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Stimulus-responsive liposomes as smart nanoplatforms for drug delivery applications

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Abstract

Liposomes are known to be promising nanoparticles (NPs) for drug delivery applications. Among different types of self-assembled NPs, liposomes stand out for their non-toxic nature, and their possession of dual hydrophilic-hydrophobic domains. Advantages of liposomes include the ability to solubilize hydrophobic drugs, the ability to incorporate different hydrophilic and lipophilic drugs at the same time, lessening the exposure of host organs to potentially toxic drugs and allowing modification of the surface by a variety of different chemical groups. This modification of the surface, or of the individual constituents, may be used to achieve two important goals. Firstly, ligands for active targeting can be attached that are recognized by cognate receptors over-expressed on the target cells of tissues. Secondly, modification can be used to impart a stimulus-responsive or “smart” character to the liposomes, whereby the cargo is released on demand only when certain internal stimuli (pH, reducing agents, specific enzymes) or external stimuli (light, magnetic field or ultrasound) are present. Here, we review the field of smart liposomes for drug delivery applications.

Graphical abstract

Smart liposomes can release their contents in response to a stimulus, which can either be internal or external. The figure shows an example of the latter whereby a beam of light can polymerize a chemical group on the outside of the liposome, thus disrupting the lipid bilayer and releasing the cargo.

Keywords

Liposomes; nanocarriers; drug delivery; smart stimulus-responsive; external/internal stimuli

1. Introduction

Advances in nanotechnology have made a substantial impact in many different fields and research areas, particularly in biomedicine and pharmaceuticals [1–6]. Studies of nanoparticles as vehicles for drug and gene delivery and for controlled release have been carried out for the last 20 years [7–10]. Among many different types of nanoparticles, liposomes are one of the most attractive types for use as drug carriers due to their dual hydrophilic-hydrophobic domains [11]. In 1965 Bangham *et al.* [12] reported the first “swollen” phospholipid system that formed distinct layers, and during the following years, a novel drug delivery system based on liposomes was developed. Liposomes are self-assembled, totally-enclosed spherical vesicles composed of non-toxic phospholipids which are capable of encapsulating both hydrophilic and lipophilic cargo molecules [13].

Liposomes have several special advantages for drug delivery: enhancing the solubility of hydrophobic drugs; the ability to incorporate both hydrophilic and lipophilic drugs at the same time; lessening the exposure of host organs to potentially toxic drugs; and allowing modification of the surface by a variety of different chemical groups [14]. The ability to design smart vehicles that can lead to accumulation of drugs or other therapeutic agents specifically at a diseased site, has resulted in the development of the concept of active targeting [15]. Surface modification of liposomes can be carried out by attachment of antibodies (or other ligands that are recognized by over-expressed receptors) for site-specific targeting. Also chemical moieties can be attached to the surface of the liposomes (or to the constituent building blocks) that will respond to various different stimuli. These “smart liposomes” can undergo triggered drug-release based on various physiology dependent properties. The stimuli can be either internal (e.g. enzyme activity, pH changes, or the presence of reducing agents) or they can be external stimuli applied from outside (e.g. temperature, light, magnetic field, or ultrasound) [16]. Release of drug from liposomes based on triggering by external stimuli, provides better accuracy concerning the timing and location of release, and better control over the delivery and dosage of the drug [17].

Over last few years many different FDA approved liposomes have been introduced for clinical applications, and many others are in different phases of clinical trials. (Table 1).

2 Temperature-responsive liposomes

Temperature plays a critical role in the metabolic activity of cells. The normal mammalian body temperature of 37°C is an ideal temperature for intracellular chemical reactions. Difficulties arise when tissue temperature exceeds the approximate limit of 43–45°C, hyperthermia, since the cells will not be able to readily maintain their normal activity [41, 42]. The utilization of mild hyperthermia (HT), a few degrees higher than physiological temperature, has long been applied together with radiotherapy and chemotherapy in order to boost the efficacy of these treatments with minimal side effects [43, 44]. Temperature-

sensitive or thermo-sensitive liposomes (TSLs) have been considered as one of the most efficient types of nanocarriers for site-specific drug delivery applications [45–47].

HT, which can be applied by using different modalities, combined with temperature-sensitive liposomes can enhance therapeutic efficiency as follows [48]:

1. Increasing the accumulation of liposomes in the tumor by increasing tumor vascular permeability
2. Controlling the release of therapeutic agents from temperature-triggered liposomes into the tumor vascular and interstitial space
3. Modulating the target cells with enhanced permeability and susceptibility to released drugs
4. Increasing blood flow at the area exposed to heat
5. Exerting direct cytotoxicity to cancer cells while sparing normal cells

The combination of TSL therapy with external HT can be carried out with three different liposome formulations namely: traditional TSLs (TTSLs); lysolipid-containing TSLs (LTSLs); and polymer modified TSLs (PTSLs). The design, drug release behavior, mechanisms of thermally-triggered release, and the clinical potential of each type have been reviewed by Ta and Porter [48]. Here, we briefly summarize each category and its properties, and highlight recent advances in that particular area.

TTSLs contain traditional phospholipids and undergo a phase transition such as a gel-to-liquid crystallite transition, or a structural transition from lamellar to hexagonal morphology [45, 48]. These types of liposomes were introduced for the first time in 1978 by Yatvin *et al* [49, 50]. These authors used dipalmitoyl phosphatidylcholine (DPPC) for the primary lipid with a transition temperature of 41 °C, and added distearoylphosphatidylcholine to DPPC in order to adjust the DPPC transition temperature to the desired value and release the cargo, neomycin. Since then, a number of studies have focused on the preparation of DPPC-based liposomes in combination with other lipids and polyethylene glycol-(PEG)-lipid conjugates in order to enhance the permeability of the liposomal membrane and producing long-circulating (stealth) TTSLs [51–53]. Levachea *et al* [52] described a DOX-loaded temperature-sensitive liposome (DOX-TL) composed of a combination of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSP), distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide-PEG-2000-ammonium salt] (DSPE-PEG-2000, and cholesterol. This liposomal formulation was triggered in response to local hyperthermia while it remained stable at physiological temperature. By optimization of the liposomal composition, they successfully improved the stability and inhibited premature drug release in antitumor therapy.

LTSLs were first reported by Anyaramabhatla and Needham [54] in 1999 to reduce the phase transition temperature, and boost rapid drug release over a period of tens of seconds. Since then, lysolipid formulations have undergone further development and has shown improved properties in comparison with TTSLs. Studies suggest that presence of the lysolipid in a relatively low molar percentage in the primary lipid (DPPC) bilayers causes

the stabilization of defects in the lipid membrane through the phase transition. The optimized LTSL formulations for rapid drug release and stable liposomal membranes are tailored to have a transition temperature in the range of 39–40°C [48].

Along with the advantages of lysolipids, there is also a drawback consisting of the possibility of the lysolipids leaking from the liposomal shell and degrading the bilayer stability. This led to the idea of synthesizing PTSLs, which have received considerable amount of interest from researchers. The incorporation of polymers can lead to structures that can be either completely or partially degraded, or undergo a phase transition, and disrupt the liposomal membrane in response to heat. These polymers exhibit a lower critical solution temperature (LCST), and an upper critical solution temperature (UCST), below and above which the polymers are soluble, and near to these temperatures the polymers undergo a coil-to-globule transition [45, 48, 55]. Among different types of temperature-sensitive polymers poly(N-isopropylacrylamide) (pNIPAAm) has been extensively studied [56, 57]. In a recent study, Pippa *et al.* [58] synthesized novel temperature-sensitive liposomes by coating DPPC with an end-functionalized C₁₂H₂₅-PNIPAM-COOH copolymer. This polymer was temperature-sensitive leading to membrane disruption, and the polymer chains aggregated at temperatures above 32°C. The composition and the molar weight of the PNIPAM chains played an essential role in the thermo-tropic properties of the mixed liposomal membrane. Also, it was shown that by tailoring the DPPC/PNIPAM ratio in the components, drug release from this nanocarrier could be effectively controlled. Turner *et al.* [59] engineered a thermo-sensitive-liposome formulation containing DPPC, soyPC, and cholesterol with optimized molar ratios. Then, the liposome was coated with PEG and dextran, and they compared the stability and the release behavior of the liposome with these two different coatings. They concluded that dextran and PEG had similar release properties. However, *in vitro* macrophage uptake was greater with dextran. Another example of polymer-modified thermo-sensitive liposomes was described by Guo and Kim [60]. They produced an electrostatic complex between cinnamic acid (CA) and polyethyleneimine (PEI), and the PEI-CA conjugate was immobilized on the surface of an egg phosphatidylcholine (EPC) liposome formulation. The PEI-CA conjugate could be disassembled above its UCST (hindering drug release), and remained assembled below its UCST, which triggered drug release by increasing stress on the liposomal membrane (Fig. 1). This PEI-CA conjugate could change its configuration in response to a temperature below or above its UCST thus controlling drug release.

Wang and Kim [26] modified the block co-polymer Pluronic F127 by attaching cinnamoyl groups (CF127) and immobilized it on the surface of EPC liposomes resulting in triggered released of its water-soluble payload in response to a temperature change. The drug release could be triggered by the phase transition of CF127 on the surface of the liposome. When the aqueous solution was heated up, the CF127 underwent micellization and gelation as a result of phase transition, which triggered the drug release from the liposomal membrane. Another approach for producing polymer-modified thermo-sensitive liposomes was recently described as so-called “polymer-caged nanobins” (PCNs). In this study, a hydrogel network was integrated over the surface of a liposomal carrier. Cholesterol-terminated poly(acrylic acid)(chol-PAAc) chains were immobilized into the bilayer membrane of the drug-loaded liposome and then chol-PAAc chains were cross-linked with telechelic diamine linkers. This

gel-like three-dimensional network of polymers was thermo-sensitive, which induced drug release from the liposomal core [61].

In addition to the three above-mentioned extensively-studied categories (TTSLs, LTSLs, and PTSLs), other novel liposomal formulations have been reported. “Nanohybrid liposomal cerasomes” (NHLC) are one of these formulations [62–65]. NHLC are liposomal bilayers with an inorganic silicate framework on the surface. This system is biologically stable. However, slow drug release from this carrier can be considered to be one of its drawbacks. Recently, Liang *et al.* [66] developed a stable NHLC formulation, thermosensitive cerasome (HTSC), that displayed rapid thermo-sensitive drug release upon exposure to high-intensity focused ultrasound (HIFU). This system consisted of four types of lipids, each of which played a distinct role in the formulation, including N-[N-(3-triethoxysilyl)propylsuccinamoyl]-dihexadecylamine (CFL), DPPC, mono C17 lipid (MSPC), and DSPE-PEG-2000. Different molar ratios of CFL to DPPC were explored to enhance serum stability and thermal sensitivity. This formulation was prepared using a thin-film hydration method in and a self-assembly process leading to sol-gel reaction. In this system, the presence of DSPE-PEG-2000 induced rapid drug release. Fig. 2a, b, and c illustrate the formation of the afore-mentioned nanosystem, its drug release behavior, and a comparison to conventional low temperature-sensitive liposomes (LTSLs).

Another approach that can be applied for further enhancing the therapeutic efficiency and bioavailability of TSLs is active targeting using cell-specific ligands such as antibodies, folate, and peptides, which can be attached to the surface of TSLs. Some degree of targeting can also be achieved by incorporating cationic lipids into the bilayer [44, 67–69]. Kono *et al.* [67] synthesized thermo-sensitive liposomes with target specificity. Different types of PEGylated liposomes were functionalized with thermo-sensitive poly (2-2-ethoxy) ethoxyethyl vinyl ether) chains and conjugated to a monoclonal antibody, trastuzumab (Herceptin) that recognizes the HER2 receptor which is over-expressed on some breast cancers. The liposomes were loaded with indocyanine green for near-infrared fluorescence imaging. Owing to the specific interaction between the antibody and its target cells, the liposomes accumulated efficiently at tumor sites.

3. Light responsive liposomes

Among diverse external stimuli, the use of light as a triggering agent for smart drug delivery systems (DDSs) has been extensively reviewed in the literature [4, 5]. Light is particularly attractive as a wide variety of parameters, such as wavelength, duration, the intensity of beam and beam diameter, can be tailored to achieve the desired release profile and tissue penetration. Furthermore, non-invasive light activation can be exerted with high temporal and spatial control [70]. Electromagnetic light wavelengths below 650nm cannot penetrate deeply through tissue (> 1cm) because of scattering and absorption by endogenous chromophores (like water, lipids, and hemoglobin) [71], thus although UV light can trigger a lot of chemical reactions, it can be employed as a triggering agent only for superficial treatments applied to the skin and mucosa [72]. The main strategy to obtain deeper light penetration is the use of near-infrared (NIR) light within the wavelength range of 650–1000nm, which also causes less damage to living cells [73].

Liposomes are amongst the most studied types of light responsive NPs. The key element in light responsive NPs is the necessity for a chromophore (a chemical moiety that can capture light energy) [4]. Many mechanisms have been developed to induce light-triggered release from NPs, such as photo-crosslinking [74], photo-un-crosslinking [75], photochemical hydrophobicity switch [76], and photo-induced cleavage of chemical bonds [77]. Mechanisms used in light-sensitive liposomes can be categorized into three groups, photo-crosslinking, photochemical triggering, and photo-isomerization [78]. Each mechanism works on the basis of a light-triggered change in chemical structure of a specific molecule. Some important photo-sensitive chemical groups are azobenzene and spiropyran (that undergo a photo-isomerization transition), and coumarin (that undergoes a photo-crosslinking reaction) [79].

3.1 Photo-crosslinking (photo-polymerization)

Although photo-crosslinking or photo-polymerization has been used as a means for formation of NPs, this same mechanism can also be employed as a means to trigger drug release from liposomes. Photo-polymerization causes short or long-lived pores to occur in the liposome membrane by polymerizing double bonds in components in the hydrophobic part of the liposome bilayer, leading to shrinkage of the bilayer, and consequent drug release (Fig. 3) [80]. Recent studies have also employed photosensitizers in the liposome structure to increase the sensitivity and to shift the wavelength of light absorption into the visible region [81]. Yavlovich *et al.* [82] prepared photo-triggerable liposomes from photo-polymerizable diacetylene phospholipid and dipalmitoyl phosphatidylcholine, which released calcein (a fluorescent dye entrapped in liposome) upon treatment with UV light (254 nm).

3.2 Photochemical activation

Many mechanisms can lead to the disturbance of structured assembly of the lipidic components of the liposome bilayer, and the consequent release of its contents. Photosensitization-induced oxidation (photodynamic), triggering with “photo-acid generators”, and photo-deprotection of fusogenic lipids [73, 83]. An example of the use of a photosensitizer in photochemical triggering is the activation of plasmenylcholine by photosensitizers activated by NIR light. Photosensitizers including octabutoxyphthalocyanine, Zn-phthalocyanine and bacteria-chlorophyll- α , which absorb light between 630–820 nm, can lead to the photo-triggering of liposomes and the release of payload [83].

3.3 Photo-isomerization

Another approach to develop light responsive liposomes is to use polymers that have been modified by attaching chromophores that undergo conformational changes upon light irradiation [84]. Lipids can be modified with light-responsive chromophores such as azobenzene, spirooxazine, stilbene, fulgide or spiropyran groups. These all contain a double bond, which can undergo trans-to-cis isomerization in their structure (Fig. 4-a). Azobenzene is amongst the most attractive chromophores due to its favorable properties, including high stability, bright color, low flammability and reversible and fast isomerization [85]. The mechanism involves switching from nonpolar and more stable trans-isomer, to the more

polar cis-isomer upon excitation with visible, UV or even NIR wavelengths leading to drug release [86–88] (Fig. 4-b and c)

The most attractive feature of photo-isomerization is its reversibility, i.e. the conversion of cis back to trans is a relaxation back to the more stable state, and can be achieved by irradiation with longer wavelengths of light or by thermal relaxation[89]. Many studies have investigated azobenzene-modified liposomes; for example, Ishii *et al.* introduced azobenzene into the amphiphilic region of the 1,2-dioleoyl-sn-glycero-3-phosphocholineliposomal membrane in order to change the membrane permeability by light irradiation [90]. Chi Yao *et al.* [87] reported a NIR light-responsive azobenzene-liposome carrier, loaded with DOX. It was found that > 90 wt% of DOX was released under 7.8 W/m² power density (Fig. 4–c).

3.4 Photo-induced thermo-responsive release

In contrast with conventional bulk gold metal salts, gold nanostructures (NPs, nanoshells, nanorods, nanocages etc.) can absorb light in the NIR region and in the visible region (5 times higher absorption coefficients than common photo-absorbing dyes) [91]. The absorption occurs due to the surface plasmons (quasi-particles of plasma oscillation) whereby the conductance electrons possess a high resonance with certain electromagnetic frequencies [92]. The photo-absorption by gold nanostructures increases the temperature by forming a “heated electron gas” which loses its energy rapidly (within 1ps) by heat exchange with the nanostructure lattice. Thereby the surrounding medium is heated locally within a time scale of 100ps [93]. This thermal property can be employed to trigger release of the drugs entrapped in liposomes (Fig. 5) [94]. Moreover gold NPs can be used on their own in laser-induced hyperthermia-based cancer treatment, in which tumor tissue is targeted by Au-NPs to kill the cancer cells [95, 96]._ENREF_23

Wu *et al.* [97] prepared DPPC liposomes loaded with hollow gold NPs and 6-carboxyfluorescein (a fluorescent dye used as a soluble model drug). Here, hollow gold NPs had a maximum absorption at 820 nm and maximum drug release occurred at 4.3 W/cm² power density. Recently, Lajunen *et al.* [98] described a liposomal DDS loaded with gold NPs prepared in nanostar and nanorod shapes. The release profile of the calcein payload, was investigated as a function of temperature (Fig. 6).

4. pH-sensitive liposomes

Although significant developments have been achieved in addressing the challenges related to liposomal drug delivery, there are still some major problems [99]. One of the most important challenges is the ability of the delivery system to selectively enhance the bioavailability of the drugs at the target site, as well as to maintain its stability in the blood circulation until it reaches its destination. Currently, one of the most common strategies to overcome the aforementioned problem is “pH-sensitive liposomes”. This strategy is frequently used to target cancer since relatively low pH is one of the most common features of solid tumors. Moreover release of liposomal contents inside the acidic compartments of cells (endosomes or lysosomes) can also be achieved. The principle mechanism of these NPs is the destabilization of the structure under acidic conditions, which in turn leads to the

release of their contents. In other words, the acidification of the surrounding medium induces selective destabilization of the liposomal membrane [99, 100].

However, the aforementioned strategy has two major limitations. Firstly, in the case of tumor therapy, the acidic sections of tumors are located remotely from the microvasculature, since that is where hypoxia is most pronounced leading to accumulation of lactic acid due to the cellular metabolism switching to glycolysis (Warburg effect). This remoteness from the blood supply makes it difficult for IV injected liposomes to efficiently accumulate. Secondly, since the tumor pH range rarely reaches lower than pH 6.5, it is technically difficult to fabricate engineered liposomes that are capable of efficiently responding to such a small pH change (~1 pH unit) [99, 101].

The structure of pH sensitive liposomes generally consists of a neutral lipid, which is usually a phosphatidylamine derivative such as dioleoylphosphatidyl-ethanolamine (DOPE), dioleoylphosphatidyl choline or N-succinyl-DOPE and a weakly acidic amphiphile, such as cholesteryl hemisuccinate (CHEMS) [102–104]. The negatively-charged group of the phospholipid undergoes destabilization in an acidic environment, leading to enhanced liposomal fusion with the cell membrane in tumors) or with the endosomal membrane (inside cancer cells) and ultimately release of its contents [99, 105]. Researchers have been trying to introduce novel lipids as a replacement for DOPE. Several approaches such as the combination of cationic/anionic lipids, a lipid diolein with CHEMS, and a formulation containing egg PC combined with Tween-80 have also reported [106, 107]. These new formulations have shown much better pH-responsive characteristics compared with DOPE liposomes.

Liposome recognition by opsonins (naturally occurring serum proteins designed to remove foreign bodies from the circulation) and subsequently their endocytosis and clearance by the reticulo-endothelial system (RES) are other hindrances to the implementation of liposomal drug delivery [108]. Moreover, the liposomal carrier and its contents can be totally degraded by various enzymes after its delivery to lysosomes after endocytosis occurs into target cells (Fig 7) [109]. Therefore, efficient strategies must be developed to design liposomes, which can release their contents into the cytosol (so the drugs can reach the nucleus which is often the final site of action) before they are degraded in lysosomes. One useful technique applied for reducing RES uptake and prolonging circulation time is the incorporation of PEG-lipid conjugates into the liposomal bilayer [110, 111]. However, the problem with this approach is that PEGylation may interfere with the membrane fusion of the liposome and may even cause membrane destabilization [112]. One suggested solution to this problem was to develop liposomes that were capable of losing their coating polymer before they reached their target sites. This approach resulted in the introduction of liposomes with a cleavable linker between their coating polymer chain and the hydrophobic part of the liposome bilayer. Through surface modification, the stability and cellular uptake efficiency of liposomes can be enhanced; for example via exploitation of novel materials such as maleimide [113, 114].

One of the new strategies of designing pH-sensitive liposomes is the modification of the liposome surface with pH-labile polymers [115–117]. Accordingly, Yuba *et al.* designed pH-labile polymers using dextran derivatives containing 3-methylglutaraldehyde residues

(MGLu-Dex). The surface modification of egg yolk phosphatidylcholine (EYPC) liposomes with MGLu-Dex was reported to show significant destabilization in weakly-acidic environments (Fig 7) [118]. In another study, Zong *et al.* reported a controlled release liposome platform using polydopamine (PDA)-coated liposomes as pH-sensitive carriers. They used a chemotherapeutic agent, 5-fluorouracil (5-FU) as the liposomal cargo. It was demonstrated that 5-FU-loaded pH-sensitive liposomes showed improved performance compared to free drugs at tumor pH (pH 6.87) [119].

Another important class of pH-sensitive liposomal delivery systems have been attached to target-specific ligands such as folate and transferring, or to cell-penetrating peptides such as TAT [101]. These ligands are capable of recognizing and binding to specific receptors over-expressed on the target cells. This method is known as “active targeting” in contrast with “passive targeting” in which liposomes accumulate at target sites merely according to their size (enhanced permeability and retention effect) [101, 120]. The combination of surface modification to target folate receptors (FR) (over-expressed on many different types of solid tumors) together with a pH-responsive liposome strategy improved the performance of FR-targeted anticancer drugs since FR-receptor mediated endocytosis leads to endosomal localization. Other important ligands that are used for surface modification of liposomes include monoclonal antibodies that are against antigens such as H-2K^k (expressed in different types of tumor cell), E-selectin (on activated vascular endothelial cells), p-glycoproteins (on endothelial cells), CD-19 (on B-lymphoma cells), CD (on T-leukaemia cells), and so forth. Immuno-liposomes are also well-investigated, which use antibodies as their surface modification on the pH-sensitive liposomes. Immunoglobulins (IG) of IgG class and their derivatives are extensively exploited as targeting moieties that can be incorporated to the surface of liposomes with no undesired effects on the liposomal integrity [121–126].

Although the main focus of most studies carried out on pH-responsive liposomes, has been the pH-controlled disturbance of the membrane structure, there are studies on different mechanisms based on internal acidification of liposomes [127, 128]. More recently there have been several attempts performed to develop pH-labile liposomes for addressing multidrug resistance of cancer cells. Liu *et al.* prepared novel pH-labile liposomes loaded with DOX using a NH₄HCO₃ GRADIENT method. The bicarbonate ion (encapsulated within the liposome structure) reacted in acidic environments to produce copious quantities of CO₂ gas which subsequently disturbed the liposome membrane and allowed drug release (Fig 8) [129, 130].

Generally, pH-sensitive liposomes have demonstrated a variety of applications including: (1) delivery systems for chemotherapy. (2) pH-sensitive immunoliposomes (3) tumor diagnostic tools and (4) biological macromolecules (vaccine/DNA/gene/oligonucleotide) delivery systems [105, 131–141]. Table 2 summarizes applications of anticancer drug delivered via pH-responsive liposomes.

5. Ultrasound-responsive

Ultrasound (US) can be simply described as acoustic waves with frequencies higher than 20 kHz, which is higher than the audible range of human hearing [157]. Common medical applications of US include imaging, tissue ablation, kidney stone disruption, physiotherapy, and so forth [158]. However, the original idea of using US as a method for drug delivery, dates back to the era when biochemists began using US to rupture biological cell membranes to remove the cell contents before purification [159]. One of the advantages of using US for drug delivery, is that it can be focused from outside to any organ almost regardless of depth, which makes it reasonably suitable for the targeted delivery of anticancer drugs/genes to tumors located in the pancreas, brain as well as any other organs such as the liver [160].

The role of US in drug delivery nanocarriers such as liposomes is to rupture the structure of the phospholipid bilayer. As the pulses of US waves propagate through tissue some physical phenomena take place: cavitation, hyperthermia and acoustic streaming [161]. It is currently understood that cavitation is the main factor that governs the response of liposomes and other lipid-based nanocarriers subjected to US [162]. Gas bubbles (which may be present already) or which can form as the pressure drops below the vapor pressure of the surrounding medium, will undergo rapid oscillations, i.e. expansion and contraction, as the pulses of US arrive. These oscillations in gas bubbles are called cavitation bubbles. Depending on the frequency and amplitude of US waves as well as the size and properties of the bubbles, there are two types of cavitation: internal and stable [158, 161] (Fig. 9). At lower US intensities, stable cavitation occurs, while at higher US intensities, internal cavitation takes place. Internal cavitation can eventually lead to bursting of the bubbles, and hence drug release occurs [163]. Studies on delivering doxorubicin (DOX) encapsulated in micelles suggested that low frequency US would cause more efficient drug release than high frequency US [164]. The mechanism of drug release is due to the internal cavitation of gas bubbles occurring near these micelles [161] (Fig. 10A). Cavitation in liposomes, which have an additional layer of phospholipids compared to micelles, either occurs near the liposomes (like micelles) or can occur inside their lipid bilayers [161] (Fig. 10B). Low frequency US waves also show better results in rupturing liposomes [165]. It has been also shown that using low frequency US on liposomes did not alter the chemical properties of the encapsulated drug or destroy its activity [166].

Microbubbles (MB), were originally designed as a contrast agent for US imaging. However, they are also able to function in drug delivery applications [167]. MBs consist of a gas-filled core that can contain air, nitrogen or perfluorocarbons (PFC) which is surrounded by a layer/ layers of lipids, proteins or polymers [167]. PFC compounds loaded into MBs have a pronounced ability to dissolve oxygen. Accordingly, Chang *et al.* investigated delivery of oxygen along with a fluorescent dye [1,10-dioctadecyl-3,3,30,30-tetramethylindocarbocyanine iodide] (DiI), a model drug, loaded into liposomal MBs. Using US and fluorescence imaging in *ex vivo* tissue models, it was found that multifunctional microbubbles could provide simultaneous controlled delivery of oxygen and therapeutic agents [168]. Generally speaking, MBs themselves have a limited drug loading capacity. This limitation requires using highly active drugs [169]. Although some drugs such as DOX can be loaded onto the coated lipid bilayer of MBs via electrostatic attraction and

hydrophobic forces (only for lipophilic drugs), liposome-encapsulated drugs that are conjugated or attached to external MBs can have a higher loading efficiency [170]. Yu *et al.* performed studies on combining liposomes with MB for delivery of DOX utilizing US. As demonstrated in Fig. 11, this method led to increased loading of the drugs, thus achieving improved results. Their results showed that it was possible for a MB to carry about 1600 liposomes. Furthermore, low pressure US pulses resulted in more efficient drug release [170].

Recent advancements in the field of US-responsive liposomal DDS have focused on designing so-called “sonosensitive” materials to be employed in liposomes to achieve better results. These efforts include a study conducted by Evjen *et al.* who found promising results using liposomes composed of 1,2 distearoyl-sn-glycero-3-phosphatidylethanolamine (DSPE), and liposomes comprised of dioleoylphosphatidylethanolamine (DOPE) [171, 172]. *In vitro* study of DSPE showed about 70% release of DOX within 6 minutes of US exposure, which was a 7-fold improvement compared to standard PEGylated liposomal DOX [172]. DOPE *in vitro* showed even better results: releasing 95% of DOX from liposomes in 6 minutes of 40 kHz US exposure; a 35% increase compared with using DSPE, and a 9-fold increase compared to standard PEGylated liposomes [171]. Other than using the aforementioned sonosensitive agents to modify phospholipid bilayer, it is also possible to use bile salts (BS) to make vesicles with higher sensitivity to an US stimulus. It has been reported that BSs destabilize the structure of the phospholipid bilayer [173]. Recently Mujoo *et al.* attempted to use BSs (e.g. cholate, chenodeoxycholate, ursodeoxycholate, glycocholate and taurocholate) to make improved US-sensitive phospholipid bilayers. They also demonstrated significant responses to low frequency US; whereas non-significant responses were obtained for high frequency US. They also found that liposomes made of taurocholate and DOPE showed less sensitivity to high frequency US than liposomes containing only DOPE. It was speculated that this phenomenon was because of a preferred shape arising from the combination of taurocholate and DOPE in the phospholipid bilayer [174].

Sonoporation is another effect that can be exerted by US action on nanocarriers, which is defined as the formation of temporary pores in the cellular membrane. The generation of these pores is an opportunity for the penetration of drugs into the cells; especially for delivery of drugs into the brain where the blood brain barrier (BBB) is the most important barrier against drug uptake. The underlying mechanism for such pore formation is mainly due to cavitation and also hyperthermia associated with MB US activation [163, 175]. For example, Lin *et al.* studied cationic liposomes (CLs) for delivery of DOX to brain tumors using focused US. The idea of using cationic liposomes originated from the general characteristic of tumor cells; i.e. their more negatively charged surfaces. Their investigation on glioma, a common brain tumor, showed promising results where tumors became smaller and animal survival was increased [176].

As mentioned previously, MBs generally can be used for delivery of highly active drugs. However, siRNA, miRNA and plasmid DNA also have the potential to produce a biological effect at comparatively low doses. This non-viral gene delivery can also be combined with US. Grayburn *et al.* applied this method with cationic liposomes for diabetes treatment using RIP 3.1, an insulin specific promoter, to trigger expression of gene NeuroD. Their

histological studies on diabetic rats showed the regeneration of pancreatic islets employing this technique [169].

In spite of many studies on US for drug delivery, it is still difficult to be quantitatively precise concerning US parameters. This is mostly because different US methods use different frequencies and materials. Moreover, the fact that cavitation is also accompanied by hyperthermia makes it difficult to study the effect of cavitation alone on lipid-based nanocarriers. One method to study cavitation effects more specifically is using different frequencies and investigating the effects of temperature and/or US pulses on drug release [161].

6. Magneto-responsive

In recent years, lipid-based NPs that also contain magnetic substances, so-called magnetic/magneto-liposomes (MLs) have been developed for diagnosis and therapy of miscellaneous diseases. As an example, metallic ions and magnetic NPs (MNPs) can be bound to, or encapsulated into, liposomes during the synthetic processes. Metallic ions such as Gd (III) have been used as a contrast agent (CA) for magnetic resonance imaging (MRI). In comparison with metallic ions, MNPs can not only be used as CAs, but also have other applications such as magnetic targeting, therapeutic hyperthermia, and magnetically triggered drug release [177]. Passive and active targeting of magnetic liposomes are potentially applicable for delivering other therapeutic agents at the same time. This ability can lead to the design of multimodal systems for theranostic purposes.

The combination of various methods such as hyperthermia and photodynamic therapy (PDT) is an interesting strategy to enhance therapeutic efficiency, and ultramagnetic liposomes can be employed for this purpose. In one study, the bilayers of liposomes that were highly loaded with MNPs in the hydrophilic core and also contained a photosensitizer (FOSCAN^{TM}) in the hydrophobic region were used for cancer therapy. Stimulation with an alternating magnetic field raised the temperature of the MNPs. Besides, magnetic NPs also played a role as MRI CA. Laser excitation activated the photosensitizer and surrounding oxygen was changed into a reactive form (singlet oxygen). Also, the fluorescence emission of the photosensitizer could be used for imaging. The coupling of magnetic hyperthermia and PDT potentiated the tumor destruction caused by the singlet oxygen. The mechanism of this process was triggering apoptotic pathways that led to more complete cancer cell and tumor regression *in vivo*. This approach could avoid surgical damage and preserve surrounding normal tissues [178].

Magnetic-fluid-loaded liposomes (MFLs) have been reported to address the problem of inefficient chemotherapy of malignant tumors in the brain and central nervous system. In one study, labeled PEGylated liposomes loaded with maghemite crystals could pass the blood brain barrier (BBB). To further investigate this approach, glioblastoma cells were implanted intracerebrally in nude mice. Then, MFLs were intravenously injected. Because of the vasculature permeability induced by a magnetic field gradient applied to the head of mice, the tumor was targeted both by EPR and magnetic targeting. Thus, the concentration

of MFLs was selectively increased in the malignant area. Also, a 7T small animal MRI system was used to monitor the process at the same time [179].

An anti-EGFR antibody conjugated to magnetic liposomes was used as an active targeting system, for co-delivery of drugs and genes. In this platform, DOX was loaded into mesoporous silica magnetic NPs coated by a liposomal bilayer. Mesoporous silica not only facilitated high drug loading (98% efficiency), but also enhanced the interaction of water molecules with the magnetic core, which enhanced the MRI contrast. Here, Plk1 siRNA was first treated with protamine to form a complex, afterwards the complex was incorporated into the liposomal system by ammonium bicarbonate gradient technique. pH-sensitive drug release was shown at acidic pH values. These multifunctional theranostic particles were tested as image-guided therapy on BxPC3 pancreatic cancer cells [180].

The increased temperature resulting from magnetic stimulated MLs can damage healthy surrounding tissue. To prevent this side effect, ~5 nm nitrodopa-palmityl stabilized magnetic NPs were embedded into liposome bilayers. Magnetic stimulation increased the temperature to the melting point of the liposome bilayers, which caused a change in the permeability. Controlled release and optimized drug loading were the most significant achievement [181]. Another research demonstrated that simultaneous encapsulation of SPIONs and methylene blue dye into lipid bilayer-coated mesoporous silica NPs (MSNs) could function as a remote smart triggered drug delivery system (DDS). MSNs provided higher encapsulation efficiency and allowed interaction of the SPIONs with lipid bilayers, and the effect of alternating magnetic force on the melting temperature of lipid bilayers made the gates stimulus-responsive [182]. Elsewhere, magnetite NPs were deposited on the surface of melamine formaldehyde particles that were coated by polyelectrolyte. The core was dissolved to form a microcapsule structure. Layer-by-layer assembly led to a covering of temperature-responsive lipid bilayers on the surface of the microcapsules. Under a magnetic stimulus, the microcapsule could trap and release the dye [183]. Recently, Salvatore *et al.* reported a new type of MLs that contained a fluorescent hydrophilic drug combined with a single stranded oligonucleotide (ON) conjugated to a cholesteryl unit of the liposome membrane. Two types of MNPs were employed in this platform. A hydrophobic MNP was located in the liposome bilayer, and an ON-decorated hydrophilic gold core-shell MNP that was connected to the single stranded ON on the surface. Controlled and sequential release could be obtained by the frequency changes in an alternating magnetic field (AMF). Five min of 3.22 kHz AMF caused the release of a model drug whereas fifteen min of 6.22 kHz AMF released the DNA because the temperature increased to the melting point of the DNA (Fig. 12) [184].

MRI is a non-invasive diagnostic tool, where the excitation of nuclear spin leads to an energy transition, and time for the relaxation of the nuclei can be detected by MRI [185]. In recent years, DDSs have been suggested to simultaneously serve as drug carriers and CAs for enhancing MRI images. This aim could be achieved by increasing longitudinal relaxivity. The study of abnormal muscle contraction or investigating brain physiology is facilitated by MRI. Fluctuations in cellular calcium (Ca^{2+}) can be used in an intelligent CA for MRI. This probe has features such as specific local tissue targeting, improvement in retention time, and modifying and amplifying the MRI signals. To this end, a formulation of a novel amphiphilic ligand in the liposome structures that contained a Gd(III) complex was reported

as a CA. Ca^{2+} binding to magnetic liposomes changed the coordination of the Gd-chelate. In fact, Ca^{2+} increased hydration and reduced local motion of the Gd(III) complex, and the increase in relaxivity could be detected even at low magnetic field values. Therefore, this type of smart CA triggered by molecules or ions involved in neuronal signaling can help neuroscience studies for better understanding of brain function [186]. Aptamer-conjugated thermosensitive liposomes encapsulating Gd-DTPA were employed as an MRI probe. These biocompatible MLs enhanced cellular uptake, due to active targeting whereby the Gd CA was released at 42 °C via mild hyperthermia. Such a CA delivery system could improve early cancer diagnosis [187]. Another study by Zhang *et al* showed that the encapsulation of Mn^{2+} in liposomal NPs not only could be monitored by MRI but also facilitated high encapsulation of As_2O_3 in the liposomes. Actually, the reaction of Mn^{2+} and As_2O_3 produced a complex structure (Mn-As complex). The complex formation and deformation affected the darkness and brightness of the MRI image contrast. Mn^{2+} alone was a dark T_2 -weighted CA whereas the Mn-As complex was a bright T_1 -weighted CA. This convertible MRI platform could provide drug release inside glioblastoma cells and furthermore, a high dosage of As overcame the temozolomide resistance of glioblastoma [188].

Carbon nanostructures are promising materials, and can be combined with MLs. In one study, buckyball (fullerene C_{60}) was utilized for surface decoration of magnetic NPs and also for the adsorption of hydrophobic drugs or fluorescent dyes by a π - π stacking mechanism. Folate-targeted thermosensitive liposomes encapsulated PEG-functionalized C_{60} - Fe_3O_4 nanocomposites and docetaxel (DTX) as a multi-functional system. Herein, the fullerene hybrid system was shown to increase the temperature after radio-frequency radiation. Fullerenes could be used for radiofrequency-triggered release of DTX, and with increased accumulation of the fullerenes and DTX in tumor tissues. Histological studies showed that the coupling of radiofrequency and magnetic field targeting of this multifunctional system caused cell necrosis, lysis, and fragmentation [189].

Recently, a new type “nanorobots” was introduced where the combination of liposomes, carbon nanohorns (CNH) and magnetic iron NPs were investigated as a nanotransporter. This structure allowed monitoring of cancerous cells even at the single cell level. The liposome structure encapsulated fluorescein di- β -D-galactopyranoside (FDG) as a quenched non-fluorescent agent that could be activated by β -galactosidase, that was conjugated with a “teddy bear”-shape, made of avidin-biotin-polyethylenimine (PEI)-MAG functionalized-CNHOx hybrid. These hybrids were investigated as a novel theranostic platform (Fig. 13-d). Confocal microscopy showed the magnetic field effect to improve transfection yield in HeLa cells *in vitro* (magnetotransfection). Cytotoxicity tests confirmed the biocompatibility of the nanohybrids in the presence of the magnetic field. Finally, the nanotransporter was applied for remote control of enzymatic reactions *in vivo* (Fig. 13). To illustrate this ability, FDG encapsulated nanotransporters injected into transgenic mice that expressed human β -galactosidase in the whole body. Simultaneous magnetic field and laser induction were used to trap the nanotransporters in the vessels of the mouse ear, and release the FDG. Due to β -Gal enzymatic reaction, the green fluorescence of fluorescein was produced by hydrolysis of FDG and could be directly observed *in vivo* [190].

7. Other stimulus-responsive smart liposomal carriers

7.1 Matrix metalloprotease 2 (MMP2)

Novel multifunctional DDSs for cancer targeting have been developed that are responsive to matrix metalloprotease 2 (MMP2) which is often upregulated in a tumor environment. In a recent study, a liposomal nanocarrier was designed comprised of polyethylene glycol (PEG) (providing elongated blood circulation and preventing non-specific interactions) on the liposomal surface, which was also conjugated to an antinucleosome monoclonal antibody (mAb2C5). Because of the EPR phenomenon, this liposomal nanocarrier accumulated in the location of the tumor (due to the nanocarrier size) and also exhibited active targeting by the mAb2C5. Therefore, in the microenvironment of the tumor, the MMP2-sensitive linker between PEG and lipid was cleaved because of up-regulated extracellular MMP2, thus the protective long chain PEG was removed. This led to the exposure of hidden surface-functionalized cell-penetrating peptides (TATp), which facilitated intracellular delivery of the drugs. Therefore, targeted and enhanced cell internalization of the nanocarriers was obtained [191].

In another study, copolymers based on the self-assembly of PEG-phosphoethanolamine (PEG-pp-PE) demonstrated MMP2-sensitive drug delivery to tumor cells, and the drug efflux mediated by P-glycoprotein (P-gp) was prevented. In this research, different homologues of PEG-pp-PE were studied. This investigation proved that the total structure of PEG-peptide-lipid was crucial for inhibition of P-gp, and the equilibrium between its hydrophilic and lipophilic parts was important. Here, PEG2k-pp-PE gave higher inhibition of P-gp. This copolymer exhibited a promising tumor-targeted drug delivery, which was connected with the enhancement in plasma membrane fluidity and the inhibition of the activity of P-gp ATPase [192].

7.2 Redox

A study by Noyhouzer *et al.* demonstrated controlled payload release of a ferrocene-modified phospholipid unilamellar liposome as a drug delivery vehicle by a redox active mechanism. This takes advantage of the fact that the redox potential is higher inside cancer cells due to a higher concentration of glutathione. Here, the exposure of the ferrocene groups on the surface of the vesicle were shown, thus providing for triggering the redox reaction. Therein, the results of flow cytometry evaluating the drug release in HeLa cells indicated a 200-times stronger signal for the cells treated with redox active vesicles. This implicated an efficient redox-sensitive delivery, and provided specificity to the cancer cells [193].

8. Dual-sensitive liposomes

Stimuli-responsive characteristics are among efficient approaches to improve the precision of drug delivery, thus to date, diverse stimuli-responsive systems have been designed [194–196]. Liposomes can discharge their cargos in response to temperature, pH, light, redox signals. Normally, the stimulus results in alterations in the structure of the lipid, as well as the destabilization and leakage of the liposome contents. In this section we will cover liposomes that respond to a combination of these different stimuli [197, 198]. The most

common format of dual-responsive liposomes is an “AND” logic gate where both stimuli must be present at the same time to get drug release.

8.1 pH/Temperature-responsive

Combined temperature-responsive and pH-sensitive liposomes have been investigated as they show controlled release when exposed to increased temperature and an acidic surrounding milieu [68, 135], thus the design of nanocarriers responsive to both temperature and pH changes provides new opportunities for controlled drug delivery [199, 200]. In one study, dual-stimuli responsive liposomes reacting to both pH and temperature were fabricated with biocompatible hyperbranched poly (glycidol) (HPG) containing pH-labile succinyl moieties and temperature-responsive oligo (ethylene glycol) (OEG) moieties. This nanoplatform switched from hydrophilic to hydrophobic in response to pH and temperature changes of the surrounding environment and the NPs were destabilized [199]. In another study, Chen *et al.* synthesized a liposomal gel formulation, sensitive to alterations in both temperature and pH. This platform was used for vaginal administration of arctigenin employing a cleavable methoxy polyethylene glycol 2000-hydrazoncholesteryl hemisuccinate (mPEG-Hz-CHEMS). Thus, high stability was found at neutral pH, but in acidic conditions (pH 5) the platform was destabilized via bond cleavage. The results showed that the vaginal gel loaded with arctigenin was an effective formulation for the treatment of vaginal candidiasis with less toxicity [200]. In another study, Kono *et al.* synthesized hyperbranched poly (glycidol) containing N-isopropylamide (NIPAM), as a thermo-responsive moiety, and succinylate groups as the pH-labile component. In response to a decrease in pH and increase in temperature, the polymer NP altered from hydrophilic to hydrophobic [201]. Such polymers have been also incorporated onto stable EYPC liposomes to generate dual sensitive liposomes and could deliver payloads into the cytosol of target cells under an acidic environment and local heating [202]. Furthermore, Wu *et al.* demonstrated that an MSN as the drugencapsulatingcore and a copolymer-lipid layer comprised of phospholipids and poly (N-isopropylacrylamide-methacrylic acid-octadecyl acrylate) as the dual-responsive shell could allow the delivery of a drug such as DOX. Here, changes in temperature and pH could each trigger the release of drug in response to more acidic and higher temperature of cancerous cells, respectively[203].

8.2 Temperature/Ultrasound responsive

Thermo-sensitive liposomes (TSLs) experience a phase alteration when they are heated and this phenomenon makes them permeable inducing released oftheir cargo [204]. Conventional TSLs are activated between a temperature range of 42°C to 45°C, and release their cargo over 30 min, while low temperature sensitive liposomes (LTSLs) discharge their drugs at a temperature range of 39°C to 40°C in a few seconds [205].

High intensity focused ultrasound (HIFU) is able to ablate tumors by employing rather long and continuous exposures to produce high temperatures for tumor thermal ablation [206]. Dromi *et al.* have combined pulsed- HIFU with LTSLs to elevate local delivery of DOX into tumors. The results showed the improvement of the antitumor effects of the drugs [207]. Elsewhere, Chen *et al.* designed a curcumin-encapsulated liposome microbubble gel comprised of N-cholesteryl hemisuccinate-O-sulfate chitosan (NCHOSC). This liposomal

microbubble had a high loading efficiency for curcumin. The temperature-responsive CS/GP gel containing liposomal microbubbles assumed a gel format body temperature where the initial release of curcumin induced by ultrasound was found to be 85%. In Fig. 14a schematic image of the process and the release diagram is shown. The results implied the fact that the double responsive curcumin-loaded liposomal microbubble gel served as a potential ultrasound and temperature dual-responsive drug delivery system [208]. Oerlemans *et al.* developed fluorescent liposomes (FCLs) that mediated ablation with the aid of MR-HIFU for the treatment of non-palpable breast lesions. FCLs could liberate their fluorescent contents after 30 seconds exposure to MR-HIFU. After exposure and release of the contents, the treated lesions could be imaged by fluorescence excited by UV light. PEG and cholesterol were included in the liposome to provide more stability and elongated circulation time. Through introducing thermo-sensitive phospholipids like 1,2-dipalmitoyl-sn-glycero-3-phosphocholine into the liposomes, MR-HIFU-mediated fluorescein release from the liposome could be accomplished [209].

8.3 pH/Redox-responsive

Combination of pH and redox is another category of dual-response liposomes. It is known that the pH of the tumor microenvironment is between 6 and 7 which is lower than physiological pH. It also becomes more acidic (e.g. pH between 5 and 6) inside intracellular endocytic vesicles [210]. Additionally, glutathione (GSH) has a significantly higher concentration inside cells, making it a promising option for smart intracellular drug delivery. Recently, Zhang *et al.* developed a cleavable polymerized liposome (CPLs) for delivery of pharmaceutical agents. A gallate-derived group with three propargyl moieties was joined to palmitoyl oleoyl phosphoethanolamine (POPE). To create large unilamellar vesicles, the mentioned anionic lipid was formulated with other lipids including palmitoyl oleoyl phosphatidyl choline. Employing the Cu(I)-catalyzed click reaction between the propargyl moieties and the azides in the cross-linker resulted in the polymerization of the unilamellar vesicles. The polymerized liposomes equipped with a disulfide or ketal group in the cross-linker could be depolymerized and discharge their payload through the addition of a reducing thiol or under acidic conditions, respectively [211]. Elsewhere, a PEGylated nano-sized polymeric lipid vesicle (PPLV) loaded with DOX exhibited dual pH and reduction responsiveness. The system enabled efficient antitumor drug delivery via triggered drug release, as well enhancing tumor cellular internalization. The PPLVs were reported to have good uptake by tumor cells under acidic conditions. On the other hand, an accelerated DOX release at pH 5 as well as with 10mM of GSH proved its promising potential [212]. In a similar study, another dual-responsive DDS containing folate-PEG-coated polymeric lipid vesicles (FPPLVs) was constructed showing an enhanced DOX release profile under acidic conditions in comparison to neutral pH values due to the loss of PEG in acidic conditions, which in turn made it easy for tumor cells to take up the NPs. Fluorescence microscopy of HeLa cells also showed that disrupting the FPPLV structure in response to 2–10 mM intracellular GSH levels triggered drug release in tumor cells [213].

Xu *et al.* designed acid/redox dual responsive liposomes, comprised of soy phosphatidylcholine (SPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine [DOPE], and a synthetic functional lipid, 2-[2-(2-carboxylcyclohexylformamido)-3,12-dioxy-1-(1H-

imidazolyl-4)-7,8-dithio-4,11-diazapentadecylamide]-glutaric acid ditetradecanoldiester (HH-SS-E2C14). HH-SS-E2C14 contained histidine, an amino acid moiety, and acid-cleavable hexahydrobenzoic (HHB) amide, as a hydrophilic block, and two tetradecyl alkane chains as a hydrophobic block. A disulfide bond was integrated into the structure as a redox-labile linkage between the two blocks. When this formulation was administered intravenously, it had a negative charge but after the EPR enabled accumulation in tumors, the surface charge became positive due to interaction with cell membranes, which were negatively charged. Subsequently, after endocytosis, the imidazole group was protonated and promoted the proton influx into the endocytic vesicle followed by an increase in osmotic pressure and rupture of the endo-lysosomes, allowing endo-lysosomal escape of the cargo [214].

8.4 Other dual responsive liposomes

8.4.1 pH/enzyme—Zhang *et al.* created a dual stimuli-responsive liposome, responsive to pH and esterase for the delivery of camptothecin and siRNA for treatment of cancer. This dual CPT-PCB (poly carboxy betaine)/siPIK1 lipoplex preparation showed a high capacity for loading of CPT and could boost the serum stability of the liposomes and provide controlled release of CPT and siRNA, due to the protonation of PCB in endosome/lysosomes, and esterase and pH sensitivity of the CPT-PCB prodrug. This system was reported to be a promising delivery platform for cancer therapy and co-delivery of CPT and siPIK1 [215].

8.4.2 Dual redox—Moreover, a co-assembly system comprised of block copolymers containing diselenide and polymeric lipid could show dual redox responsiveness, because it could be disrupted when exposed to either low concentration of hydrogen peroxide (as an oxidant) or to glutathione (as a reductant). In the presence of 0.05 mM GSH or 0.1% H₂O₂, most of the co-assembly system was disrupted. Also, the results illustrated that hydrophobic interactions provided the driving force of the co-assembly [216].

8.4.3 Redox/ultrasound—In 2013, Nahire *et al.* showed that polymer-coated echogenic lipid NPs could release their content via redox triggering and in response to diagnostic frequency ultrasound. As a result, a 3 MHz ultrasound combined with a reducing agent increased the drug release up to 96%. Such lipid NPs were stable in oxidizing environments, and released their content in the reducing environment of the cytosol. The rate of drug release was boosted by simultaneous application of redox trigger and ultrasound [217].

9. Conclusion

Liposomes have long been one of the most studied types of drug-delivery nanosystems. In addition to the other advantages described in some detail above, another overriding reason lies in their remarkably low toxicity. Widespread concern about possible hazardous effects of diverse nanostructures such as carbon nanotubes, fullerenes, noble metal nanostructures, various inorganic nanoparticles, quantum dots, and so forth has led to the establishment of a brand new area of toxicology called “nanotoxicology”. In contrast to many of these nanostructures, liposomes are principally composed of naturally occurring lipids that are

naturally found as constituents of cell membranes that compose all the cells of our bodies. The body is well used to dealing with the basic components of liposomes, so they can be regarded as the ultimate in biocompatibility and biodegradability. This is probably why there are more liposomal preparations that have gained regulatory approval for medical applications, than any other class of nanoparticles. Now that liposomes have almost progressed to being the “standard of care”, the road is open for researchers to improve upon them by active targeting strategies, and by engineering various stimulus-responsive and smart characteristics.

Smart modification of liposome is an opportunity to impart a stimulus-responsive moiety to the structure. Herein, encapsulated cargos can be released through activating the stimuli-responsive part followed by alterations in the structure of liposome, e.g. conformations, or bond cleavage and the structural destabilization of the carrier, thus leading to the leakage of the cargos. These alterations can be triggered on-demand and with highly precise control via internal stimuli (pH, reducing agents, specific enzymes) or external stimuli (light, magnetic field or ultrasound). In this regard, smart stimuli-responsive liposome vehicles are opening new horizons for drug/gene delivery applications and innovative therapies for various diseases and disorders, especially in clinical evaluations and exploitations.

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Biographies

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Mahdi Karimi received his BSc degree in Medical Laboratory Science from Iran University of Medical Science (IUMS), in 2005. In 2008, he achieved the MSc degree in Medical Biotechnology from Tabriz University of Medical Science and joined Tarbiat Modares University as a PhD student in nanobiotechnology field and completed his research in 2013. During his research, in 2012, he affiliated with the laboratory of Professor Michael Hamblin in the Wellman Center for Photomedicine at Massachusetts General Hospital and Harvard Medical School as a visiting research, where he contributed to the design and construction of new smart nanoparticles for drug/gene delivery. On finishing the study, he joined, as Assistant Professor, the Department of Medical Nanotechnology at IUMS. His current research interests include smart nanoparticles' design in drug/gene delivery and microfluidic systems. He has established a scientific collaboration between his lab and Professor Michael Hamblin's lab to design new classes of smart nanovehicles in drug/gene delivery systems. Dr. Karimi was recipient of several Grants from Iran Science Elites Federation (ISEF), and Iranian Nanotechnology Initiative Council. He was also Ranked 34th among all faculty members in Iran, by Iran Science Elites Federation (ISEF) in Iran and selected as the best young researcher of Iran University of Medical Sciences in 2016.

Parham Sahandi Zangabad received his M.Sc. in Nanomaterials and Nanotechnology from Sharif University of Technology (SUT), Tehran, Iran. As well, since 2012 until Jan 2015 he was a research assistant at the research center for Nanostructured and advanced materials (RCNAM) at SUT working on nanocomposites and their mechanical properties. Since late 2014, he joined Professor Karimi's Advanced Nanobiotechnology & Nanomedicine Research Group (ANNRG) in Iran University of Medical Science, Tehran, Iran, having collaboration with Prof. M.R. Hamblin from Harvard Medical School, Boston, MA, US. To research in nanomedicine, smart drug delivery systems and biosensors was the aim of this collaboration, resulted in several high impact review article, and books. Afterwards, since February 2016 he became a research assistant in Research Center for Pharmaceutical Nanotechnology (RCPN), Tabriz University of Medical Sciences, Tabriz, Iran, working on nanocarriers for anticancer drug delivery systems. This led to beneficial experiences in design and applying smart nanocarriers in biomedical applications. He was then research assistant in Cellular and Molecular Research Center in Iran University of Medical Sciences, since 2017, working on drug/gene delivery systems.

Shayan Shahsavari is Member of Nanoclub Elites Association (NEA) and CEO at Mataab Company, at Tehran, Iran. He has joined as a member of NEA at Iran Nanotechnology Initiative Council, as an expert nanobiotechnologist. In 2014, he established Mataab Co to manufacture and commercialize high-tech products at biotechnology incubator, Pasteur Institute of Iran. His fields of research are theranostics and tissue engineering. Recently, he has joined ANNRG, Iran University of Medical Sciences to collaborate with prof. Mahdi Karimi in the related fields of nanomedicine including nanobio-sensors and drug delivery systems.

Figure 1. Schematic of Temperature-dependent behavior of liposome conjugating PEI-CA, (A) above UCST, and (B) below UCST.

Figure 2.

a) Schematic of the formation of drug loaded nanohybrid liposomal cerasome and its drug release on exposing the system to HIFU [66]. The release behavior of one of the synthesized HTSCs formulations (HTSCs-3) compared to the component lipids of conventional low temperature-sensitive liposomes (LTSLs) is shown in Fig. 2c. It was concluded that below 41°C, LTSLs released calcein (used as a model drug) more rapidly than HTSCs; however, above 41°C, LTSLs and HTSCs-3 exhibited similar release profiles. Accordingly, HTSCs-3 showed a more narrow temperature responsive range, which was better for drug release in the hyperthermia range. This formulation overcame one of disadvantages of temperature-sensitive liposomes, *viz* variable drug delivery efficiency due to heterogeneous heat distribution in tumor tissue after mild hyperthermia (that occurs due to variations in tumor vascularity) b) Release behavior of one of the HTSCs' formulations compared to LTSLs. Reprinted with permission from ref. [66], copyright 2015, American Chemical Society.

Figure 3.
Schematic of light activated-polymerization of liposomes, leading to the release of cargos.

Figure 4.

a) Schematic of a liposomal membrane equipped with azobenzene moieties (left), and the polarity alterations during the reversible cis-trans isomerization of azobenzene (Right) [86] (Open Access), b) Schematic of photo-isomerization mechanism occurred in a liposomal carriers, c) drug release curves under 980 nm irradiation (Release obtained in buffer and under the irradiation duration time of 30 minutes). Reprinted with permission from ref. [87], copyright 2016, John Wiley & Sons, Inc.

Figure 5. Schematic of gold nanoparticles being excited by NIR radiation leading to drug release from thermosensitive liposomes. Reprinted with permission from ref. [94], copyright 2015, Elsevier.

Figure 6.

Curves indicating Calcein release profiles (DLD: 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine, 1-stearoyl-2-hydroxy-sn-glycero-3-phosphocholine, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)] and DD: 1,2-distearoyl-sn-glycero-3-phosphocholine, 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine). Reprinted with permission from ref. [98], copyright 2015, Elsevier.

Figure 7.

Design of MGluc-Dex-modified liposomes as a vaccine for induction of antigen-specific immunity. MGluc-Dex-modified liposomes are taken up by dendritic cells (DC) via endocytosis and trapped in endosome. The weakly acidic DC cytosol induce the formation antigen-specific cytotoxic T lymphocytes (CTL) via presentation by MHC class I, resulting in induction of cellular immunity (Th1 response). Antigen molecules in the endosomes undergo presentation by MHC class II and induce antigen-specific Th2 cells that lead to induction of humoral immunity. Reprinted with permission from ref. [118], copyright 2014, Elsevier.

Figure 8.

Gas generating based pH-labile liposome. A) A novel DOX-encapsulated liposome with NH_4HCO_3 will produce CO_2 in acidic environment leading to drug release. B) Quantitative DOX release from pH-sensitive liposome (Lipo-c) and normal liposome (Lipo-s). C) The resistant MCF-7 cells viability after treatment with DOX-encapsulated liposomes. Reprinted with permission from ref. [129], copyright 2012, Royal Society of Chemistry.

Figure 9. Stable and internal cavitation. (A) Stable cavitation: Bubble oscillations due to low power US pulses which are non-resonant. (B) Internal cavitation: high power pulses will lead to amplified resonance and finally collapse of the bubble.

Figure 10.
Proposed mechanism for (A) cavitation effect on micelle rupture (B) liposome collapse

Figure 11.
Potential ability of microbubbles to carry a large number of liposomes and hence increasing the drug loading capacity. (See text)

Figure 12. Schematic illustration of sequential controlled release of dual MNP-equipped liposomes. Reprinted with permission from ref. [184], copyright 2016, American Chemical Society.

Figure 13.

In vivo remote control of enzymatic reactions using nanotransporters. a) Schematic image of the experiment 1): injection of nanotransporters, 2): Nanotransporters accumulated by a magnet, 3): Manipulation and observation of physicochemical aspects of nanotransporters with NIR radiation. b) Accumulation of nanotransporters in the vessels by magnetic field. c) *In vivo* β -Gal reaction observed in the blood vessel of a living mouse. d) Nanotransporter structure Reprinted with permission from ref. [190], copyright 2016, John Wiley & Sons, Inc.

Figure 14.

a) Schematic illustration of Cur-LM-G and the external morphology of the blank liposome microbubble gel is shown at the middle: A) under the temperature of gelation, B) gelation process, C) the formed Cur-LM-G under the temperature of gelation, and D) gelation, b) the percentage of curcumin which is released from Cur-LM-G in the presence and without the presence of ultrasound is shown. Reprinted with permission from ref. [208], copyright 2013, Elsevier.

Table 1

FDA approved liposomal drug delivery systems used in clinical application.

Product	Released Drug	Treatment	Ref
Doxil	Doxorubicin (DOX)	Kaposi's sarcoma Breast Cancer	[18], [19]
Myocet	DOX	Metastatic Breast Cancer	[20], [21]
Lipodox	DOX	Breast Cancer, Kaposi's sarcoma Ovarian cancer	[22] [23]
Daunoxome	Daunorubicin	Kaposi's sarcoma	[24, 25]
Marqibo	Vincristine	Acute lymphoblastic leukemia	[26] [27, 28]
Amphotec	Amphotericin B	Invasive aspergillosis Serious fungal infections	[29, 30]
Depocyt	Cytarabine	Neoplastic meningitis and lymphomatous meningitis	[31, 32]
Estrasorb	Estrogen	Menopausal therapy	[33]
DepoDur	Morphine sulfate	Pain	[34–36]
Visudyne	Verteporfin	PDT for age-related molecular degeneration	[37, 38]
Epaxal	Inactivated hepatitis A viral strain RG-SB	Vaccine for hepatitis A	[39, 40]

Table 2

Several examples of pH-labile liposomes reported in literature for anti-cancer therapy.

Liposome formulation	Preparation method	pH sensitive moiety	Encapsulated cargo	Target site/cell line	Encapsulation/release efficiency(%)	Size(nm)	Bioassay	Reference
DOPE/HSPC/CH EMS/CHOL/mPE ⁹ 2000 – DSPE(4:2:2:0.3)			DOX	B lymphoma cells	95/-	120	<i>In vitro</i>	(142)
PEG-HZ-PE		TAT peptide	TAT peptides	H9C2 rat embryonic cardiomyocytes, LLC tumors			<i>In vitro</i> and <i>In vivo</i>	(143)
DOPE/CHEMS/D SPE- PEG		unsaturated phosphatidyl ethanolamine	Cisplatin	A549 (lung cancer) A431 (cervix cancer) GLC4 (lung cancer) Ehrlich solid tumor	26.3/- 23	110 121,130	<i>In vitro</i> and <i>In vivo</i>	(144–146)
PEG/PE, TATp		cell- penetrating TAT peptide	DOX	B16-F10,HeLa and MCF-7 cells	-	120	<i>In vitro</i>	(147)
Fplate-Poly(2- ethyl-2- oxazoline)-DSPE		FPEOz- DSPE	DOX	Human ovarian cancer cell SKOV3	91/45	100–150	<i>In vitro</i>	(148)
DOPE/DSPG/DS PE- PEG(7:3:0.53)			Methotrexate	Rat femoral artery	2.51/37	143	<i>In vitro</i> and <i>In vivo</i>	(149)
PC/DDAB/CHE MS/Tween-80/ folate–PE–PEG (25:25;49:1:0.1)	A procedure based on polycarbona te membrane extrusion	OALc- containing liposomes targeted to the FR	Cytosine- <i>b</i> -D- arabinofuranos ide	KB human oral cancer cells	15/33.83(dependent on Tween-80 concentration)		<i>In vitro</i>	(150)
HSPC/CHEM/PE G/diethylenetriam inepentaacetic acid-modified phosphatidylethan olamine			Calcein		-/>20	88–102	<i>In vitro</i>	(151)
PE(Oz)-CHEMS	lipid film hydration method		DOX	A375 cells	96/90	100–120	<i>In vitro</i> and <i>In vivo</i>	(152)
PE:Chol:OA (3:2:3)	thin-film dispersion method		Docetaxel		-86.9	277	<i>In vivo</i>	(153)
DOPE:CHEMS:P DP–PEG–DOPE (6:4:0.1)	reverse- phase evaporation vesicle method	EGFR dioleyl/phosp hatidylethano lamine (DOPE)	Gemcitabine	A549 (lung cancer)	67/-	146	<i>In vivo</i>	(154)
HHG2C18-L and PEGHG2C18-L	film dispersion method	zwitterionic oligopeptide lipids hexahydrobe nzoic amide	temsirolimus(CCI-779)	A498 (renal carcinoma) Renca (murine renal carcinoma)	93.76<1 (for <i>in vitro</i>)	104.5	<i>In vitro</i> and <i>In vivo</i>	(155)
S100PC:Chol:mP EG–Hz–Chol (90:10:3)		Hydrazone	Paclitaxel	MCF7 (breast cancer)	92.2/65.8	132.6	<i>In vitro</i>	(156)

DOPE: dioleoylphosphatidylethanolamine ; CHEMS: Cholesteryl hemisuccinate ; Chol: Cholesterol ; DSPE: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine; HSPC: Hydrogenated soy phosphatidylcholine ; PC: phosphatidylcholines ; PE: Phosphatidylethanolamine ; DSPG: dipalmitoylsuccinylglycerol ; PEG: poly ethylene glycole;DDAB: dimethyldioctadecylammonium bromide ;PEG-HZ-PE: PEG and PE being conjugated with the lowered pH-degradable hydrazone bond; HHG2C18: 1,5-dioctadecyl-lglutamy1 2-histidylhexahydrobenzoic acid; HS: hydrogenated soy; PE(Oz: poly(2-ethyl-2-oxazoline));