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Review

Current developments in drug delivery with thermosensitive liposomes



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ABSTRACT

Thermosensitive liposomes (TSLs) have been an important research area in the field of tumor targeted chemotherapy. Since the first TSLs appeared that using 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) as the primary liposomal lipid, many studies have been done using this type of liposome from basic and practical aspects. While TSLs composed of DPPC enhance the cargo release near the phase transition temperature, it has been shown that many factors affect their temperature sensitivity. Thus numerous attempts have been undertaken to develop new TSLs for improving their thermal response performance. The main objective of this review is to introduce the development and recent update of innovative TSLs formulations, including combination of radiofrequency ablation (RFA), high-intensity focused ultrasound (HIFU), magnetic resonance imaging (MRI) and alternating magnetic field (AMF). In addition, various factors affecting the design of TSLs, such as lipid composition, surfactant, size and serum components are also discussed.

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Abbreviations: (DPPC), 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine; (DSPE-mPEG2000), 1,2-distearoyl-sn-glycero-3-phosphatylethanol-amine-N-[methoxy(polyethyleneglycol)-2000]; (DSPC), 1,2-distearoyl-sn-glycero-3-phosphocholine; (MSPC), 1-stearoyl-2-hydroxy-sn-glycero-3-phosphatidylcholine; (P188), HO-(C₂H₄O)_a-(C₃H₆O)_b-(C₂H₄O)_c-H, a=80, b=27, c=80; (P188), 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphatidylcholine; (MPPC), 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphatidylcholine; (DPPGOG), 1,2-dipalmitoyl-sn-glycero-3-phosphoglyceroglycerol; (P-lyso-PC), lysophosphatidylcholine; (LTSLs), lyso-lipid temperature sensitive liposomes; sTSLs, slow release TSLs; fTSLs, fast release TSLs.

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1. Introduction

Nanotechnology has become a promising tool that can improve the pharmacokinetic behavior of drugs and enable them to have passive or active targeting to some extent [1]. Liposomes currently represent one of the best studied nanoparticle-based drug delivery systems for treating cancer and other diseases [2,3]. Liposomes have several advantages over free drug administrations, including treatment monitoring, diagnosis and drug delivery [4]. In addition, the enhanced permeability and retention (EPR) effect can also increase the accumulation of these drug-loaded liposomes at the tumor site, thereby improving their antitumor activity. Due to these advantages, the toxicity of various chemotherapeutic drugs to normal tissue has been significantly reduced by liposomal encapsulation, and several liposomes have been approved by FDA for cancer treatment [5]. However, the enhanced protective effect of liposomal encapsulation limits the timely release and uptake of the drugs at the tumor site [6].

Various efforts have been made to overcome the limitations. The drug delivery systems that respond to external stimuli such as temperature [7], pH [8], electromagnetic fields [9] and light [10] have received increasing attentions due to their ability to deliver and release payloads in an environmentally responsive manner (Fig. 1). Among these possible considerations to achieve sufficient tumor uptake of payloads, thermosensitive liposomes (TSLs) provide us a promising alternative. The first TSLs formulation reported by Yatvin et al in 1978 [11], was consisted of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) in the molar ratio of 3:1. In the presence of serum, the drug release from TSLs was 100-fold higher at 44 °C than at 37 °C. There are two major advantages of TSLs combined with hyperthermia. First, they can increase the vascular permeability and interstitial transport of the drugs within the sites of disease, while minimizing drug delivery to critical normal tissues [12,13]. On the other hand, hyperther-

mia can enhance the permeability of lipid bilayer and thus promotes the release of payload [14,15].

Due to the excellent biodegradability, drug encapsulation capacity and thermosensitivity of DPPC, the original DPPC based liposomes have been intensively studied for the drug delivery system with the characteristics of stimulus response over the past few decades. Fig. 2 depicts the enhanced tumor uptake of TSLs by mild local hyperthermia. Recently, new TSLs developed by Needham et al. [16] have been approved for human clinical trials, which can decrease the melting transition temperature (T_m) of liposome to 40–43 °C by adding a lysolipid into the DSPC/DPPC lipid bilayer. Therefore, thermal stimulus release of payloads can be achieved at temperatures as low as 40 °C. Consequently, high drug levels in tumor tissue can be achieved while reducing drug exposure to healthy tissue [17,18]. It is important that the encapsulated drugs can be rapidly released at the T_m of the lipid bilayer, which provides a means of improving the site-specific delivery when hyperthermia is applied to the tumor area [19]. At the T_m , structural changes in the lipid membrane occur as transforming from gel to the liquid-crystalline phase [20], suggesting that these liposomes undergo major morphology changes during the melting transition, including the formation of open liposomes, bilayer discs and pore-like defects [21]. It is important to ensure that the TSLs release the payload rapidly at the trigger temperature when they reach the tumor site, and that they have high stability at body temperature and storage [22]. Therefore, this review will discuss the key factors affecting the prescription design and drug release behavior of TSLs, as well as the latest heating modalities for triggered release.

2. Drug loading methods

The remote drug loading method is one of the best approaches to achieve the high encapsulation efficiency of the drug into liposomes. Various drugs have been loaded into TSLs successfully by this strategy such as anthracyclines [23], irinote-

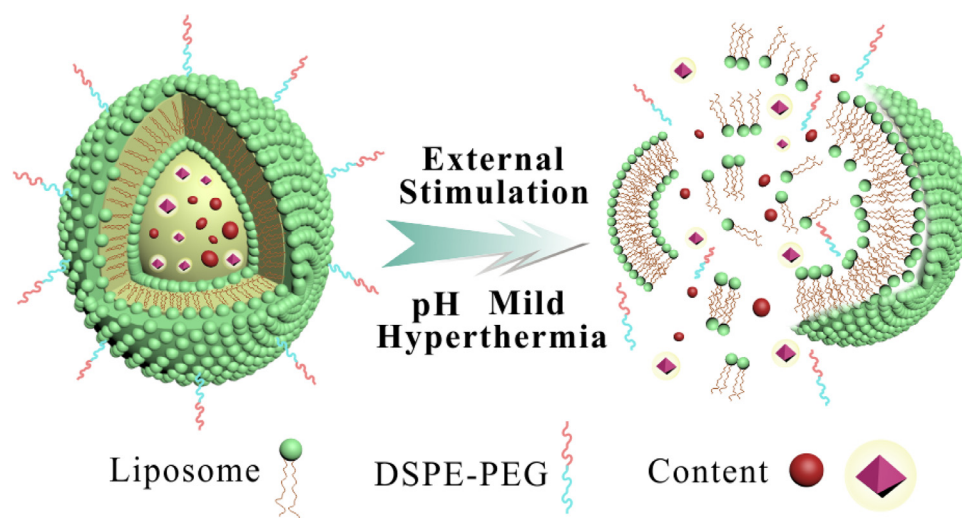


Fig. 1 – Liposomes were prepared by multifunctional biomaterials, responding to external trigger around the stimulated zone, rapidly releasing its contents.

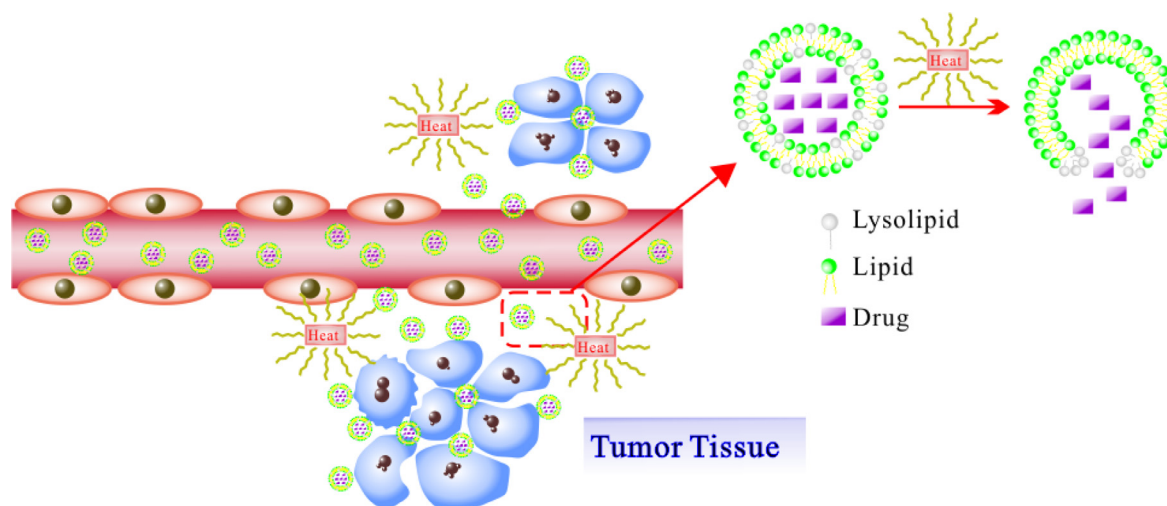


Fig. 2 – Thermosensitive liposomes passed through the tumor via the enhanced permeability and retention where the external heating locates, promoting the content drug release.

can [24,25], vinblastine and vinorelbine. The driving force of the encapsulation process includes transmembrane gradient of pH [26], ammonium [27–29], multivalent cations [28] or molecules [23], which drive the diffusion of the drug into the core of the liposomes and trap it there as a protonated form or stable complex [30].

The pH gradient method [31] is one of the most common approaches for preparing liposomal amphipathic weak acids or bases with high efficiency [32,33]. In the presence of a transmembrane pH gradient, the interior of the liposomes is acidic and the external pH is adjusted to physiological conditions. Uncharged weakly basic drug such as doxorubicin (DOX) incubated with these liposomes diffuse into the vesicles and becomes protonated within the vesicle. The positively charged DOX can no longer pass through the bilayer and is trapped in the liposome. The standard pH gradient-based loading method relies on incubation temperature which is above the phase transition temperature (T_m) of the bulk phospholipid to promote drug loading, therefore it will not be of particular interest for thermosensitive liposomal formulations. In the absence of cholesterol, the permeability of liposomes increases at incubation temperatures above T_m , which in turn can lead to collapse of the gradient and drug loading failure. But the lack of cholesterol is more beneficial to the sensitive of temperature. The addition of short-chain alcohols, such as ethanol, was utilized at suitable concentration to increase drug loading rates without affecting the *in vivo* behavior of the resulting liposomes, especially for TSLs formulations [34]. However, the acidic environment inside these liposomes can promote hydrolysis of the lipids during long-term storage, thus resulting stability problems [35]. For highly membrane permeability drugs, such as anthracyclines and irinotecan, it is certainly helpful to employ a drug loading method which promotes the formation of a multivalent complex of the drug inside the core of the liposomes [36]. The central idea behind this technique is to incorporate multivalent molecules or ions inside the inner phase of the liposomes, which facilitates

coordination with multiple drug molecules. The formation of stable complex in the liposomal core can minimize the drug leakage and toxicity. This method involves the use of gradient produced by Cu^{2+} [37,38], polyphosphate, polysulfate and EDTA [23,39].

By employing a multi-compartment model, sensitivity analysis has revealed that TSLs properties, especially the release rate during hyperthermia (HT), had the greatest influence on peak intracellular drug concentration [40]. Therefore, it is important to design the TSLs to achieve the desired release rate. Drug release from TSLs has a saturated effect on peak intracellular drug concentration, and no further gain could be achieved for release rates greater than 0.1018 s^{-1} . A similar effect has also been found for heating duration. Therefore, the results obtained can be applied to guide the design and optimization of TSLs parameters as well as treatment regimens.

3. The materials of thermosensitive liposomes

Needham et al. [16] first developed a TSLs formulation with a T_m just above physiological temperature, leading to a significant drug release triggered by phase transitions. The original TSLs consisted of DPPC ($T_m = 41.4^\circ\text{C}$) alone or with DSPC with a T_m range of $42.5\text{--}44.5^\circ\text{C}$. The chemotherapeutic drugs encapsulated in the TSLs can be released at elevated temperatures. Therefore, TSLs mainly depends on the thermo sensitive materials (Table 1).

3.1. Lysolipids


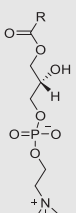
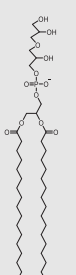
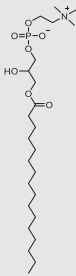

Lysolipids are the main molecular components used in the development of TSLs. Adding small amounts of lyso-lipids (such as 10% 1-myristoyl-2-stearoyl-sn-glycero-3-phosphocholine (MSCP)) with DPPC liposomes, it is postulated that lyso-lipids

Table 1 – The effect of the lipid composition on the physicochemical properties of a liposomal carrier containing drugs and in vitro antitumor activity on a tumor model.

Material	Structure	Formulation molar ratio	Phase transition temperature (T _m)	Tumor	Result	Reference
DPPC		LTSLs DPPC/MSPC/DSPE- mPEG2000 90:10:4	~42 °C		Statistical analysis imply that, poor retention of lysolipids in the LTSLs membrane could also affect drug release characteristics of LTSLs <i>in vivo</i> .	[40] [46]
		DPPC/P188 3:0.4	~42 °C	A549 cells	DOX released from DPPC/P188 liposomes with an encapsulation efficacy above 90%, at 42 °C exhibited lower cytotoxicity compared with free DOX solution,	[27]
DSPE- mPEG2000		s TSLs DPPC/DSPC/DSPE- mPEG2000 55:40:5	~45 °C	nu/nu mice with Human BLM melanoma cells 0.3 mg/kg DOX, in a water bath	Dox-sTSLs showed a much slower release rate at 42 °C than Dox-fTSLs reaching 72% release within 1 h, but significantly improved Dox retention in serum at 37 °C	[34] [20] [35]
		fTSLs DPPC/DSPC/DSPE- mPEG2000 80:15:5	~42 °C			
		DPPC/DSPC/DSPE- mPEG2000 80:15:5	~42 °C	A dorsal skin flap window chamber models implanted with human BLM melanoma	TSLs with 5 mol% DSPE-mPEG2000 were stable at 37 °C, while released 60% CF in 1 min and almost 100% CF in 1 h at 42 °C.	[50] [54]
DSPC		DPPC/DSPC/DSPE-PEG 6:3.5:0.5 using the approach of ammonium EDTA remote drug loading	~42 °C	In human BLM melanoma-bearing NMRI nu/nu mice 1.5 mg/kg idarubicin with local mild HT	Approximately 100% of encapsulation efficiency is obtained.	[25] [50] [55] [108]
MSPC		LTSLs DPPC/MSPC/DSPE- mPEG2000 86:10:4	~43 °C	A mouse Model iv 3 mg DOX/kg In water bath maintained at 43 °C; Cell based assays	HaT approximately increased 1.4-fold drug delivery to the locally heated tumor (~43 °C) than LTSLs-DOX, enhanced the drug release rates at 40–41 °C compared to LTSLs	[18] [22] [26] [53]
		HaT formulation DPPC/Brij78 96:4				

(continued on next page)

Table 1 (continued)

Material	Structure	Formulation molar ratio	Phase transition temperature (T _m)	Tumor	Result	Reference
HSPC		DPPC/HSPC 4:1	~44 °C	Statistical analysis	Complete release at 42 °C the significant increase of calcein release at T _m and decrease at 37 °C were observed	[16] [44] [48]
MPPC		Add 10% DSPE-mPEG2000 DPPC/MPPC 1:10	~42 °C	Coarse-grained molecular dynamics (CGMD) model	Arsenic released 55% from DPPC/MPPC liposomes	[41] [109]
DPPGOG		DPPC/DSPC/DPPGOG 50:20:30	~42 °C.	Statistical analysis	DPPGOG improves the <i>in vitro</i> properties in TSLs formulations compared to DSPE-mPEG2000, increases the content release rate without negative effect	[43]
P-lyso-PC		P-lyso-PC/DPPC/DSPC/DPPGOG 1:4:2:3	~42 °C	BFS-1 fibrosarcoma and C6 glioma cells	HePC is as effective as P-lyso-PC in accelerating the content release rate of DPPGOG-based TSLs	[42]
HePC		HePC/DPPC/DSPC/DPPGOG 1:4:2:3				

tend to stabilize pores in the lipid bilayer as it undergoes phase transition from gel to liquid, and increases the drug release rate of DPPC liposomes at 39–42 °C [41]. Incorporation of lysolipids such as monopalmitoylphosphatidylcholine (MPPC) into TSLs formulations further accelerates the rapid release of the drug cargo under mild hyperthermia (40–42 °C) [42,43]. The release of arsenic from pure DPPC liposomes is comparable at 37 °C and 42 °C, indicating that the presence of lysolipid is necessary for a significant enhancement of the release rate [44]. Upon heating the liposomes rapidly to 42 °C, both the TSLs containing 5 mol% and 10 mol% MPPC showed very large amounts of arsenic release within the first hour. However, the drug release of pure DPPC TSLs was almost unchanged, indicating that the presence of lysolipids is necessary to significantly increase the drug release rate. Hexadecylphosphocholine (HePC) is structurally related to lysolipid, which has a reduced metabolism and high accumulation in tumors and other tissues. Lindner et al. [45] investigated whether HePC was as effective as lysolipids in accelerating the content release rate of dipalmitoyl-sn-glycero-3-phospho-glyceroglycerol (DPPGOG) based TSLs. As expected, HePC increases the release rate of TSLs similar to lysolipids. And the TSL based on HePC incorporation showed cytotoxicity in a thermally induced manner. The phase transition temperature of the liposome can be controlled by adjusting the ratio of the compound phospholipid, which can enhance the stability of the liposome at 37 °C and can reduce the leakage of the drug loaded liposome before reaching the target organ [45]. Hossann et al. [46] demonstrated in their study that DPPGOG not only increased the *in vivo* half-life of TSLs (DPPC/DSPC/DPPGOG, 50:20:30 molar ratio), but also enhanced the cargo release rate without the side effects. To date, Sandstrom and Eleftheriou group respectively imitated lysolipids, imparting monoalkyl chains into on the thermo-responsive properties of DPPC liposomes. Platelet activating factor (PAF) has a structure of monoalkyl chain, thus it is a naturally occurring lipid and does not impart adverse pathological effects, and exhibits more favorable release properties than the commonly used DPPC: lysolipid formulation. The results indicated that lysolipid/PAF is more effective in inducing thermo-responsive properties [43,47]. Alavizadeh et al. [48] hypothesized that the addition of hydrogenated soy phosphatidylcholine (HSPC) to cisplatin loaded TSLs formulation would improve the plasma stability of liposomes. Their studies showed that the addition of HSPC increased the T_m , leading to the improved *in vivo* stability of TSLs.

3.2. 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethyleneglycol)-2000] (DSPE-mPEG2000)

Pegylated liposomes have a long circulation half-life from minutes to days due to the employment of PEG-lipid (or “stealth”) technology [49]. Although stealth liposomes extend the circulation time and enhance accumulation at the site of tumor, they do not guarantee the delivery of drugs to tumor cells at therapeutic and bioavailable concentrations. It has been demonstrated that the presence of low concentration of PEG-lipids reduces the size of vesicles without damaging their structure [50]. Banno et al. [41] investigated the role of DSPE-

mPEG2000 in lysolipid-containing TSLs (LTSLs) formulation and whether this component can promote the release characteristics of LTSLs. The study proposed that although lysolipids are more important components in determining the release behavior of LTSLs, they also stabilize the edges of capillary lipid bilayers. Though the addition of 4 mol% DSPE-mPEG2000 increased the T_m by about 1 °C, the inclusion of 5.0, 9.7, 12.7 and 18.0 mol% MSPC slightly lowered the T_m back to 41.7 °C. Needham et al. [51] also demonstrated this standpoint that the binding of lysolipid to DSPE-mPEG2000 in TSLs induced sustained nanopores with a diameter of about 10 nm in the lipid bilayers, thus resulting in rapid release. Unfortunately, this formulation is still not optimal due to premature leakage under physiological conditions [52]. At the same time, Li et al. [53] studied and conformed that 5 mol% of DSPE-mPEG2000 can not only protected TSLs from leakage in plasma at 37 °C, but also has a good content release profile upon mild hyperthermia therapy. So far, DSPE-mPEG2000 has always been the favorite of preparing liposomes [49].

The temperature sensitivity of TSLs can be successfully converted between 41 and 42 °C by carefully selecting the lipid composition of TSLs. The initial TSLs consists of DPPC alone [54] or with DSPC in the T_m range of 42.5–44.5 °C [55]. Lu and his coworkers investigated the rapid release of TSLs at certain DPPC/DSPC ratios during the phase transition, and proposed different release kinetics with the aim of applying TSLs more widely in clinic [56]. The release of carboxy fluorescein and DOX from these liposomes was rapid and quantitative within seconds when exposed to a temperature of 4 °C [13,53]. However, this formulation also displayed considerable leakage (up to 30% after 1 h) under physiological conditions. Lokerse et al. [57] studied the promoting effect of various lipid composition on the release of TSLs, indicating that an increase in the proportion of DSPC as compared with DPPC not only increased the T_m value, thereby affecting drug release and retention, but also prolonged the half-life of blood circulation. These advantages are attributed to the increase in membrane rigidity that successfully avoided serum opsonins. Moreover, the increase in DPPC content brought about decreased burst release. The slow release rate of encapsulated drug, and sometimes it is difficult to apply hyperthermia locally, limited its application [28]. To improve the release kinetics, Needham et al. [16] used lysolipids which have low T_m (e.g., MPPC or MSPC), lipid-grafted PEG and DPPC, to generate a new, low temperature sensitive liposomal DOX formulation (DOX-LTSLs). This formulation, commercially named ThermoDox[®] (currently in phase III clinical trials). Lu et al. developed a novel and well-designed idarubicin TSLs formulation to unravel the underlying ultrafast release mechanism at 42 °C, and on the optimal therapeutic efficacy of idarubicin-TSLs in different solid tumors [26].

3.3. Elastin-like polypeptides

In addition to elastin-like recombinamers, there are several classes of recombinant polymers, such as collagen-like peptide, that are as responsive components in thermo-responsive self-assembly of well-defined nanovesicles [58]. Park et al. [59] optimized the composition of elastin-like polypeptide (ELP)-TSLs, in which cholesterol acts as membrane stabilizer

and ELP as heat-triggered moiety to liposome. By considering the main effect of cholesterol and ELP in the amount and temperature of drug release, the optimal formulation was finalized as the liposome composed of DPPC/DSPE-PEG/cholesterol/ELP (55/2/15/0.41, molar ratio) for stable blood circulation and effective drug release under mild hyperthermia.

3.4. Surfactants

Polyoxyethylene (20) stearyl ether (Brij78) is a non-ionic surfactant consisting of PEGylated acyl chains, so it can replace the functions of DSPE-mPEG2000 in PEGylated phospholipids, thereby reducing opsonization and improving its pharmacokinetics. Tatsuaki et al. developed a TSLs formulation composed of DPPC and Brij78 at molar ratio of 96:4, and compared their drug release rate with TSLs containing lysolipids (DPPC:MSPC:DSPE-mPEG2000 = 86:10:4, molar ratio). The results showed that compared to lysolipid-TSLs, the novel TSLs formulation displayed an increased drug release rate at 40–41 °C and similar stability at 37–38 °C [18]. Tagamii et al. [38] post-inserted 16 mol% Brij78 to improve the release kinetics of DOX (100% drug release in 15–40 s at 40–42 °C), while as the stability at 37 °C was maintained with only 5% drug loss in 30 min. However, the lipid membrane could only accommodate Brij78 to a certain extent. For example, incubation of preformed Cu-TSLs with 24 mol% of Brij78 impaired the bilayer stability, induced significant drug loss (> 20%) and produced an unstable formulation. Tagami et al. incorporated Poloxamer 188 (P188) into DPPC liposomes and found that calcein released faster at 42 °C than that at 37 °C [28]. The mechanism is that the critical micelle concentration (CMC) of P188 converted from monomers to micelles is very sensitive to temperature, and small changes in temperature can change the CMC by several orders of magnitude. Therefore, P188/DPPC possible release calcein at 42 °C. P188 also has a protective effect on injured cells and tissues and holds promise for medical applications. Zeng et al. [60] added poloxamer to the TSLs formulation using oxaliplatin (OXP) as model drug. The results showed that OXP-TSLs had the best stability at body temperature and had rapid release at the trigger temperature. In summary, DPPC/P188 liposomes exhibit advantages both *in vitro* and *in vivo*, and have great promise for the future treatment of cancers [28,61].

Obviously, the possibility of improving the TSLs efficiency does not end and novel appropriate materials have been developed. The ideal system is to combine advanced materials with delivery in the sense that the system is inactive until a certain pathological condition is detected, at which point the drug is released timely. If we remains to be interested in determining the roles of components in the drug retention and release attributes of LTSLs, so as to establish parameters for further optimization of the LTSLs composition for *in vivo* use, and ultimately for future clinical applications [62–64].

4. Influence of serum components on TSLs

Upon parenteral administration, plasma opsonin proteins, lipoproteins and so on would like to interact with liposomes. Mononuclear macrophage system would recognize liposomes

with various serum components, and lead to the liposomes be degraded [65]. Hossann et al. [66] made a research into the influence of blood components and designed four different TSLs. They used a thin film dispersion method to load calcein (CF) into the TSLs as a marker for fluorescence spectrum detection. The results showed that (1) the release of CF in the serum of normal saline or low molecular weight is low, the serum component of polymer can increase the membrane permeability of tumor cells, which is beneficial to increase the rate of heat sensitive drug release. (2) Human serum protein (HSA) can significantly increase the release rate of CF at the T_m of lipid, while below T_m it can improve the stability of the membrane bilayer. (3) Immunoglobulin (IgG) can only increase the release rate of CF in the anion sensitive liposome and the effect is not significant. (4) Cholesterol is an essential component of serum and can be exchanged from the vesicles to the membrane of the liposome, thereby altering the membrane permeability and improving CF release. In a case study, a cholesterol anchor (chol-pHPMALac) included in thermosensitive liposomes, displayed a rapid release to a temperature increase when content of chol-pHPMALac is 5 mol% [67]. Sadazuka et al. [68] reported the stability of PEG modified liposomes for protein adsorption in serum. PEG modified liposomes circulated for a long time in the bloodstream and drug accumulation in tumors enhanced. The PEG chains on the surface of the liposomes diminished the adsorption of opsonizing proteins with the increase of water binding ability of the PEG chains because of serum proteins cannot bind to the water gathered on the surface of the liposomes.

5. Influences of size on TSLs

The determination of T_m alone is not sufficient to predict the drug release from TSLs. It is well known that vesicle size may be considered firstly to decrease clearance by organs of the mononuclear phagocyte system, affect drug release rate [69], pharmacokinetic parameters and therapeutic efficacy of liposomes. Smaller particles generally have an increased rate of drug release due to the increase in specific surface area. Hossann et al. [70] studied the influence of size (50–200 nm) on intravenous application of TSLs and found that the T_m of lipid bilayer was not affected by the size of vesicles within the test range. However, the vesicle size has a significant effect on the *in vitro* release characteristics of TSLs at temperatures ranging between 30 and 45 °C. In general, the vesicle size is inversely proportional to the content release properties. As the vesicle size decreases, the content release rate increases. Compared to TSLs containing lysolipids, the size dependence of the content release from 1,2-dipalmitoyl-sn-glycero-3-phosphoglyceroglycerol (DPPG2) contained TSLs is generally less affected in the range of 100–150 nm. Independent from gadodiamide release, vesicle size influenced the signal intensity of DPPG2 contained TSLs also at temperatures below T_m due to improved water exchange for smaller vesicles. Liposomes around 100 nm are routinely used *in vivo*, hence a quality control for TSLs preparations is required prior to be used. Even small changes in size or a wider size distribution might affect stability and release properties and thus yield in decreased efficacy or unwanted side effects of drug loaded TSLs

during *in vivo* applications. Galloway et al. [71] used a particle molding technology called Particle Replication in Non-wetting Templates (PRINT) to fabricate highly controllable and monodisperse particle-based vaccines that provided a platform for exploring particles which can be generated through a broad range of target sizes. Also, Chu et al. [72] capitalized on PRINT which produced size and interpreted the role of size on destiny of liposomes *in vivo*. Thus, we could optimize the size of liposomes basing on PRINT as a reference, further facilitating the related liposomes formulation. In addition, different interior buffers show different release. DOX-loaded liposomes with ammonium sulfate as the interior buffer did not release DOX at 42 °C, whereas liposome with citric acid as the interior buffer released DOX as rapidly as calcein-loaded liposomes. Therefore, Tagami et al. [28] speculated that gelation may inhibit drug release from DPPC/poloxamer 188 hybrid TSLs containing ammonium sulfate.

6. Advanced TSLs systems

6.1. Polymer-modified TSLs

It has been shown that lysolipids are often rapidly desorbed from the lipid bilayer of TSLs, resulting in a loss of thermal sensitivity both *in vitro* and *in vivo*. In fact, premature leakage of payloads *in vivo* has been observed that more than 50% of loaded drugs were released within 5 min at 37 °C. To address these issues, the TSLs has been attempted to conjugate thermo sensitive polymers such as poly(N-isopropylacrylamide) onto lipid bilayers, which exhibit a lower critical solution temperature (LCST) and provide a temperature-sensitive functionalities to the liposomes. These polymers are soluble in water and take on a hydrated coil state below their LCST. However, they become water-insoluble and take on a dehydrated globule state above the LCST. When the liposome membranes are fixed with thermo sensitive polymers, they are stabilized by the hydrated polymer chains below the LCST. However, destabilization of the liposomes occurs above that temperature due to interaction between the lipid bilayers and the hydrophobic polymer chains, resulting in release of the payloads. Terence et al. [100] developed a novel, dual-sensitive TSLs with polymer modification (pTSLs), which is capable of releasing drug in response to both temperature and pH value. They suggested that pTSLs released a greater amount of drugs at tumor position in response to heating, thereby reducing the dosage required. It also has a lower onset temperature for drug release, thereby reducing the required thermal dose and improving the safety profile of these formulations. In addition, it possesses a pH-sensitivity that responds to the slightly acidic tumor micro-environment, thus promoting drug release in the absence of externally applied heating. Their most important finding was that pTSLs in combination with MRgFUS (43 °C, 5 min) showed the greatest reduction in tumor growth and outperformed all other treatment methods including free drug, TSLs plus focused ultrasound (43 °C), and pTSLs plus mild focused ultrasound (40 °C). Especially, poly [2-(2-ethoxy) ethoxyethyl vinyl ether (EOEOVE) blocked octadecyl vinyl ether with LCST around 40 °C was shown to provide highly temperature-responsive

drug release functionality when attached onto the liposome surface [73]. When the EOEOVE copolymer-modified pegylated liposomes loaded with DOX was injected into tumor-bearing mice, and subsequently heated at 44–45 °C for 10 min at 6–12 h of administration, significant tumor suppression was caused. Kono et al. [74] sought to combine the EOEOVE modified pegylated liposomes with herceptin and indocyanine green (ICG). The results showed that the liposomes had a high-temperature responsive release function and could inhibit tumor growth by heating at 44 °C. The advantages of the polythermal sensitive liposomes are wide selection of membrane materials, and the thermal properties of liposomes are less affected by particle size. However, there are still some problems that need to be solved. For example, the copolymerized N-isopropylacrylamide (NIPAAm) has a cloud point temperature of about 35 °C, which is lower than 41 °C. So it is necessary to develop more suitable thermal sensitive polymers, and the toxicity of these thermal polymers needs further study. Hyperthermia combined with drug delivery systems [75] has been tried clinically to treat solid tumors, in combination with, because it can synergistically induce tumor cytotoxicity in combination with chemotherapy and radiotherapy.

6.2. TSLs combined with new heating modalities

Radiofrequency ablation (RFA) is useful in the treatment of a variety of malignancies [76]. The basic principle is to make use of high frequency current, which will cause friction heat of charged particles in biological tissue, inducing tumor coagulation, irreversible cellular damage and cell degeneration. RFA is non-invasive, safe, easy, and quick, so it is widely used in clinical therapy [77]. RFA combined with long-circulating liposomal doxorubicin (Doxil; Alza Pharmaceuticals) increased the extent of local tumor destruction and survival in an animal tumor model, inducing tumor coagulation with drugs, and followed by improved therapeutic efficacy [52,78]. It will inhibit tumor at relative low temperature with no common severe toxicities. RFA combined therapy effectively inhibited tumor growth and increased the survival rate of animals with medium-sized tumors significantly, which had been validated in clinical trials. Lencioni et al. [79] demonstrated that the combination of RFA and TSLs-DOX increased survival compared to the standardized RFA alone. Poon et al. have described the clinical findings of RFA plus TSLs-DOX. They concluded that this combination therapy was safe and improved DOX release at tumor site, thereby further improving the therapeutic index of the drugs to solid tumors [80].

Accurate control of the temperature in an narrow window of 41–42 °C is one of the most crucial prerequisite as vascular shut-down may occur above 43 °C. the high-intensity focused ultrasound (HIFU) technology employed in cancer therapy is useful due to its ability to initiate hyperthermia or ablative therapy for tumors. HIFU ablation treatment is based on the power of a focused ultrasound beam to locally heat biological tissues over a necrotic level with minimal impact on the surrounding tissues. A HIFU treatment can be applied percutaneously to induce lesions (*i.e.*, localized tissue necrosis) at a small, well defined region (approximately 1 mm) deep within tissue, while leaving intervening tissue between the

HIFU transducer and the focal point substantially unharmed [81]. Escoffre et al. [82, 83] revealed that *in vitro* HIFU induced a significant release of DOX from TSLs without affecting the cellular uptake and cytotoxicity of the released DOX. For applications in hyperthermia, the HIFU technology needs to be adapted to maintain homogeneous heating of the tumor tissue over 30–60 min at 41–42 °C, which requires a robust temperature feedback to cope with problems coming from. Although the concept of temperature-triggered drug delivery was proposed more than thirty years ago, several chemical and technological challenges had to be addressed to advance this approach towards clinical translation. In particular, non-invasive focal heating of tissue in a controlled fashion remained a challenge. With the propagation of ultrasound in the body tissues, some physical effects such as hyperthermia occur, which can be used as a trigger for drug release from TSLs [83,84]. Rational design of an ultrasound-triggered delivery vehicles usually involves the design of materials in response to hyperthermia [85].

For the latter, HIFU allows completely non-invasive heating to establish hyperthermia (40–45 °C) of tumor tissue over time [86,87]. Magnetic resonance imaging (MRI) [88] plays a pivotal role in this procedure, which thanks to its superb spatial resolution for soft tissue as well as the possibility to acquire 3D temperature information [89,90]. MRI was used to monitor the *in vivo* release of Gd-HP-DO3A following laser-based heating of the tumor implanted on the left hind limb [91,92]. Consequently, MRI systems emerged with an HIFU ultrasound transducer embedded in the patient bed (MR-HIFU) [93], where the MRI is utilized for treatment planning, and provides spatial and temperature feedback to the HIFU [94]. For tumor treatment, the lesion is heated to 42 °C using HIFU [95]. MRI-based temperature and release monitoring can provide valuable insights into the potential transport mechanisms of drug delivery [96]. Recent preclinical studies have successfully used MRI-guided HIFU in combination with TSLs in tumor-targeted therapy [97,98]. *In vivo* studies had found that PTSLs slowed tumor growth more effectively than free DOX and TSLs when administered in combination with MRI-guided HIFU [94].

Recently, a series of further optimized DOX-TSLs formulations have been reported, showing desired triggered release by mild hyperthermia and favorable stability at physiological temperature [99]. Tai et al. encapsulated magnetic nanoparticles (MNPs) and hydrophilic drugs into TSLs [100]. MNPs generates heat when exposed to an alternating magnetic field (AMF), a new imaging method called magnetic particle imaging (MPI) that uses the nonlinear response of MNPs to detect their presence in AMF. AMF is referred here as the drive magnetic field and experimental results suggested that MPI can be used to enhance the effectiveness of chemotherapy based on the drug release from TSLs without damaging surrounding normal tissues [101]. Dou et al. [102] summarized the potential causes of the failure of the ThermoDox® in clinical trials, which would provide guidance for the ongoing clinical translation of TSLs. In the future, heating method have to be considered when comparing the efficacy of thermal induced drug release in tumor suppression experiments. HIFU or RF may be employed as clinically relevant heating method to non-invasively reduce the tumor volume.

6.3. Smart thermosensitive liposomes

Many researchers have developed thermosensitive nanovehicles to control drug release within the diseased region under locally-treated mild hyperthermia. Although TSLs could improve the targeting drug delivery to some extent by physical heating, there were still some problems need to be solved, such as active transporting of drugs to specific tumor sites after intravenous injection. To address this problem, introduction of various biological ligands or antibodies into the drug delivery systems provides a possible strategy to selectively deliver drugs to tumor cells.

On the basis of the above background, Guo et al. hypothesized to construct a smart-triggered drug delivery system (IR780-BTSLs-FA) which integrated targeting, therapeutic and diagnostic functions together, providing many potential advantages [103]. The smart liposomes contain NH_4HCO_3 which quickly decomposes to generate CO_2 bubbles when heated to a high temperature (40–42 °C). Fig. 3 gives a brief description of this mechanism. Accordingly, the lysosomal membranes were disrupted to release the contained drugs. IR780-mediated photothermal heating could trigger the drug release from the TSLs as well as *in vitro* and *in vivo* imaging, where folate ligand was used to modify the surface of TSLs to elevate its targeting ability to tumor cells [104]. The experiment has shown that it could strongly suppress tumor growth under photothermal heating when injected intravenously into tumor-bearing mice [103]. As epidermal growth factor receptor (EGFR) is overexpressed in a variety of cancer cells, Haeri et al. [29] developed TSLs with anti-EGFR ligands for targeted delivery and localized triggered release of chemotherapy. With the prolonged duration of hyperthermia, the heat penetration increased, resulting in the activation of more EGFR targeted nanomedicines, thus releasing higher doses of drug than non-targeted TSLs. When the TSLs are used in combination with local hyperthermia, they have the potential to provide site-specific triggered drug release and therefore enable precise spatial and temporal control of therapy. It might prove that it is promising as a drug delivery system for imaging-guided tumor therapy. Additionally, researchers had reported that non-spherical, such as rod-like nanoparticles, could penetrate tumors more rapidly and accumulate at higher levels than size-matched spheres [105]. Agarwa et al. [106] used gold nanorods (GNRs) to work synergistically with TSLs. GNRs are photothermal nanoparticles based on NIR in the 650–900 nm range, which are induced to a depth of 10 cm in the soft tissues where the tumor is located [107]. As the non-spherical character of GNRs alters the liposomes, it leads to the co-aggregation with drug loaded liposomes due to the amplified EPR effect [15]. Fig. 4 depicts this phenomenon roughly.

Inspired by flagellar propulsion by bacteria such as *E. coli*, artificial bacterial flagella (ABFs) are capable of performing precise three-dimensional navigation in fluids under low-strength rotating magnetic fields, making them attractive tools for targeted drug delivery [108]. Qiu et al. [109] reported the successful functionalization of titanium-coated ABFs with DPPC based TSLs, known as "smart" drug carriers. They showed the ability to load both hydrophilic and hydrophobic drugs, and release calcein (drug model). The results

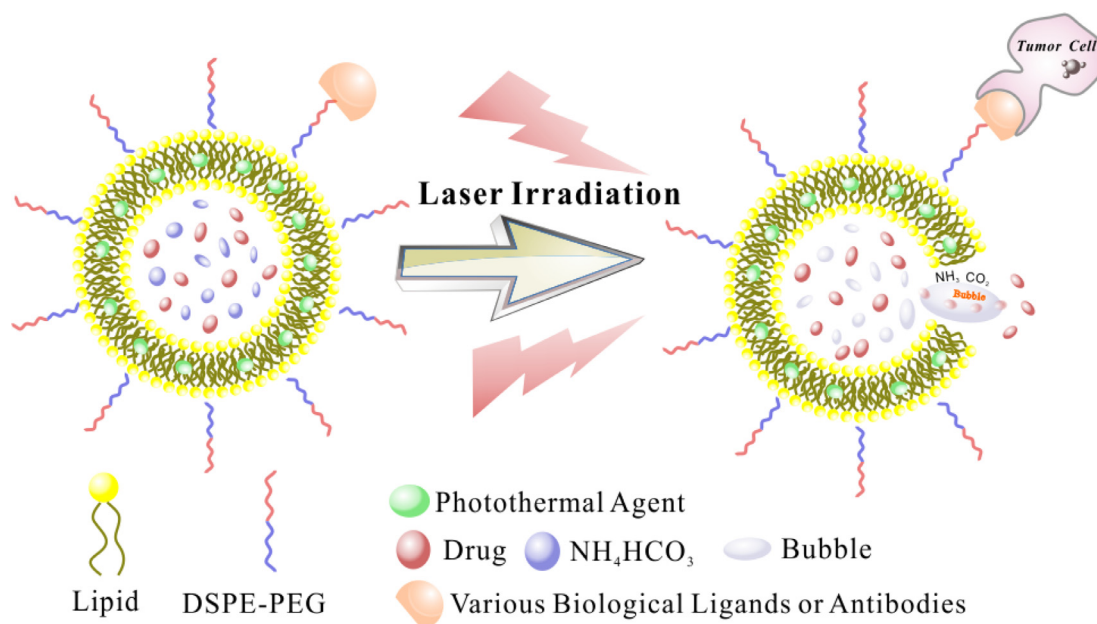


Fig. 3 – Bubble thermosensitive liposomes encapsulate ammonium bicarbonate (NH_4HCO_3) and drugs. NH_4HCO_3 generated CO_2 bubbles when exposed to laser irradiation, where to a high temperature ($40\text{--}42^\circ\text{C}$) from radiant heat. These modifications would enhance permeability and retention effect.

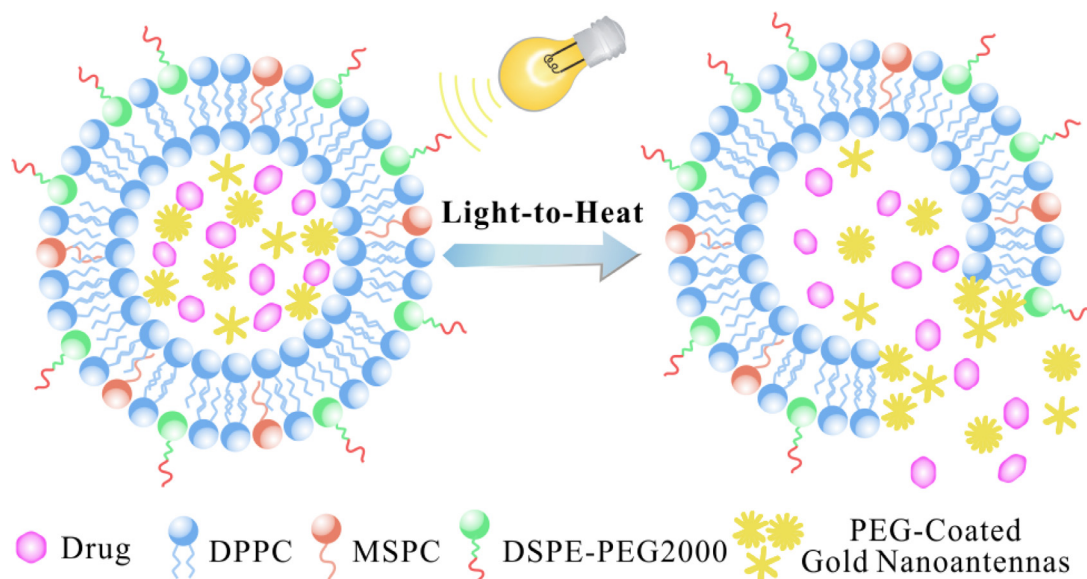


Fig. 4 – Gold nanoantennas has unique geometry, which could absorb near-infrared light, efficiently converting light to heat and effectively releasing drugs from low-temperature-sensitive liposomes.

showed that calcein was released at 39°C , and the release efficiency of calcein reached $73\% \pm 15\%$ at 41°C . These artificial bacterial flagella functionalized with TSLs are called “smart” drug carriers and can be used for targeted and triggered drug delivery, microfluidic devices and biosensing.

In an effort to develop the next generation of liposomes for localized drug delivery, Pradhan et al. [110] described that folate receptor targeted thermosensitive magnetic liposomes

(MagFolDox) achieved more effective therapy. They attributed this phenomenon to the fact that the folate receptor is over-expressed in tumor cells. Magnetic liposomes are magnetized with a permanent magnetic gradient field to penetrate the tumor tissue, and the temperature-induced drug release combined with the hyperthermia induced by the alternating current magnetic field provides an ideal formulation for treating tumor. Smith et al. demonstrated that the combination of TSLs

with HER2-specific affibody molecules (Affisomes), which are capitalized on the feature of malignancy overexpressed human epidermal growth factor receptor 2 (HER2), helps to improve the treatment of HER2 positive tumors to a great extent [111]. However, TSLs retain their triggered release properties without any significant modulation in their physico-chemical characteristics [112]. Based on the understanding of the role of Affisomes, they successfully deliver drugs to HER2 positive tumors.

Wang et al. [113] designed DOX-loaded leucine zipper-consisted temperature-controllable drug delivery system. The lipo-peptide resembles an on/off switch that can form dimers by the hydrophobic force. It can be dissociated into single random coils when the helix structures are destroyed above the melting temperature, thus successfully achieving a reversible and highly responsible drug release behavior under intermittent heating. It was possible to tune the release of chemotherapeutic drugs and offer immense potential for advancing drug delivery systems. Besides, Dicheva et al. [114] measured and tested the pharmacokinetic profile, biodistribution and therapeutic effect of cationic thermosensitive liposomes (CTSLs) encapsulating doxorubicin (Dox) upon mild hyperthermia, and validated their targeted and drug release functions. Smart thermosensitive liposomes (STSLs) represent an interesting tool that can significantly improve the efficiency and accuracy of treating a broad category of diseases.

7. Conclusions

The early TSLs consist mainly of lipids that can undergo gel-liquid phase transitions at response temperatures, such as DPPC with a T_c of 41 °C. Since pure DPPC liposomes inevitably lead to incomplete drug release, other phospholipids such as DSPC and HSPC are usually added to increase the drug release rate. However, the T_c of liposome is also increased to 43–45 °C, resulting in the need for higher thermal doses to trigger drug release, which may bring a risk of necrosis to the normal tissues around the tumor. In the subsequent efforts to improve the thermal response sensitivity of conventional TSLs, addition of lysolipids was proposed. Incorporating 10 mol% MPPS into the PEGylated TSLs could reduce the T_c to 39–40 °C and accelerate the release of drugs upon mild heating. However, more and more studies have shown that lysolipids are easily desorbed from the lipid bilayer to neutralize the stability and thermal sensitivity of TSLs. As an alternative, the combination of TSLs with external energy sources for local thermally induced drug release has shown great promise for improving intratumoral drug concentrations, and the efficacy and potential adverse effects of the method are currently evaluated in clinical trials [29].

To the best of our knowledge, TSLs in combination with regional hyperthermia or high-intensity focused ultrasound and other heat means in the clinical setting is a promising tool for targeted and triggered drug delivery to solid tumors as well as achieving controllable drug release. The current status in the development of a broad variety of TSLs represents a pathway towards the design of nanocarriers with dramatically enhanced efficiency. This review sheds light on our understand-

ing of TSLs functional properties as well as further strategies to improve the design of the TSLs formulation.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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