Thermosensitive Liposomal Drug Delivery Systems: A Review

Abstract:

Thermosensitive liposomes are a promising tool for external targeting of drugs to solid tumors when used in combination with local hyperthermia or high intensity focused ultrasound. The overall objective of liposomal drug delivery is to selectively target drug delivery to diseased or damaged tissue, while minimizing drug delivery to critical normal tissues. The purpose of this review is to provide an overview of temperature-sensitive liposomes. Temperature-sensitive liposomes are an especially enticing option, as tumors can be heated in a controlled and predictable manner with external energy sources. Traditional thermosensitive liposomes are composed of lipids that undergo a gel-to-liquid phase transition at several degrees above physiological temperature. More recently, temperature-sensitization of liposomes has been demonstrated with the use of lysolipids and synthetic temperature-sensitive polymers. Thermosensitive liposomes (TSL) in combination with regional hyperthermia represent a powerful tool for tumor specific drug delivery. Hyperthermia increases the efficiency of various chemotherapeutic drugs and is administered as an addition to chemotherapy for the treatment of cancer patients. The temperature-dependent effect can be strongly increased by the use of temperature-sensitive liposomes in combination with regional hyperthermia, which specifically releases the entrapped drug in the heated tumour tissue.

Introduction:

Liposomes are spherical vesicles formed by a membrane bilayer usually composed by of phospholipids (Figure 1). The membrane encloses an aqueous core that can be used to encapsulate hydrophilic drugs, whereas lipophilic drugs can be incorporated into the membrane. Several methods are available for the preparation of liposomal formulations, ranging from laboratory scale to Good Manufacturing Practice production for clinical batches.[1] Loading of drugs can be achieved by active (Figure 2A) or passive (Figure 2B) loading methods(Figure 3).

Flow chart 1. Protocol for liposome preparation

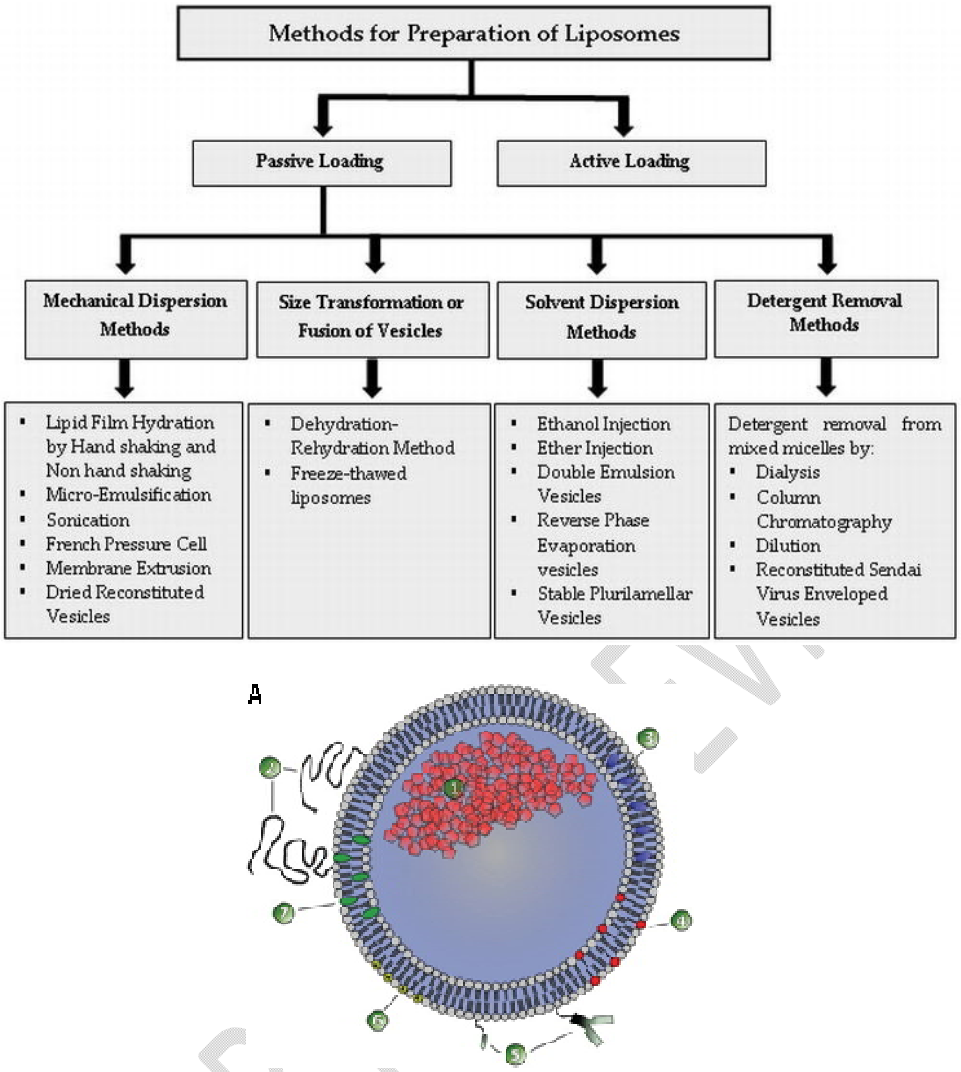

Figure 1 Structure of liposomes

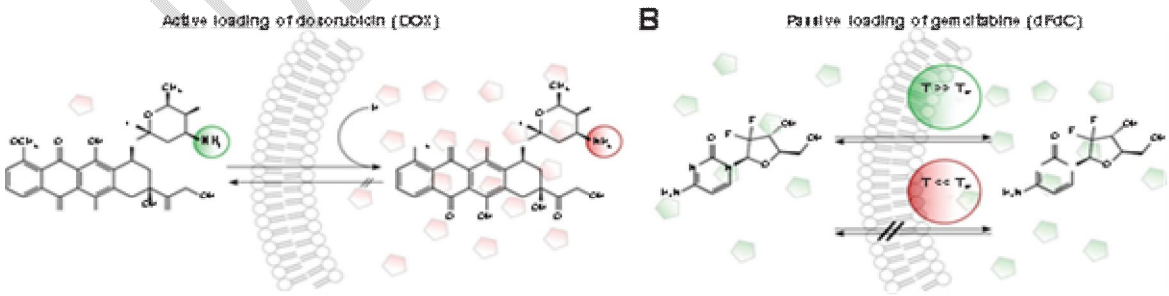

Figure 2 Loading of drugs

Stable encapsulation of a drug inside a liposomal formulation increases its half-life in ~~the~~ circulation after intravenous administration by avoiding rapid metabolism. Moreover, unwanted distribution in different compartments of the body is avoided, so the risk of drug-related side effects is reduced. The versatility of liposomal drug delivery systems reflects the fact that their biophysical characteristics, e.g., vesicle size, lamellarity, surface charge, membrane fluidity, and surface, can be modified by the lipid composition and/or preparation

method used. Since naturally occurring molecules like (phospho)lipids and cholesterol are used as the main components, liposomes are in general classified as biocompatible. In 1965, Bangham et al described the spontaneous formation of liquid crystals after dispersing lecithin in an aqueous medium.[2]The purpose of this review is to provide an overview of temperature-sensitive liposomes in general and the Low Temperature-Sensitive Liposome (LTSL) in particular. This LTSL was designed to release the drug rapidly upon a temperature trigger using mild hyperthermia at 41–42°C. Its basic design to load and retain drugs while evading the body's ~~defences~~ defenses is based on a wealth of information about liposomes that was gained ~~over a period of~~for 40 years. These studies include basic research and preclinical and clinical investigations that have led to the most advanced nanoscale drug delivery system in clinical therapy.[3]Heat-triggered drug release from liposomes can also be achieved by adding thermosensitive polymers to the formulation.~~[4]However~~ [4]However, in the present review, we focus on formulations where thermosensitivity is achieved by the bio-physical properties of the membrane-forming phospholipids and highlight the influence of lipid composition on the in vitro and in vivo behavio~~u~~r of the TSL formulations currently under investigation.[5]

Novel paradigm of drug targeting: intravascular temperature-triggered drug release by external targeting

Classical PEGylated long-circulating doxorubicin formulations like Doxil/Caelyx have been designed to exploit the enhanced permeability and retention effect (Figure 3A),[6] and passively accumulate inside tumor tissue because of the leaky tumor vasculature. Nevertheless, passive drug targeting has failed to achieve increased clinical efficacy in humans when compared with the free drug as a result of several shortcomings. Accumulation depends on the specific structure of the tumor vasculature and might be increased by heating the tumor tissue.[7,8] However, extravasation of vesicles is the rate-limiting step, and nanoparticles have to circulate for days to accumulate in sufficiently high concentrations[6] because accumulation in tumor tissue competes with uptake in the liver and spleen, and less than 10% of the injected dose accumulates in the tumor.[9]A promising alternative, reported by Manzoor et al is external targeting achieved by temperature-triggered, localized intravascular drug release from TSL with focused heating (Figure 3B).[10] After reaching the heated tumor tissue, doxorubicin was released directly into the bloodstream, generating a high intravascular drug concentration.[10,11] This led to a significantly increased penetration depth of bioavailable doxorubicin into the tumor tissue when compared with that in animals treated with free doxorubicin or Doxil/Caelyx.~~[10]Using~~ [10] Using this approach, poorly perfused tumor areas, which are known to be more difficult to treat due to a hypoxia-mediated resistance mechanism, could also be reached. The concept of intravascular drug release was then extended to targeting ~~of~~ more hydrophilic drugs, such as gemcitabine.[12]Temperature-triggered drug targeting by TSL has the advantage of being able to externally control drug release spatially and temporally by steering the heating focus and heating power. Applicators for regional or localized heating of even deep-seated tumor tissue are well established in clinical practice~~,~~ and are used to heat tumor tissue to temperatures of 42°C (mild hyperthermia).[13] Therefore, commonly used TSL are designed to release the encapsulated drug between 39°C and 42°C.

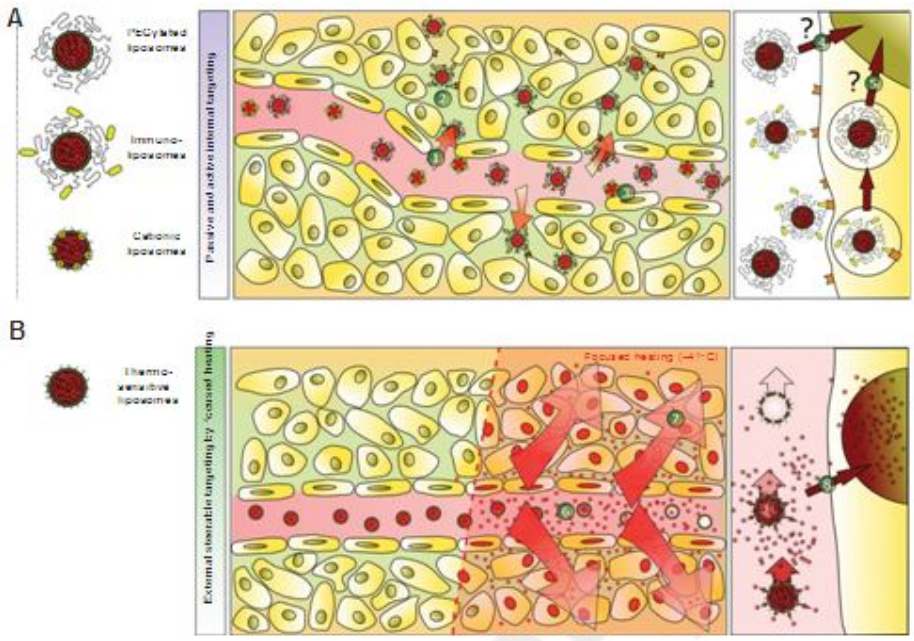

Figure 3:Schematic illustration of targeting concepts with liposomes.

Influence of lipid composition on drug release

Encapsulated hydrophilic drugs are released from TSL at the melting phase transition temperature (T_m) of the lipid bilayer. At T_m, the structure of the lipid bilayer changes as a transfer from a solid gel phase (L_β) to a liquid-crystalline phase (L_α) occurs (Figure 4). The membrane is more permeable to water and hydrophilic drugs in the liquid-crystalline phase than when in the gel phase.[14,15]The permeability of hydrophilic drugs is highest at temperatures around the Tm because of the coexistence of membrane areas in both phases forming grain boundaries.[16,17]

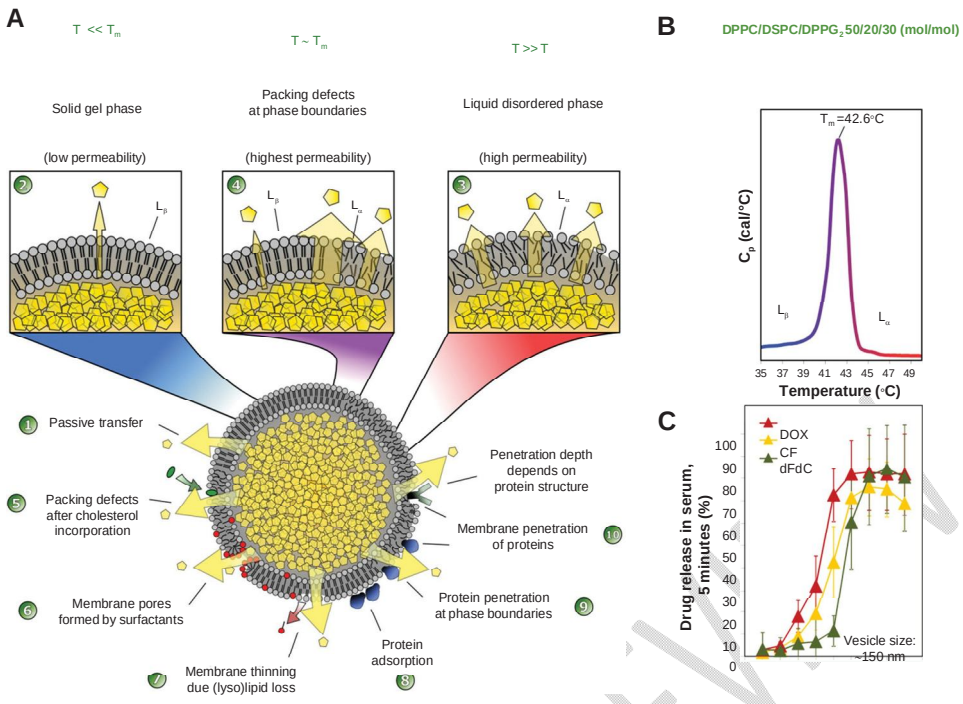

Figure 4:Factors affecting drug release from thermosensitive liposomes.

Traditional temperature-sensitive liposomes

In 1995, Gaber et al reported the effect of cholesterol and PEG-phosphatidylethanolamine ~~with regard to~~concerning stabilizing TSL formulations in vitro.[18]Incorporation of 30 mol% cholesterol into TSL formulations eliminated Tm by changing the phase state of the membrane to a liquid-ordered phase. A traditional temperature-sensitive liposome (TTSL) formulation with ~~co~~encapsulated doxorubicin and a gadolinium-based contrast agent for MRI-guided delivery of doxorubicin is currently under investigation.[19–22] The TTSL formulation has been used in these studies because of its higher stability when compared with lysolipid containing low temperature-sensitive liposome (LTSL) formulations.[19]

Lysolipid-containing low temperature-sensitive liposomes

The breakthrough in the development of clinically usable TSL formulations was the incorporation of lysolipids into the membrane bilayer, as described by Needham et al in 2000.[23]LTSL was the first TSL formulation suitable for use in the intravascular drug release approach. This formulation is characterized by ultrafast drug release upon heating, although the incorporation of surfactants in the formulation decreases vesicle stability around Tm.[24] Moreover, approximately 70% of lysolipid was found to dissociate from the formulation within one hour post injection.[25]

$DPPG_2$ -thermosensitive liposome

In 2004, a new liposomal formulation ($DPPG_2$ -TSL) composed of the phospholipids DPPC, DSPC, and1,2-dipalmitoyl-sn-glycero-3-phosphodiglycerol ($DPPG_2$), was reported by Lindner et al.[26]$DPPG_2$ is a synthetic phospholipid with a molecular weight close to that of natural occurring 1,2-dipalmitoyl-sn-glycerol-3-phosphoglycerol, because only one additional glycerol molecule is bound via an ether bond to the head group.[26] The molecular class of

oligoglycerols was developed to increase the circulation half-life of vesicles in the same way as for PEGylated lipids. ~~Incorporation~~ The incorporation of $DPPG_2$ led to a prolonged circulation time in non-thermosensitive[27] and thermosensitive[28,26]formulations. The plasma half-life of carboxyfluorescein encapsulated into $DPPG_2$ -TSL was reported to be 9.6 hours in hamsters and 5 hours in rats.[26] ~~In~~ In contrast with the LTSL formulation, incorporation of surfactants into $DPPG_2$ -TSL was avoided. However, the release rates of carboxyfluorescein and doxorubicin from $DPPG_2$ -TSL were as fast as measured with the LTSL formulation,[29] but drug release from the $DPPG_2$ -TSL formulation started at approximately one-degree higher temperature.[29] $DPPG_2$ -TSL showed improved in vitro stability in complete serum when compared with LTSL.[30] ~~The~~ The presence of serum components at 37°C stabilized the formulation over time, whereas the opposite was found for LTSL.

Stealth TSL

A sterically stabilized TSL formulation (Stealth TSL; Table 1) was developed from the original Yatvin formulation by adding DSPE-PEG2000 for improved stability and a better in vivo half-life when compared with the LTSL formulation,[29,31] and enabled passive accumulation of TSL in tumor tissue.[32] Li et al compared Stealth TSL and LTSL~~,~~ and found that the former had superior in vitro stability at 37°C in serum.[27] The maximum release of doxorubicin from Stealth TSL was at 42°C.[27] In comparison with LTSL, the release of doxorubicin from Stealth TSL starts at higher temperatures (39°C versus 37°C).[27]

Hyperthermia-activated cytotoxic formulation

Another TSL formulation with encapsulated doxorubicin currently under investigation is the hyperthermia-activated cytotoxic (HaT) liposome formulation described by Tagami et al (Table 1).[33] HaT is composed of DPPC and the non-ionic surfactant, polyoxyethylene (20) stearyl ether (Brij78; Figure 5A). Brij78 consists of a PEGylated acyl chain, so it was hypothesized that Brij78 could replace the function of lyso-PC and DSPE-PEG2000 in the LTSL formulation.[33,34] The HaT formulation showed 100% doxorubicin release within 3 minutes at a temperature of 40°C–42°C in the buffer.[34] In comparison with LTSL, HaT showed enhanced drug release rates at 40°C, with similar blood pharmacokinetics.[33] For both formulations, a blood circulation half-life of approximately 0.5 hours was observed after injection.[33] A single intravenous treatment with HaT at a doxorubicin dose of 3 mg/kg body weight in combination with local hyperthermia showed enhanced tumor regression when compared with LTSL.[33]

STL formulation

In 2013, Park et al reported another stabilized formulation composed of DPPC, DSPE-PEG2000, cholesterol, and fatty acid-conjugated elastin-like polypeptide 55:2:15:0.4125 (mol/mol) (STL) with encapsulated doxorubicin (Table 1).[35] Pharmacokinetic studies in mice showed plasma half-lives of 2.03 hours and 0.92 hours for doxorubicin encapsulated in STL and LTSL,[35] respectively. In combination with ~~high~~ high-intensity focused ultrasound, STL achieved significantly better tumor growth delay 7 days after injection when compared with LTSL.[35]

Thermosensitive liposomes for MRI-guided drug delivery

MRI is the method of choice for image-guided drug delivery with TSL. Its abilities ~~with regard to~~concerning morphological and functional tumor characterization without exposure to

ionizing radiation are well known, and it is a standard method in clinical use. Further, MRI thermometry is established for the control of thermotherapies, such as radiofrequency hyperthermia and high intensity focused ultrasound. Dedicated hybrid systems have already been introduced into clinical applications.[15,36,37] Localized drug release from TSL has been demonstrated in rodents,[38,39] and nonrodents,[40–42] using MRI for the control of hyperthermia. Beyond controlling the volume of heating, encapsulation of MRI contrast agents in TSL formulations allows additional characterization of the drug delivery only accessible in humans when using MRI.

Signal mechanism

Paramagnetic gadolinium chelates[54,41,43–45] and manganese ions[46-49] are typical MRI-active contrast agents for encapsulation in TSL formulations (Table 1). The nuclear magnetic resonance of water protons is the primary origin of the MRI signal and not the contrast agent itself. MRI contrast agents are only visualized by their ability to accelerate the water proton relaxation in the vicinity of the contrast agent molecules. This indirect ~~signal~~ signal-forming contrast process is only effective if the contrast agent molecule is allowed to interact with a large number of water protons. For visualization of temperature-induced release, the contrast agent has to be encapsulated inside the TSL.[50–54] Below the Tm, the agent interacts mainly with the water present inside the TSL~~,~~ because water exchange with the exterior of the TSL is limited. As a result, the visibility of the contrast agent is reduced when compared with free contrast agents. When approaching the Tm, the increase in water exchange results in a signal increase in T1 -weighted images.[49] Around the Tm, the contrast agent is released and the signal change is maximal and comparable with the signal change achieved with the free contrast agent.[53] This makes it possible to strongly change an MRI signal by altering temperature.[54]

Table 1 Overview of thermosensitive liposomes

Abbreviation	Membrane composition	First publication	T_m (encapsulated doxorubicin)	Encapsulated drugs	Encapsulated MRI contrast agent	Targeting principle
First TSL	DPPC/DSPC 3:1 (mol/mol)	1978[9]	–	Neomycin[9] Methotrexate[9]	–	
TTSL	DPPC/HSPC/Chol/DPPE-PEG 50:25:15:3 (mol/mol)	1995[45]	40.9°C[54]	Doxorubicin[45,54]	Gd-based[54]	Passive targeting before heat-triggered, interstitial drug release
LTSL	DPPC/*lyso*-PC/DSPE-PEG_{2000} 90:10:4 (mol/mol)	2000[11]	40.8°C[10]	Doxorubicin[11] Cisplatinum[65]	Gd-based and Mn-based[54,87-89]	Intravascular drug release
$DPPG_2$-TSL	DPPC/DSPC/ $DPPG_2$ 50:20:30 (mol/mol)	2004[67]	41.9°C	Doxorubicin[46] Gemcitabine[28] Miltefosine[71]	Gd- and Mn-based[84-86,90,93]	Intravascular drug release
Stealth TSL	DPPC/DSPC/ DSPE-PEG_{2000} 80:15:5 (mol/mol)	2007[46]	43.0°C[72]	Doxorubicin[27,72]	–	Passive targeting before heat-triggered, interstitial drug release
HaT	DPPC/Brij78 96:4 (mol/mol)	2011[73]	41.0°C[74]	Doxorubicin[73] Gemcitabine[75] Oxaliplatin[75]	Gd-based[97]	Intravascular drug release
STL	DPPC/DSPE-PEG_{2000}/Chol/ELP 55:2:15:0.4125 (mol/mol)	2013[77]	–	Doxorubicin[77]	–	Intravascular drug release

Abbreviations: Brij78, polyoxyethylene (20) stearyl ether; DPPE-PEG, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-methoxy(PEG); $DPPG_2$, 1,2-dipalmitoyl-*sn*-glycero-3-phosphodiglycerol; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; $DSPE\text{-}PEG_{2000}$, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-methoxy(PEG)-2000; ELP, fatty acid conjugated elastin-like polypeptide; Gd, gadolinium; HaT, liposome hyperthermia-activated cytotoxic formulation; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; HSPC, hydrogenated soy phosphatidylcholine; LTSL, lysolipid-containing low-temperature sensitive liposomes; Mn, manganese; MRI, magnetic resonance imaging; TTSL, traditional temperature-sensitive liposome; TSL, thermosensitive liposome; Chol, cholesterol; T_m, phase transition temperature.

Applications

TSL can be applied with an encapsulated contrast agent to distinguish heated from unheated tissue[39,53] or to quantify absolute temperatures complementing traditional MRI thermometry methods,[54,55] thus serving as a tool for quality assurance in thermotherapy in patients.

The major drawback of the above approach is the toxicity related to manganese (II). To overcome this, other researchers are using clinically approved gadolinium-based contrast agents. Hossann et al investigated six of these contrast agents for encapsulation in DPPG2 - TSL, and considered a non-ionic contrast agent with a low contribution to osmolality to be optimal.[40] Two strategies of encapsulation are possible using gadolinium-based contrast agents, but the release kinetics and signal mechanisms for both the contrast agent and drug have to be considered. One strategy is to combine two subsets of TSL, with one encapsulating only the contrast agent and a second encapsulating only the drug.[56] This strategy allows a higher amount of contrast agent and drug to be encapsulated whilst avoiding osmotic effects.[40] The second strategy is to coencapsulate both drug and contrast agents in the same TSL,[57]which limits the amount of both components in each TSL. Nevertheless, for both strategies, it has to be ensured that the temperature-dependent drug release rate and MRI signal change are correlated.[57]

Conclusion

TSL are is a promising tool for external targeting of drugs to solid tumors in combination with local hyperthermia or high intensity focused ultrasound.

Several formulations have been developed, with one currently under clinical investigation. In vivo results show strong evidence that external targeting is superior over passive targeting of highly stable long-circulating drug formulations. Moreover, MRI-guided drug delivery adds the possibilities of online monitoring of the heating focus, calculating locally released drug concentrations, and externally controlling drug release by steering the heating focus and power. The combination of external targeting with TSL and MRI-guided drug delivery will be the unique characteristic of this nanotechnology approach in medicine.

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