubicin- loaded liposome–quantum dot (L-QD-Dox) hybrids.

Liposome: A Clinically Established Drug Delivery System

Liposomes are proven candidates for delivery of a wide range of therapeutics, since their payload can be encapsulated in their internal aqueous compartment or embedded within the phospholipid bilayer. Clinical applications of liposomes in the delivery of anticancer agents for the treatment of different cancer indications are well-established. Stealth liposomes can passively accumulate in solid tumors due to their inherently leaky vasculature and defective lymphatic drainage. Doxil, Caelyx, and Myocet are nanometer-sized liposome systems (encapsulating doxorubicin in their aqueous core) which have been used for Kaposi's sarcoma, ovarian cancer, and multiple myeloma.[4–6] DepoCyt (cytarabine-containing multivesicular liposomes) with a sustained-release profile has also been approved for cancer therapy.[7]

New generations of liposome systems, including those responsive to external or environmental stimuli (e.g., pH, temperature, enzymes) to trigger drug release at specific and controlled sites, have been developed. ThermoDox is a temperature-sensitive nanometer-sized liposome that is currently in clinical trials to be used in combination with hyperthermia treatment in oncology.[8]

Liposome: A Soft Spherical Nanoscale Platform

Lipid bilayer vesicles were first used as a model mimicking biological membranes.[9] The flexibility offered in manipulating the size and composition of lipid vesicles has led to their use as model plasma membranes, subsequently used to mimic living cells and to understand a variety of physiological phenomena, such as diffusion across membranes and responses to various biological and pharmacological agents. Moreover, the possibility to reconstruct proteins in the lipid membrane has been heavily exploited in the design of

biosensors to detect and study the interaction between small molecules and ligands with plasma membrane receptors.[10]

Liposomes have also been explored as "chemical containers", designed to serve as nanoscale reaction vessels for remotely controlled reactions.[11] The ultrasmall dimensions of the reaction volume can lead to rapid diffusional mixing that permits the study of fast chemical kinetics. These technologies are also well suited for the study of reaction dynamics between biological molecules within lipid-enclosed environments that mimic cell membranes.

Liposome–Nanoparticle Hybrids

The versatility of the liposomal structure lies in its capacity to cargo drug molecules and biological macromolecules that are either hydrophilic, therefore entrapped in the liposome inner aqueous core, or hydrophobic, therefore incorporated within the lipid bilayer. Early forms of liposomal hybrid systems were introduced by Ringsdorf with the description of liposome–polymer hybrids and their ensuing assembled structures.[12] In the past few years, with the advent of nanotechnology, there has been a dramatic increase in the development of novel nanoparticulate materials. Nanometer-sized particles such as superparamagnetic iron oxides (SPIOs), gold, and semiconducting nanocrystals (quantum dots, QD) possess novel magnetic and optical properties that can be used as imaging probes. However, their surface hydrophobicity or poor colloidal stability at physiological conditions frequently renders them inappropriate for clinical use. A few years ago, we presented the concept of *liposome–nanoparticle hybrids* as a general methodology, taking advantage of the much more developed and sophisticated liposome technology, to be used as a platform for the delivery of novel nanoparticles.[13] Encapsulation of various types of nanoparticles within liposomes can lead to enhanced nanoparticle hydrophilicity, stability in plasma, better control of the pharmacological fate, and an overall improvement in their biocompatibility. Encapsulation of SPIOs, gold, silver nanoparticles, polystyrene nanospheres, lipid vesicles, and many others into liposomes has been achieved. The three different approaches for the engineering of liposome–nanoparticle hybrids are schematically shown in Figure 1. Hydrophobic nanoparticles can be embedded in the lipid bilayer, whereas hydrophilic nanoparticles can be encapsulated within the internal liposome aqueous core. Alternatively, various types of nanoparticles can be chemically or physically adsorbed onto the external liposome surface.

Table 1 summarizes the liposome–nanoparticle hybrid systems that have been described today, classified according to these three engineering approaches, highlighting their intended applications. As can be seen, most of the liposome–nanoparticle hybrids have been designed for use as diagnostic probes. In addition, various types of liposome–nanoparticle hybrids have shown promise in significantly stabilizing colloidal dispersions of otherwise unstable nanoparticle systems in vitro and in vivo. Recently, many studies have shown that incorporation of metallic nanoparticles in liposomes can also trigger release of encapsulated contents using external stimuli, such as magnetic fields, laser irradiation, or electromagnetic radiation at different radiofrequencies. Lastly, only a few studies have described loading of liposome–nanoparticle hybrids with therapeutic agents to be used as theranostics. Two such examples from our own research will be described in following sections.

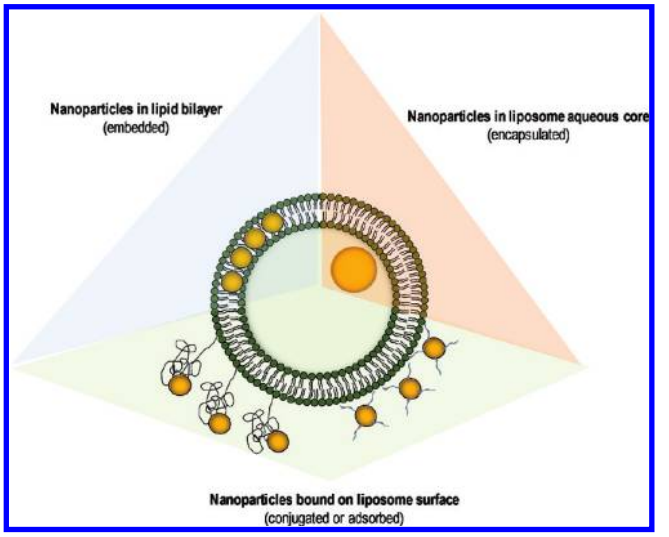

FIGURE 1. Schematic diagram of three different approaches to engineer liposome–nanoparticle hybrids. Hydrophobic nanoparticles embedded in the lipid bilayer (left); hydrophilic nanoparticles encapsulated in the aqueous core (right); and nanoparticles chemically conjugated or physically adsorbed/complexed to the liposome surface (bottom). Diagrams are not drawn to scale.

Liposome–Nanoparticle Hybrids for Theranostic Applications

There are multiple examples of liposome systems with diverse characteristics and capabilities that incorporate therapeutics or imaging agents.[14] However, only a few studies have described combinatory systems with therapeutic and imaging capacity using liposomes. Grange et al. showed combined delivery and magnetic resonance imaging (MRI) of doxorubicin-containing liposomes in a Kaposi's sarcoma model in vivo.[15] MRI was exploited not only to track the liposome tissue distribution but also to monitor drug

TABLE 1. Liposome–Nanoparticle Hybrids[a]

		Nanoparticle type	Lipid composition	Hybrid average diameter	Hybrid Functionality	Theranostic activity	Ref
Bilayer Embedded NP	Gold	Dodecanethiol coated Au NP (2nm)	PC	50-60nm	Cell membrane probe	No	19
		Hexanethiol capped Au NP (2.5nm)	DSPC:DPPC	200-500nm	UV light-induced drug release	No	20
		Stearylamine coated Au NP (3-4nm)	DPPC	20-200nm	Stabilize liposome membrane	No	21
	Iron	Oleic acid coated SPIO (5nm)	DPPC	150-200nm	Radiofrequency-induced drug release	No	22
	QD	TOPO-capped CdSe QD (2-4nm)	DMPC:DOTAP:DPPE-PEG_{2000}	20-100nm	QD solubilization Cell labeling *in vitro*	No	23
		TOPO-capped CdSe/ZnS QD (2-4nm)	DOTAP:DOPE:Chol DSPC:Chol:DSPE:PEG_{2000}	80-100nm	Cell labeling *in vitro* and *in vivo* Cell imaging and drug delivery	Yes (Doxorubicin)	24,25
Encapsulated NP	Gold	Gold nanoshell (100nm)	PC:Chol:DPPE-PEG_{2000}	N/A	Phototherapy-induced hyperthermia	No	26
		Hollow gold nanoshell (30-40nm)	DPPC	400-500nm	Laser-induced drug release	No	27
	Ceramic	$Y2O3:Er^{+3}$ (150nm)	DPPC:Chol:DPPG	500nm	NIR imaging	No	28
	QD	COOH-PEG-QD (25nm)	DOPC:DC-Chol DSPC:Chol:DSPE-PEG_{2000}	80-100nm	Cell labeling and imaging Tumor targeting	No	29,30
	Iron oxide	Magnetite (Fe_3O_4)	TMAG:DLPC: DOPE	N/A	Cell sorting and gene delivery	No	31
		Dextran Magnetite (Fe_3O_4) (5-10nm)	SPC:Chol:PS	N/A	Targeted drug delivery	No	32
		Citrate stabilized Maghemite (γFe_2O_3) (7.7nm)	EPC: DSPE-PEG_{2000}	200nm	MRI imaging	No	33
	Lipid	DSPC:Chol liposomes (50nm,200nm)	DPPC, DSPC	0.3-2μm	Drug delivery	No	34
	Polystyrene	Sulphate and amidine polystyrene NP (100-300nm)	DODAB, DODAC, DHP, PC	100-200nm	Nanoparticle stabilization Biosensor constructs	No	10,35
		Nanoparticle type	**Lipid composition**	**Hybrid average diameter**	**Hybrid Functionality**	**Theranostic activity**	**Ref**
Surface conjugated NP	QD	Streptavidin-QD	DOTAP:DOPE:$DSPE_{2000}$-biotin	100nm	Multicolor cell imaging	No	36
		Carboxylated CdSe/ZnS Qd chemically linked to amine functionalized PEG_{2000}-DSPE (4nm)	DSPC:Chol:DSPE-PEG_{2000}	200nm	Imaging and therapeutic modalities	Yes (Doxorubicin)	37
	Gold	Citrate coated Au NP (13nm)	EYPC:DDAB EYPC:DSPE-PEG_{2000}	200nm	Increase liposome colloidal stability	No	38
		DPPE-Nanogold (1.4nm)	DPPC:Chol	90 nm	Drug delivery and imaging system	No	39
		DPPE-Nanogold (1.4nm)	DSPC:DPPC	200-500nm	Light-induced drug release	No	20
		Hollow gold nanoshell (30-40nm)	DPPC	400-500nm	Laser-induced drug release	No	27
		PEG-maleimide-functionalized Au NP (64nm)	SOPC:DOPE	120-620nm	Cell membrane probe	No	40
Surface adsorbed/complexed NP	QD	DNA-QD conjugate	Lipofectamine2000	N/A	Cell labeling and gene delivery	Yes (pDNA)	41
		PEG-QD	Lipofectamine2000	N/A	Co-delivery of siRNA and QD	Yes (siRNA)	42
	Gold	COOH-Au (4nm)	EPC:DOTAP	92nm	Stimuli-responsive (acid) NP-stabilized liposomes	No	43
		Hydrophilic Au NP (300nm aggregates)	DPPC:DOTAP:Chol	5μm	NIR-induced drug release	No	44
		DDAB coated Au NP (9nm)	DOTAP, Lipotap	N/A	Gene delivery	Yes (pDNA)	45
	Polystyrene	COOH- polystyrene NP (20nm)	DLPC	N/A	Liposome stabilization	No	46

[a]TMAG, *N*-(α-trimethylammonio-acetyl)-didodecyl-D-glutamate chloride; DLPC, dilauroylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; SPC, soy phosphatidylcholine; PS, phosphatidylserine; DPPC, dipalmitoylphosphatidylcholine; DSPE-PEG_{2000}, distearoylphosphatidylethanolamine polyethylene glycol of MW 2000; EYPC, egg yolk phosphatidylcholine; NIR, near infrared; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DOPC, dioleoylphosphocholine; DOPS, dioleoylphosphatidylserine; DHP, dihexadecylphosphate; DODAB, dioctadecyldimethylammonium bromide; DODAC, dioctadecyldimethylammonium chloride; PC, phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; Chol, cholesterol; DSPC, distearoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; DC-Chol, 3 [*N*-(*N*,*N*-dimethylaminoethane)-carbamoyl] cholesterol; SOPC,1-stearoyl-2-oleoylphosphatidylcholine; $Y2O3,Er^{+3}$, rare earth dopped ceramic; DDAB, dimethyl-dioctadecyl ammonium bromide; SPIO, superparamagnetic iron oxides; TOPO, trioctylphosphine oxide; CdSe, cadmium selenide.

delivery and release. Encapsulation of doxorubicin and MRI contrast agents in temperature-sensitive liposomes allowed noninvasive and dynamic imaging of drug release during hyperthermia application.[16,17] In these studies, the MRI imaging agents were either ProHance (Gd(HPDO3A)-(H(2)O)) or manganese sulfate ($MnSO_4$); therefore, no nanoparticles were used. Previously, cationic magnetoliposomes (iron oxide nanoparticle-containing liposomes) were used for gene delivery, since they were able to complex nucleic acids (plasmid DNA), and consequently to allow the isolation of the transfected cells using a magnetic field.[18] In other studies, magnetolipsomes were used to accumulate the vesicles to a desired tissue or to induce hyperthermia in response to a magnetic field . Despite the fact that such liposome–nanoparticle hybrids exhibited therapeutic activity, the magnetic nanoparticles were not used as imaging agents. In our research, we sought to combine liposomes with nanoparticles with the intention to design liposome–nanoparticle hybrids that could achieve therapeutic and imaging capabilities.

Liposome–Viral Nanoparticle Hybrids: Engineering Artificial Viral Envelopes

Viruses traditionally have a major role in the study of infection and disease, while more recently genetic engineering technologies have allowed their utilization as compelling gene therapy vectors.[47] With the advent of nanotechnology, viruses have also been explored in the context of nanomedicine, specifically due to their nanoscale dimension (most virions are between 20 and 100 nm in diameter), unique size distribution, well-characterized surface structure, and high surface area-to-volume ratio that allows for attachment of multiple moieties at specific sites on the viral particle surface.[48,49] Recently, several investigations have recognized the viral capsid as a versatile building block that can serve as a scaffold for the fabrication of novel nanomaterials. A range of minerals, such as cobalt, nickel, and gallium, have been deposited on the viral template.[50] Besides their use as a template for mineralization, viruses have been used as a scaffold for fluorescent molecules or other nanoparticles exploiting the conjugation capabilities offered by the lysines and/or cysteines of the viral capsid. Quantum dots (QD),[51] super paramagnetic iron oxide particles (SPIOs),[52] platinum[53] and gold[54] nanoparticles have all been chemically conjugated to virus nanoparticles to design novel biosensors, electronic memory devices, and multimodal imaging agents.

Apart from designing biosensor devices and diagnostic agents, adenovirus (Ad) and adeno-associated virus (AAV) are human viruses that have been heavily explored in gene therapy.[55] Despite their high gene transfer and expression efficacy, Ad and AAV suffer from a variety of issues that have precluded their widespread clinical translation, including rapid blood clearance (requiring multiple administrations), tissue toxicity (liver in the case of Ad), and activation of severe and complex immune responses. In order to alleviate some of these side effects, many reports have described genetic modifications of viral capsids[56] and chemical conjugation of hydrophilic polymers, such as polyethylene glycol (PEG) and poly *N*-(2-hydroxypropyl)methacrylamide (HPMA) on both Ad and AAV, to prolong their blood circulation and reduce their immunogenicity and toxicity.[57] Despite some success in partially shielding the virus from the immune system, all such strategies suffered a significant reduction in gene transfer efficacy and a dramatic increase in the overall size of these vectors that affected their pharmacokinetic profile.

Our group has described an alternative approach by engineering a liposome–virus hybrid system. The Ad virions are seen as nanoparticles that can be entirely encapsulated within liposomes, in that way allowing the construction of artificial (lipid bilayer) viral envelopes by self-assembly (Figure 2A).[58,59] In these studies, viral particles could be enveloped with cationic, zwitterionic, and PEGylated lipid bilayer envelopes. That illustrates the design flexibility offered by such a hybrid system in terms of the possible resulting physicochemical and pharmacokinetic properties. The lipid envelope tightly wrapped around the Ad surface as shown by transmission electron microscopy (TEM) and atomic force microscopy (AFM) (Figure 2B and C, middle), and the presence of artificial envelopes altered the biological behavior of the virus in vitro and in vivo. Cationic lipid envelopes dramatically reduced the transfection capability of Ad in vitro (Figure 2E) due to their failure to escape the endosomal compartments following endocytosis (Figure 2D, middle). In contrast, PEGylated lipid envelopes prolonged the Ad blood circulation and reduced their liver gene expression and toxicity following systemic administration, allowing for passive targeting to solid tumors.[59]

More recently, in order to improve on the poor gene transfer efficiency of the artificially enveloped viruses, a pH-sensitive envelope (DOPE:CHEMS) was allowed to self-assemble around the Ad nanoparticles and pH-sensitive

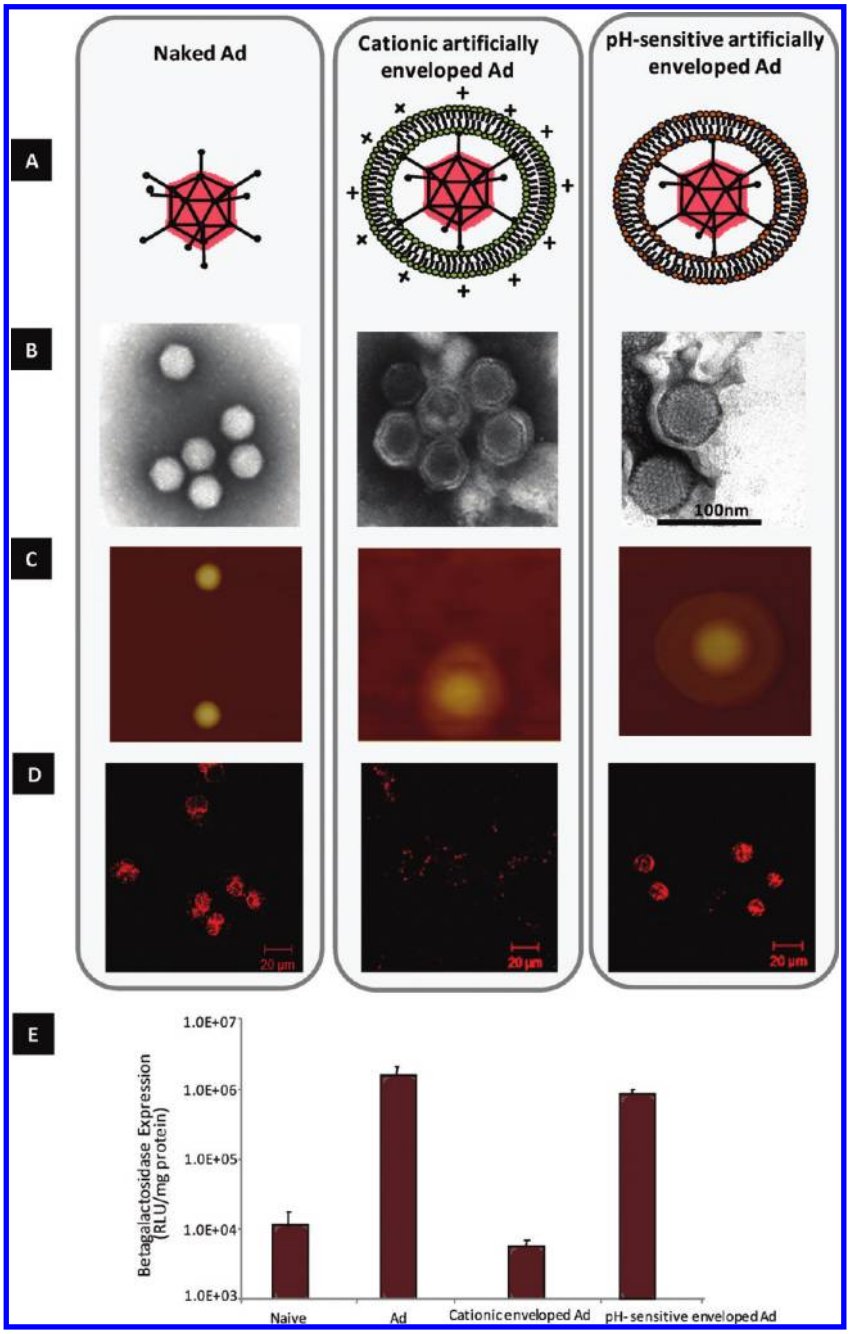

FIGURE 2. Liposome–virus hybrids: artificially enveloped adenoviruses (Ad). (A) Schematic depiction; (B) transmission electron microscopy; (C) atomic force microscopy (height images) of naked Ad, enveloped Ad in cationic (DOTAP:Chol), and pH-sensitive (DOPE:CHEMS) bilayers (left to right). (D) Intracellular trafficking of fluorescently labeled Ad in A549 (CAR+) cells. Confocal laser scanning microscopy images of Cy3-labeled, naked, and enveloped Ad in cationic and pH-sensitive envelopes (left to right). (E) In vitro (β-gal) gene expression of cationic and pH-sensitive enveloped Ad compared to naked Ad.[58−60]

enveloped Ad hybrids were successfully engineered (Figure 2B and C, right).[60] These liposome–virus hybrid systems showed similar levels of gene expression as those of naked Ad (Figure 2E) in vitro. Intracellular trafficking of fluorescently labeled Ad confirmed that both Ad and pH-sensitive enveloped Ad successfully escaped the endosomes and trafficked to the perinuclear region, in contrast to Ad enveloped in cationic envelopes where clear endosomal localization was observed (Figure 2D). The high level of gene expression of pH-sensitive lipid enveloped Ad was also maintained in vivo following intratumoral injection, which offers encouragement for further development. This hybrid system has been designed with potential theranostic capabilities, whereby the encapsulated virion is

the (gene) therapeutic component and imaging probes can be incorporated either on the viral capsid or on the lipid bilayer wrapping it. So far, we have only shown that the Ad capsid can be conjugated with organic fluorescent probes (Cy3) to allow optical tracking of the Ad; however, further modifications with a variety of diagnostic probes[49] prior to liposome encapsulation are deemed feasible with minimum disruption of the overall hybrid structure. Such engineering flexibility can allow the theranostic applications of artificially enveloped Ad to be easily tailored and should be further investigated.

Liposome–Quantum Dot Hybrids for Cancer Theranostics

Semiconducting nanocrystals known as quantum dots (QD) are fluorescent nanoparticles, which offer distinct spectrofluorometric advantages over traditional fluorescent organic molecules. QD exhibit fluorescence characteristics that are 10–20 times brighter than conventional organic dyes and offer greater photostability. Due to these photophysical characteristics, they are being explored as potential imaging agents primarily in optical (fluorescence-based) diagnostic applications.[61]

Most QD types are originally prepared in organic solvents; therefore, their hydrophobic shells compromise their water solubility and consequently their compatibility with the biological milieu. The most successful approach has been to functionalize QD with polar moieties and ligands with specific receptor-recognition signals (such as peptides and monoclonal antibodies or their fragments).[62] However, this surface modification often leads to significant reduction in QD fluorescence intensity and photostability. We have recently proposed engineering of liposome–QD hybrids that follows the approach shown in Figure 1 (left) by embedding hydrophobic QD within a variety of lipid bilayers (L-QD hybrids) (Figure 3A; right).[24] Structural characterization by cryo-transmission electron microscopy (cryo-TEM) and atomic force microscopy (AFM) of such L-QD hybrid bilayer vesicles confirmed the incorporation of QD in the liposome bilayer (Figure 3B and C; middle). L-QD hybrids allowed hydrophobic QD nanoparticles to be used in aqueous (i.e., biological) environments, while their incorporation in the acyl environment of the lipid bilayer significantly enhanced QD optical stability during storage and exposure to UV light.

The hybrid L-QD bilayer vesicles are thought to constitute a novel delivery system that offers the potential for transport of combinatory therapeutic and diagnostic agents to cancer cells in vitro and in vivo. To prove this concept, doxorubicin (Dox) was loaded inside L-QD hybrids of different lipid compostions (Figure 3A; right).[25] Successful Dox loading was confirmed by observation of doxorubicin crystals inside the hybrids (Figure 3B and C; right). The effect of QD on the vesicle integrity was shown to be highly dependent on the lipid components used. Minimum drug leakage was obtained using high phase transition temperature (T_m) bilayers (consisting mainly of DSPC lipids) compared to a low T_m bilayer (made mainly of EPC) (Figure 3D). More interestingly, these stable, Dox-loaded L-QD (L-QD-Dox) hybrids were uptaken by cells and were able to release Dox intracellularly, as evidenced by a significant enhancement in the cytotoxicity obtained, almost to the same level as the free drug (Figure 3E). In an alternative approach (according to Figure 1; bottom) to design liposome–QD hybrid systems with theranostic capabilities, Weng et al. covalently conjugated hydrophilic, polymer-functionalized QD at the outer tips of the polyethylene glycol chains coating the surface of liposomes and then loaded Dox into the aqueous core of these vesicles.[37] Prolonged blood circulation half-life and therapeutic efficacy compared to Dox alone was reported for this hybrid system; however, conjugation of the polymer-coated QD onto the outer liposome surface almost doubled the average size of the hybrid (from 112 to 212 nm) and greatly lowered Dox loading efficiency (30%). In comparison to previous attempts to design drug-loaded liposome-QD conjugates, we have demonstrated that incorporation of hydrophobic QD into the lipid bilayer of liposomes maintains the mean vesicle diameter consistently between the desirable 100–120 nm range, allows high loading efficiencies of Dox (>95%) using the established osmotic gradient technique, and has minimal effect on the Dox fluorescence characteristics.[25] Preservation of high Dox loading efficiencies, in particular, that can be achieved with this L-QD-Dox hybrid vesicle system are considered essential to maintain therapeutic efficacy comparable to the clinically used liposomal doxorubicin (DOXIL).

Following the design, engineering, and characterization of various L-QD and L-QD-Dox hybrid systems in vitro, we further studied the in vivo behavior of L-QD hybrids following local and systemic administration. Similar to in vitro results, the profile of L-QD in vivo was determined by the molecular structure of the lipids used. For instance, hybrids made of

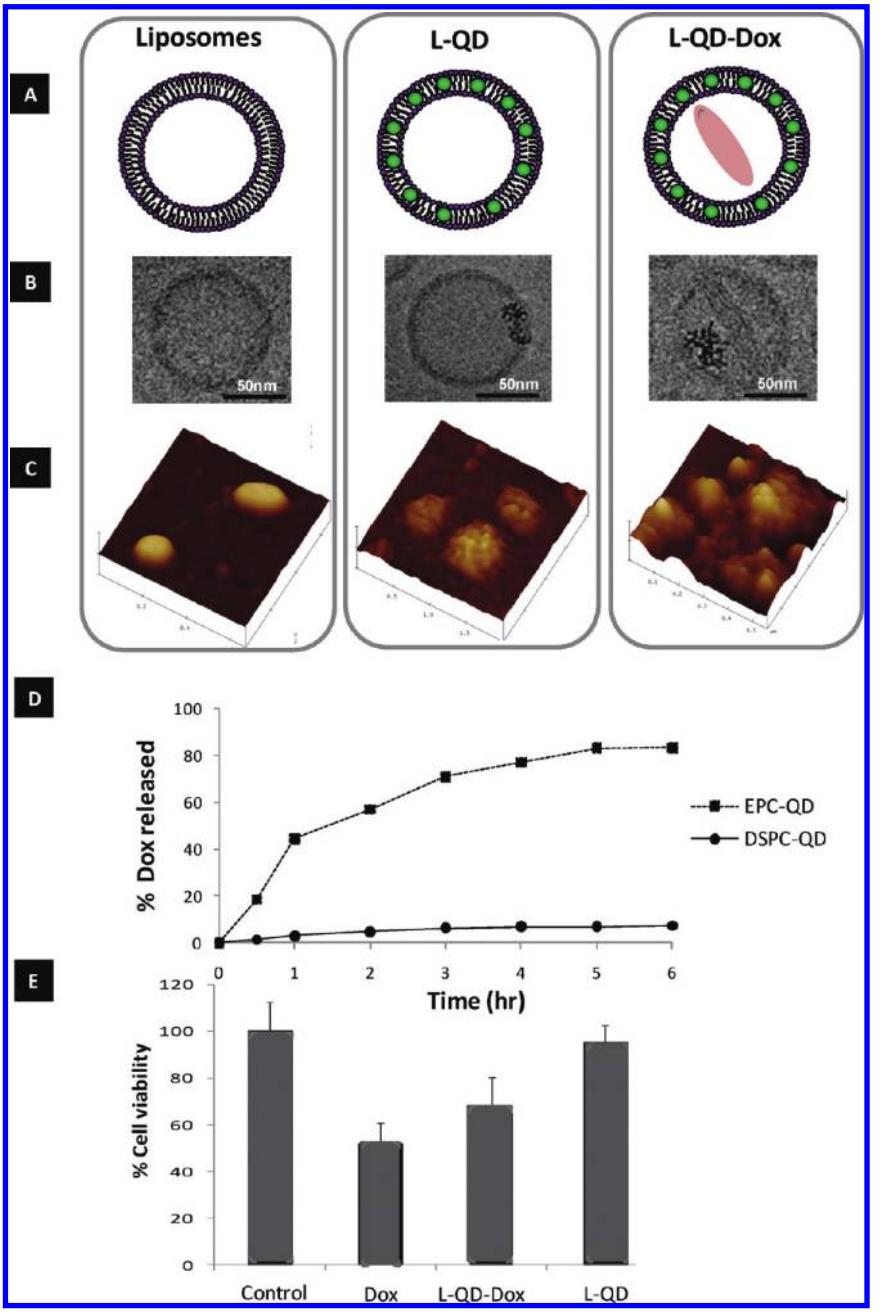

FIGURE 3. Liposome–quantum dot hybrids: lipid bilayer-embedded hydrophobic QD vesicles loaded with doxorubicin. (A) Schematic depiction of hybrids; (B) cryo-transmission electron microscopy; (C) atomic force microscopy (3D images) of empty liposomes, L-QD hybrids, and L-QD-Dox hybrids (left to right). (D) Serum stability of L-QD-Dox hybrids incubated in 50% mouse serum. QD were embedded in EPC:Chol:DSPE-PEG_{2000} and DSPC:Chol:DSPE-PEG_{2000} liposomes. Dox was loaded using the pH-gradient technique and Dox release was assessed by measuring Dox fluorescence. (E) Cytotoxicity of L-QD-Dox hybrids. MCF-7 cells were incubated with free Dox, L-QD-Dox, and L-QD hybrids, and cell viability was assessed using MTT assay.[25]

cationic lipids efficiently labeled tumor cells compared to zwitterionic L-QD hybrids after intratumoral administration.[24] In addition, simply by manipulating the lipid composition, we targeted L-QD hybrids to different organs following systemic administration;[63] sterically stabilized (DSPE-PEG-containing) L-QD exhibited prolonged blood circulation compared to cationic L-QD hybrids. In contrast, cationic L-QD hybrids showed high transient lung accumulation post-injection which makes them more suitable for pulmonary targeting and imaging.

In accordance to the approach in Figure 1 (right), our group has also reported the engineering of functionalized

quantum dot–liposome hybrids (f-QD-L) by encapsulation of hydrophilic f-QD in the internal liposome aqueous compartment.[29] We proposed the design of such hybrids for the delivery of hydrophobic therapeutic agents (small drug molecules, photosensitizers, etc.) along with hydrophilic imaging probes (such as f-QDs) using a single vesicle. In addition, we showed that such an approach can enhance the cellular uptake and retention of polymer-coated QD in vivo and accelerate their tumor accumulation following systemic administration.[30] Further work is being undertaken to combine imaging and therapy in vivo of both types of L-QD and f-QD-L hybrids by our laboratories and those of others. In addition, such vesicle systems can be further modified with different targeting ligands on the liposome surface to offer binding specificity and target cancer cell receptors. In that way, construct liposome–nanoparticle hybrid systems with triple capabilities (active targeting, therapy, and imaging) can be obtained.

Conclusion

Liposome (or lipid bilayer vesicle) science has developed into a mature and rich technology base, offering us a wide range of tools and components for the construction of more complex and multifunctional devices in a variety of industrial applications. In medicine, liposomes constitute one of the most successfully translated delivery systems that is currently in clinical use for a variety of indications against cancer, inflammatory, dermatological diseases and in various types of vaccines. Such established clinical profiles along with the engineering versatility of the liposome structure are considered key advantages for the further development of liposome-based systems with combinatory therapeutic-diagnostic (theranostic) capabilities. In this Account, we have elaborated on the concept of utilizing liposomes as templates for the incorporation of a wide variety of nanoparticles toward engineering liposome–nanoparticle hybrid systems of advanced, previously unattainable functionality. The rapid developments in nanomaterials synthesis and fabrication now offer a tremendous range of nanoparticles with different characteristics and capabilities that are very commonly incompatible with the biological milieu. Liposome–nanoparticle hybrids can be designed by embedding, encapsulation, or conjugation of nanoparticles onto various types of liposomes. The theranostic potential of such hybrids has been illustrated with two examples from our work: *liposome-encapsulated viral nanoparticle hybrids* capable of gene therapy (viral gene transfer) and optical imaging (probe molecules on the viral capsid), and *doxorubicin-loaded, lipid bilayer-embedded quantum dot vesicle hybrids* capable of chemotherapy (cytotoxic activity of doxorubicin) and optical imaging (embedded quantum dots). Such liposome–nanoparticle hybrid systems also offer unprecedented versatility and flexibility in the combination of physicochemical characteristics (emission/absorbance wavelength; surface; size; responsiveness to external stimuli) and biological activity profiles (gene transfer; cytotoxicity; pharmacokinetics) that can possibly be achieved with the right selection of lipid compositions and nanoparticle types to best suit a given biomedical application and pathological condition. More research from various laboratories is needed to explore this concept further in order to truly exploit our knowledge and clinical experience of liposomes in combination with novel nanomaterials toward more capable and "smarter" theranostic devices.

BIOGRAPHICAL INFORMATION

Dr. Wafa' T. Al-Jamal received her Ph.D. in drug delivery from The School of Pharmacy, University of London in 2008. She was awarded an Overseas (ORS) Scholarship from the University of London to focus on the development of novel liposome-based structures for tumor imaging, targeting, and therapy. She is a Senior Research Fellow at the Nanomedicine Lab, Centre for Drug Delivery working on the engineering and pharmacokinetics of organic/inorganic hybrids for theranostic applications for cancer. She also focuses on the development and study of temperature- and pressure-sensitive delivery systems for cancer imaging and therapy and is the Scientific Manager of the SONODRUGS project sponsored by the FP7 European Commission programme.

Professor Kostas Kostarelos is Chair of Nanomedicine at the School of Pharmacy, Head of the Centre for Drug Delivery Research, and Leader of the Nanomedicine Lab (www.nanomedicinelab.org) all at the University of London. He has been invited as a Fellow of the Royal Society of Medicine, the Institute of Nanotechnology, and the Royal Society of Arts. He obtained his Diploma and Ph.D. in Chemical Engineering from Imperial College London. His previous academic appointments include Assistant Professor of Genetic Medicine & Chemical Engineering in Medicine, Cornell University Weill Medical College, NY, USA; Instructor, Pulmonary & Critical Care Medicine, New York-Presbyterian Hospital, NY, USA; Manager, Bioengineering Core, Belfer Gene Therapy Center, Cornell University Weill Medical College, NY, USA; Deputy Director, Imperial College Genetic Therapies Centre, Imperial College London, UK. He is the Founding and Senior Editor of the journal *Nanomedicine*. In 2010 he was

awarded an Invitation Professorial Fellowship from the Japanese Society for the Promotion of Science (JSPS).

ABBREVIATIONS

DOPE, dioleoylphosphatidylethanolamine; CHEMS, cholesteryl hemisuccinate; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; EPC, egg phosphatidylcholine; Chol, cholesterol; DSPC, distearoylphosphatidylcholine; DSPE-PEG_{2000}, distearoylphosphatidylethanolamine polyethylene glycol of MW 2000; Cy3, cyanine dye 3; A549, human lung adenocarcinoma epithelial cell line; β-gal, β-galactosidase; Dox, doxorubicin; L-QD, liposome–quantum dot; L-QD-Dox, doxorubicin-loaded liposome–quantum dot; MCF-7, human breast adenocarcinoma cell line.

FOOTNOTES

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