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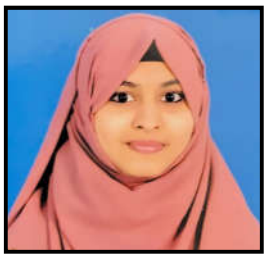

LIPOSOMES WITH THERMOSENSITIVE TERMINALS FOR TUMOR TARGETING: RECENT TRENDS

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ABSTRACT

Thermosensitive liposomes (TSLs) are the optimistic class of nanomedicine which has the potential of providing site specific delivery of anticancer drugs. They are more effective when combined with local hyperthermia or high intensity focused ultrasound in treatment of cancer, by enhancing the vascular permeability of the drug and its transport to the target site without damaging the normal tissues and they also enhance the permeability of lipid bilayers. The two important elements which has to be considered that affects efficacy are, kinetics of release and stability of plasma, thereby to improve properties the new formulations are aimed at. The usage of lysolipids and thermosensitive polymers which are naturally and synthetically derived demonstrates the temperature sensitization of liposomes. This article summarizes about the materials and methods used in the preparation of thermosensitive liposomes and also various biomedical applications of TSLs. The main objective of this article is to introduce the recent advances used in the thermosensitive liposomes like traditional thermosensitive liposomes which is composed of lipids that undergoes transition from gel-to-liquid phase at a certain point above the physiological temperature. Multi-functional thermosensitive liposomes are another approach which is used to enhance the delivery of anticancer drugs. Thermosensitive liposomes when combined with magnetic resonance guided drug delivery for external targeting, becomes an unique feature in nanotechnology in the field of medicine.

KEYWORDS

Thermosensitive liposomes, Mild hyperthermia, Transition temperature and Extravasation.

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INTRODUCTON

Liposomes were discovered by Alec Bangham in 1961. They are composed of bilayer lipid shells and they are formed when lipids are dispersed in water spontaneously. The drugs having the amphiphilic character that is both hydrophilic and hydrophobic in nature can be entrapped by using liposomes. They are usually biocompatible and biodegradable.

They are non-toxic, non-allergic and antigenic in nature. Liposomes have the property to protect the drug which undergoes premature inactivation[1].

RES is a type of mechanism which is inhibited by the liposomes where the accumulation occurs at the site of tumor. Liposomes burst out through the microvessels of tumors which are highly permeable and due to lack of functional lymphatic drainage they remain locked in the compartment of interstitial fluid. The toxic effects are minimized which usually results in the liposomal extravasation in healthy tissues is the main advantage of using liposomes as novel drug delivery system in the treatment of cancer[2].

Thermosensitive liposomes are the best choice for the uptake of tumor. To facilitate the method thermosensitive liposomes are merged with hyperthermia. They give two main benefits. Firstly, the vascular permeation is enhanced and transport of the drugs to the diseases site while the drug delivery is minimized to the critical normal tissues. Secondarily the payload is released by hyperthermia causing the enhancement in permeability of bilayer of lipids[3].

The encapsulated drug release is activated within few seconds to minutes when the thermosensitive liposomes are heated to the optimal temperature at the site of tumor[4].

To enhance the release of contents of TSLs, HIFU (high intensity focused ultrasound) shows great effects. In this process the phospholipids undergo gel-to-liquid phase transition temperature (Tm) because of the enhanced permeability of lipid bilayer[5].

The most vital option of treatment for malignant tumor is chemotherapy. To overcome the disadvantages of chemotherapeutic agents like lack of efficacy and tumor specific delivery, encapsulation of drugs in a biocompatible nanoparticle like liposomes are developed. Thermosensitive liposomes are designed in such a way that it delivers the drug content at the tumor site, where the drug enters into the tumor cells in its free form. For the treatment of sarcoma of soft tissues, the chemotherapy is combined with hyperthermia. At the clinically allowed temperature the levels of drug are increased due to hyperthermia which leads to enhancement in the perfusion rate of tissues, rate of flow of blood is enhanced and microvessels permeation is increased. The permeability of tumor vessels to the antibodies is enhanced by the hyperthermia[6].

Advantages of thermosensitive liposomes and hyperthermia

Thermosensitive liposomes are used in the combination of hyperthermia, as it intends in the direct deracination of tumors at high temperature.

The potency of chemotherapy is improved by the use of hyperthermia.

The cytotoxic effects are enhanced as the oxygenation and perfusion of the cancer cells are increased.

The susceptibility of the tumor cells is enhanced temporarily and the vasculature permeability of cancer cells are increased by the combination of hyperthermia and TLS.

TLS and hyperthermia in combination causes the liposomes bilayer to melt and convert to fluid state to release water soluble content of anticancer agents in the area of heated cancer cells[7].

Materials used in Thermosensitive liposomes

Surfactant

Polyoxyethylene (20) stearyl ether also called as (Brij 78) which comes under the classification of non-ionic surfactant can be used as a surfactant in the preparation of thermo-sensitive liposomes. This surfactant usually consists of PEGylated acyl chains with the composition of 96:4 molar ratio of DPPC and Brij78 and this formulation was compared with another TSLs containing lysolipids. It was confirmed from the results that the DPPC containing TSLs showed the highest drug release at 40-41ºC when correlated to lysolipid TSLs and the stability was found to be at 37-38ºC.

Another surfactant known as Poloxamer 188 (P188) was used by Tagami where P188was incorporated in DPPC liposomes and the results showed that the drug content was released at 42ºC instead of 37ºC[8]. This P188 has many medical applications like it has the ability of having the protective effect on damaged cells and tissues[9].

Lysolipids

Lysolipids are considered as one of the main fundamental component in the TSLs formulation, one such example used is 1-myristoyl-2-stearoyl-sn-glycero-3-phosphocholine which in small amounts

are added into DPPC liposomes causes stabilization of pores in lipid bilayer as the transition of these liposomes from gel to liquid occurs and also enhances the release rate of the drug of DPPC liposomes at the temperature of 39-40ºC[10].

One more example which is used in the preparation of TSLs is basically a lysolipid known as Mono Palmitoyl Phosphatidyl Choline (MPPC), which under mild hyperthermia undergoes the drug release in faster rate[11].

Winter N D *et al*. demonstrated that the lysolipid is essential to increase the rate of release of arsenic from DPPC liposomes which is compared at the temperatures of 37 and 42ºC. The lysolipid used here is MPPC in two concentrations of 5mol% and 10 mol%. These two TSLs formulations were heated rapidly at 42ºC, results depicted that there was a release of very large quantity of arsenic in 1st hour[12].

Another example of lysolipid is a natural component which is platelet activating factor (PAF) and this has the monoalkyl chain and doesn't have any adverse pathological events and shows better release when compared to DPPC liposomes. Results shows that PAF has the property to induce the release of the drug effectively in TSLs[13].

Polypeptides

The polypeptide such as collagen-like polypeptide are the reactive material in TSLs formulation. Park et al demonstrated that the elastin-like polypeptide is used as heat triggering agent in TSLs formulation. Membrane stabilizing agent used here was cholesterol. The last formulation was optimized which consisted the molar ratio 55:2:15:0.41 of DPPC/DSPE/Cholesterol/ELP which showed the release of the drug effectively and blood circulation was stable under mild hyperthermia[14].

PEG lipids

DSPE-mPEG2000 is a PEGylated liposomes which is used in the formulation of TSLs. PEG lipids in the low concentrations decreases the vesicle size without destruction of the structure[15]. Banno *et al*. demonstrated that the DSPE-mPEG2000 when incorporated in the formulation of lysolipid containing TSLs (LTSLs) enhances the release of the LTSLs and they also cause the stabilization of the capillary lipid bilayers edges. The results showed that when 4mol% DSPE-mPEG2000 was added into the formulation of LTSLs, the transition temperature (T□) was increased by 1ºC. The lysolipid when binds to the DSPE-mPEG2000 causes the formation of pores which are nano in size with the diameter of about 10nm which retains in the bilayers of lipid, which enhances the release rate. Li *et al* 2010 investigated that DSPE-mPEG2000 of 5 mol% protects the TSLs from leakage in the plasma at the temperature of about 37°C and the results showed that it has good release profile in mild hyperthermia condition.

Advances in thermosensitive liposomes for controlled delivery

Polymer-modified thermosensitive liposomes

Incorporation of synthetic and natural polymers in the composition of lipids is found to be most effective method. The thermal sensitivity of TSLs can be increased using thermosensitive polymers because they have the function of temperature response and this is the major advantage as compared to non-thermosensitive liposomes. LCST is the low critical solution temperature which is exhibited by the thermo-sensitive polymers which is used in the modification of liposomes. The ability of hydrogen binding is reduced above the LCST which usually leads to the formation of less saturated polymer chains and causes decreased phase separation. The LCST is dependent on the efficiency of hydrogen binding and this can be altered and optimized depending on the monomer content which can be either hydrophilic or hydrophobic. The bilayer lipid membrane undergoes membrane disruption due to the phase transition; this disruption is because of the use of polymers which are thermosensitive in nature which further leads to enhancement of drug release[16].

NIPAM is the most common polymer which is thermo-sensitive in nature and it is known as Poly(N-isopropylacrylamide), which is generated from the polymer poly (N-substituted-acrylamide). The LCST of the NIPAM is around 32°C, which is a very acceptable temperature. Because it is close to the body temperature of 37°C, it is used in biological fields[17].

Kono et al. demonstrated the release of the fluorescent dye by considering two formulations that is DPPC liposomes and non thermosensitive EPC (egg phosphocholine) in combination witha

copolymer of NIPAM and an ODA, results showed that there was increase in the fluorescent dye release which was entrapped in the liposomes whose temperature was above LCST, it was also observed that both the formulations below the LCST showed minimum release[18].

DOPE liposomes coated with poly (NIPAM-ODA) showed better release of calcein at the temperature of 40^{o}C. By using hydrated NIPAM chains the DOPE lipids can be neutralized, they have the ability to form a hexagonal phase on bilayer lipid membrane with the use of destabilizing effect. As the dehydration of polymer chain occurs above the LCST, the stability of the polymer decreases. This leads the liposomes to be unstable which ultimately leads to release of the drug.

Poly[2-(2-ethoxy) ethoxyethyl vinyl ether] (EOEOVE) is another example of the polymer used in formulation of thermo sensitive liposomes. Aoshima *et al*, studied that this polymer undergoes transition in the structure, it transforms from a coil which is hydrophilic in nature to the hydrophobic globule. At the temperature of around 40°C the transition occurs which leads to increase in the ability of the drug to sustain inside the formulation and they are found to be stable at any temperature. The results confirmed that this polymer modified TSLs shows enhanced release at the temperature above 40°C and reached the emptying completely within 1 min at around 45°C.

Copolymers were synthesized using the polymer EOEOVE, the polymer was known as poly [2-(2-ethoxy) ethoxyethyl vinyl ether-block-octadecyl vinyl ether] with 40°C LCST which acts as the anchor units whereas the polymer EOEOVE behaves as thermo-sensitive moiety[19].

Katagiri *et al* worked on the PTSL to determine its efficacy against cancer, Fe_3O_4 nanoparticles which are hydrophobic in nature were incorporated into the lipid bilayer containing the polymer poly (EOEOVE-bODVE) and it was bonded through the hydrophobic interactions. The release of the fluorescent marker was increased by heating the Fe_30_4 nanoparticles[20].

The polypeptide which is elastin in nature consists of Val-Pro-Gly-Val-Gly chain units are incorporated inside the bilayer of lipids which is known to have a property of thermo-responsive. The plasma half-life of TSLs is increased by this polymer. The hydrogen bonding between these water molecules and ELP causes the swelling below the transition temperature, and below the LCST it causes drying which decreases the flexibility of the bilayer[21].

Park *et al* developed the ELP by modifying the structure which confers the thermo-sensitivity to liposomes, this modified ELP at physiological temperature brings about a rigid membrane, enhancing the stability of vesicle during blood circulation. The bilayer of lipids which contains DPPC/DSPE-PEG_{2000}/CH liposomes is associated with the N-terminal portion of ELP linked to single stearyl group (C18). The results showed that this formulation has the highest stability and at mild heating this showed enhanced release of DOX liposomes. After At 42°C, ELP-liposomes can release more than 95 percent of their medication content in 10 seconds, according to the researchers, compared to less than 20 % in 30 minutes at 37°C in serum. The intended formulation has a plasma half-life of 2.03 hours, whereas LTSL has a half-life of 0.92 hours. After one injection intravenously, the growth of tumor was found to be delayed when ELP liposomes were used in the combination with the ultrasounds which is of high intensity when compared to LTSL[14].

Another polymer known as P(NIPAAm-co-DMAPAAm)-DOPE was synthesized, and by considering the physicochemical properties this polymer was modified to DOTAP/DOPE liposomes to enhance the release of liposomes and cellular uptake. This modified liposome is referred as thermo-sensitive liposomes. Results showed that the thickness of the hydrated layer of thermo sensitive liposomes were same as that of the PEGylated liposomes below the LCST. It was concluded that at around 40°C these liposomes were stable and there was no release. These liposomes showed enhanced cellular uptake and the liposomal contents were released in the cytosol above the LCST and the release was found to be more than the PEGylated liposomes. This modified thermo-sensitive liposome P(NIPAAm-co-DMAPAAm) is highly used as the carriers for intracellular drug- delivery[22].

Traditional thermosensitive liposomes

The T_m of the TSL must be higher than the physiological temperature to achieve the therapeutic success of the TSLs. Mabrey et al worked on the TSL formulation where the DPPC was used as one of the major compound in the formulation of TSLs. This DPPC has the T_m of about 41.4°C.

Iga *et al*, studied that the level of the cytotoxic drugs was increased at the tumor site by using the combination of TSL and hyperthermia. The therapeutic effect of formulation was also enhanced. To improve the stability of vesicles and to enhance the release rate of the drugs, mixtures of DPPC and other phospholipids were designed in the formulation of TSLs[23].

Gaber *et al* studied that the liposomal circulation is enhanced with the increase in the release of the content upon heating when the components like cholesterol (CH) and other modified-polyethylene glycol (PEGs) were incorporated into the bilayer of lipids. To optimize the stability of vesicles in serum Cholesterol was added. The cholesterol reduces the leakage of the drug at 37°C, below the T_m temperature in lipid bilayer[24].

Dicheva *et al*, investigated that the use of the cationic lipids has showed better carcinogenic activity. Cationic nanocarriers delivers the drug having anticancer activity to the cancer cells and angiogenic endothelial cells. Compared to non-cationic formulations this formulation showed better targeting to the tumor cells[25]. Pradhan *et al*. Studied about the multi-functional approach using TSL formulation. The thermo-sensitive magnetic liposomes were formulated which was targeted using folate receptors. This formulation showed active targeting of the drug and the pharmacological effect was seen which was used for the treatment of cancer using thermo-chemotherapy[26].

De Smet *et al*, reported that combination of Doxorubicin and Gadolinium (GD) was used in the diagnosis of cancer and its treatment. In order to achieve local and deep hyperthermia, the high-intensity focused ultrasound (HIFU) was combined with the magnetic resonance imaging (MRI). The results showed that when the concentration of doxorubicin was increased to 8-fold in the cancer cells, the radiolabeled TSL was increased to 4-fold[27].

Affram *et al*, formulated two TSLs delivery system with two drugs Gem and Gadolinium (GD). These two drugs were incorporated into the TSLs, Gem is poorly impermeable drug which was encapsulated into the TSL. The results showed that the anticancer efficacy of the Gem was enhanced and the distribution of Gem and GD in tissues and organs was also increased[28].

Li *et al*, formulated TSLs by incorporating DSPE-PEG_{2000} into the lipid's bilayer and the grafted polymer on the surface of the liposomes was evaluated for the optimal concentration. This was formulated to increase the efficacy of the release under mild hyperthermia. The results demonstrated that the 5% mol of DSPE-PEG_{2000} incorporated into the bilayer was enough for the stabilization of the membrane of lipids in serum at physiological temperature and the release rate was increased at 42°C. It was also studied that the DSPE-PEG_{2000} of higher density causes the collapse in the membrane integrity which leads to the release of CF. to enhance the therapeutic performance of the formulation DPPC/DSPC/DSPE-PEG_{2000}, two steps mild hyperthermia was proposed. In first step at 41°C which is considered to be mild hyperthermia enhances the permeability of tumor vasculature and it also enhances the accumulation of the liposomal drug intratumorally. In the second step the release rate of the drug is promoted which leads to minimization of the distribution of the drug through circulation. This type of approach is termed as the interstitial approach. The results showed that the accumulation of the liposomes and the bioavailability of the DOX was enhanced in the site of tumor in two step approaches as compared to the one step treatment[29].

Al-Ahmad *et al*, formulated a new type of thermosensitive vesicles in which the leucine zipper peptides were incorporated into the bilayer membrane of lipids and their *in vivo* activity was checked using two approaches. In the first approach the interstitial release method was used and in the second method the intravascular release protocol was introduced, in this method during the heating process the TSL was introduced which results in the release of the drug into the blood vessels when it comes in contact with the heating area. The results showed that both the methods were effective but it

was found that in intravascular approach the suppression of growth of tumor was higher[30].

Multi-functional thermosensitive liposomes (MTSL)

The thermo-sensitive liposomes which consist of the combination of cyclic argininеglycine-aspartic acid (cRGD) was formulated by Kim et al. Results demonstrated that the accumulation of tumor was enhanced by targeting to $\alpha_v ß_3$ integrin, this is over expressed in the vasculature and in malignant tumors. The results showed that there was about 7-fold higher cellular uptake of multifunctional vesicles[31].

Campbell *et al*, demonstrated that use of cationic nanocarriers delivered the anticancer drugs selectively to the tumor site with the electrostatic interactions with phospholipids, anionic glycoproteins and proteoglycans on the vasculature of tumor. The accumulation of the drug is facilitated by the irregular and slow blood flow and hyperthermia at the cancer site which enhances the carrier extravasation[32].

Dicheva *et al*, formulated cationic thermo-sensitive liposomes which consists of DPPC, DSPC, DSPE-PEG_{2000} and the lipid DPTAP which is cationic in nature. The results showed that these cationic TSLs have high stability and the kinetics of release is similar to non-cationic TSL. The binding levels of cationic TSL as compared to the non-cationic TSL is higher to the benign metastatic leiomyoma (BML) and human umbilical vein endothelial cells (HUVEC). Due to their smaller size the cationic TSL internalize into the tumor and the CF release is enhanced when the temperature is triggered which improves the therapeutic outcome. It was also demonstrated that encapsulation of DOX enhances the effectiveness of the TSL and also the release of the drug was triggered by mild hyperthermia. The results showed that dual targeting approach was successful[33].

In 2016 yang *et al*, formulated multi-functional thermo sensitive liposomes for the delivery of small interfering RNA (siRNA) specifically. Two main strategies were combined to give smart multifunctional TSL in a single carrier. In the first step the direct conjugation of CPP (cell penetrating peptide) with the siRNA occurs through the disuphide bonds. It is used in the determination of glutathione carrier sensitivity. In the second step TSL consisting of Asparagine-Glycine-Arginine were encapsulated by siRNA-CPPs which has the target function of vasculature. The results showed that as compared to siRNA-CPPs the siRNS-CPPs/NGR-TSL had better in vivo tumor activity and enhanced efficiency in gene silencing[34].

Negussie *et al*, formulated a novel cyclic Asn-Gly-Arg. The results demonstrated that the $CD13^+$ cancer cell was actively taken up by the peptide whereas the binding was less to $CD13^-$. The results showed that this formulation had greater affinity of about 3, 6-fold when compared with the linear form and conjugation on the liposomal surface of LSTL[35].

It has been demonstrated that the use of a single strategy is seldom sufficient to enhance the efficacy of anticancer therapy. Therefore, carriers that combine different strategies in one system, making them clinically more effective, are being designed and developed. When the active or passive targeting strategies are combined with the thermal triggering the approach has been successful[36].

Impact of lysolipids on thermo-sensitive liposomes (TSL)

The lysolipids (LPs) are phospholipids which have lost one or both of their acyl groups. They are readily incorporated into the membrane easily due to its non-cylindrical structures and the physical and chemical properties of lipid bilayers are altered. The properties like permeability of membrane, morphology and stability are modified. Due to the particular geometry of lysolipids, the incorporation of even a small amount of LP would result in a destabilization of the membrane and reduce its ability to act as a barrier[37]. The decrease in Tm must occur. Based on clinical trials, mild HT of ≤ 43°C should be used since higher temperatures may cause hemorrhages or permanent damage to the surrounding healthy tissue[38].

Anyarambhatla and collaborators proposed a lysolipid-modified TSL in 1999, which incorporated LP in a PEGylated TSL, with the objective of enhancing drug release by reducing the Tm phase transition. DPPC/MSPC/DSPE-PEG2000 is the formulation which is in the 90:10:4 molar ratio[39].

The traditional TSL were typically capable of releasing a drug after 30 minutes of contact, while

the inclusion of lysolipids into the liposome's bilayer allows the release of drug within seconds.

De Smet *et al*, studied about the simultaneous release of two components doxorubicin and gadolinium which is used in MRI as a contrasting agent. They used two different thermo-sensitive systems: a liposome-based sample of DPPC/HSPC/CH/DPPE 2000 and a liposomal system DPPC/MPPC/DPPE 2000. LTSL formulation also had a faster release rate than conventional TSL formulation at 42°C, as expected from an ideal thermo-sensitive system, but this formulation caused leakage of doxorubicin at the temperature of about 37°C[40].

The presence of lysolipids actually determines a vesicle's sensitivity to a serum by allowing the liposomal bilayer to interact with HSA or exchange and merge with the cellular membranes. Co-encapsulating MRI contrast agent showed that there was no alteration in the loading or release of DOX and this resulted in the simultaneous release[41].

Hossann *et al*, developed a new TSL formulation with a longer plasma half-life based on DPPGOG, rather than PEGylated lipids, which is a usual method. In the presence of foetal calf serum, three potential formulations were designed and compared: DPPC/DSPC/DPPGOG 50:20:30, DPPC/P-lyso-PC/DSPEPEG2000 90:10:4 and PEGylated based TSL. When compared to PEGylated liposomes, the DPPGOG-based formulation exhibited better stability at 37°C. CF was persisted in serum for up to 10 hours at 37°C in the DPPC/DSPC/DPPGOG sample, whereas PEGylated TSL became unstable after 6 hours. DPPGOG was shown to enhance membrane permeability in a way analogous to P-lyso-PC, releasing more than 70% of CF at their Tm[42].

When CF was supplanted with DOX, all of these characteristics were retained. In point of fact, DOX was held by DPPGOG-TSL in the percentage of 89 which was particularly released at the temperature of held 89% of 42°C. Finally, DPPGOG was able to improve the liposomes *in vivo* half-life, comparable to DSPE-PEG2000, and maximize drug release, equivalent to P-lyso-PC, without impacting TSL stability.

Woo *et al*, developed Cisplatin-loaded LTSL in 2008, implementing drug encapsulation procedure called passive equilibration, which combines drug loading in preformed LTSL. The cisplatin was found to be released from LTSL at 42°C in 5 mins[43].

Zhang *et al* studied the potential of LTSL for administering high molecular weight molecules. The model drug fluorescein isothiocyanate conjugate-albumin (MW 66 kDa) was encapsulated into liposomes, demonstrating not only authorized at physiological temperature, but the release behavior at 42 and 44.5°C at faster rate was also studied.

Only Brij78, according to the results, can be optimally incorporated either using the thin film method or the post-insertion technique, yielding in formulations that outperform traditional lysolipid-based thermo-liposomes. Brij78-based formulations, in instance, exhibited similar stability to LTSL at 37°C and quick DOX release at 40–42°C within 2–3 minutes[44].

New thermo-sensitive vesicles based on alkyl phosphocholines, a novel family of anticancer and antiprotozoal compounds chemically related to lysophospolipids, have recently been investigated. HePC can serve as both a drug and a carrier constituent, offering the formulation a unique and wonderful functionality, permitting the use of fewer excipients and also enhancing the biocompatibility which leads to be a suitable candidate for various biomedical applications[45].

Lindner *et al* opted to test the efficacy of the HePC to induce a burst release from DPPGOG-based TSL via mild hyperthermia in 2008. When HePC were integrated into the liposomal membrane, it elevated the CF release rate similarly to lysolipids and had a better therapeutic efficacy against cancer cells than micellar formulation[46].

Future prospects

Thermo-Sensitive Polymersomes (TSP)

Liu *et al* formulated the polymersomes which consists of hydrophilic poly (N-vinyl caprolactam) which is attached to hydrophobic core PDMS poly (dimethylsiloxane), resulting in a thermo-responsive bola amphiphile and performed a comprehensive study. When the temperature rises, the PVLC block collapses and combines, reducing the size of Ps and enhancing membrane permeability, enabling DOX to be discharged. Also essential are the hydration

and dehydration of PVLC blocks linked to the hydrophobic PDMS block, because it is predicted that hydrophilic molecules may overcome the hydrophobic PDMS layers at elevated heat due to the presence of transitory openings. The cytotoxicity of DOX was evaluated on the human alveolar adenocarcinoma A549 cell line, with cell viability lowering from 85 percent to 59 percent and from 71% to 50%, respectively, for polymersomes containing 0.1 and $0.5gmL^{-1}$ of drug. Therapeutic efficacy for 0.1 and 0.5g mL1 DOX-loaded vesicles terminated after 48 hours, while it continued for 1 and 5 g mL1 DOX-loaded vesicles until 72 hours, the cytoxicity of the cell was achieved by 75% and 97% respectively[47].

A number of n-poly(N-vinylcaprolactam) poly(N-vinylcaprolactam) poly(N-vinylcaprolactam) (dimethylsiloxane) Various PVCL proportions have been used to make 65-poly (N-vinyl caprolactam) n(PVCLn-PDMS65-PVCLn) copolymers, the stable vesicles are formed between 0.36 and 0.52 PVCL ratio. The size of polymersomes reduces as the temperature rises, depending on the length of the PVCL chain. The volume of the vesicle is decreased as the temperature increases from 35-55°C. PVCL19-PDMS65-PVCL19, on either hand, has a hydrodynamic diameter increase from 300 to 800nm. Furthermore, the researchers evaluated DOX discharge fromPVCL15-PDMS65-PVCL15 PVCL10-PDMS65-PVCL19, and PVCL15-PDMS65-PVCL10 derived formulations at the temperature of 25 and 42°C in a fluid-simulating site of tumor, attaining released drug percentages of 86, 29, and 11 percent at 42°C, respectively, and displaying a significant effect on PVLC length[48].

The use of thermo-sensitive amphiphilic polymer in the development of stimuli-responsive polymersomes to further control the release of medicines has gained significant a lot more interest. Li *et al* formulated the component known as b-poly(N-isopropylacrylamide) from poly[N-(3-aminopropyl)-methacrylamide hydrochloride]: the polymer turns insoluble in water when the temperature is elevated above the LCST of the PNIPAAM chains, and the monomers self-assemble into the vesicular form.

Methods of preparation of thermosensitive liposomes

Incubation of Blank Liposomal method (IBL)

Lipids (DPPC, DSPE-PEG2000, w/w, 10:1) were solubilized in chloroform and placed on a rotary evaporator to extract the organic solvent at a low pressure (0.1MPa) and form a film on the flask wall. With a 9 percent sucrose solution, the film was hydrated at 55°C. The suspension was sonicated at 100W for 3 minutes, resulting in a clear blank liposome suspension. To regulate the particle diameter, the liposomes were extruded via a polycarbonate membrane. The liposome was filled with L-OHP and incubated at 55°C for 3 hours. Dialysis (3500kDa) was used to remove the non-encapsulated L-OHP from the formulation for 12 hours. The final liposome (LCTL) was made with DPPC, DSPEPEG2000, and L-OHP, with a lipid to drug ratio of 7:5:1.

Reverse-Phase Evaporation method (Rev)

The most prevalent technique for encapsulating water-soluble medicines inside liposomes is REV. To begin, 6mL organic solvents (chloroform: ether=2:1, v/v) were used to dissolve lipids (DPPC, DSPE-PEG2000, w/w, 10:1). In the mentioned organic solvents, two milliliters of L-OHP solution (4mg/mL) in glucose 5% were added, and the mixture was sonicated at 200W for 5 minutes. The organic solvents in the mixture were then extracted using rotary evaporation. The most prevalent method for encapsulating platinum compounds is REV. Chloroform was used to dissolve the lipids. At a 3:1 (v/v) ratio between the organic and aqueous phases, the aqueous phase containing L-OHP (4mg/mL) dissolved in glucose 5% was added to the organic phase. The lipid-to-drug ratio was fixed at 7:5:1 (weight-to-weight). The chloroform was extracted using a rotary evaporator after the mixture was sonicated at 200W for 5 minutes. During the evaporation, a distinct liposome developed. The liposomes were extruded through a 0.22m membrane and dialyzed in the same way as IBL blank liposomes were incubated.

Applications of thermosensitive liposomes

TSL can be used in combination with the contrasting agent which is encapsulated, used to differentiate between heated and unheated tissues. Supplementing existing MRI thermometry methods

and thereby functioning as a quality assurance tool in patient thermotherapy. When the contrast agent and drug are both encapsulated in a TSL formulation, it has been demonstrated that quantitative drug release estimation based on T_1 relaxation time changes is possible, allowing "drug dose painting" "chemo dosimetry". Because MRI does not detect the drug itself, a link between the contrast agent and drug release had to be developed.

LTSL were loaded with doxorubicin actively using a manganese (II) gradient. The paramagnetic manganese (II) serves as an MRI contrast agent, and doxorubicin and manganese (II) create a stable combination. As a result, the contrast agent and the medication have the same release kinetics, allowing for a link between the change in T_1 relaxation time (as assessed by MRI). It was feasible to demonstrate that doxorubicin release in the tumor model was diverse, and that LTSL delivered during hyperthermia had the strongest anticancer effect when compared to other delivery regimens using this strategy. The above method has a huge disadvantage in terms of manganese toxicity (II).

Other researchers are working around this by employing gadolinium-based contrast agents that have been approved by the FDA. Hossann *et al* studied six contrasting agents for encapsulation in DPPG2 -TSL, and found that a nonionic contrast agent with a minimal contribution to osmolality was the best choice.

Using gadolinium-based contrast agents, two encapsulation techniques are feasible, but the release kinetics and signal mechanisms for both the contrast agent and the medicine must be considered. The two subsets of TSL were combined in which one was used by encapsulating the contrast agent and the other was used by encapsulating medication. This method allows for more contrast agent and medicine to be encapsulated while avoiding osmotic effects. The second approach is to coencapsulate both the medicine and the contrast agent in the same TSL, limiting the amount of both components in each TSL. Nonetheless, it must be ensured that the temperature-dependent medication release rate and the MRI signal change are connected for both procedures.

Nephrogenic systemic fibrosis, an uncommon side effect in patients, is a significant risk linked with the use of a gadolinium-based contrast agent in clinical practice. A combination of a lower glomerular filtration rate and a protracted retention period, as well as gadolinium transmetallation, is the pathophysiological mechanism (III)[45].

De Smet *et al* studied that the blood dynamics and biodistribution of TTSL with coencapsulated doxorubicin and gadolinium, finding substantial clearance with 0.3 percent of the administered dose in all analyzed organs one month after injection. However, more research into the dangers associated with this technique appears to be required before it can be used in humans.

CONCLUSION

As discussed above thermosensitive liposomes are targeted delivery which releases the encapsulated drug when heated to temperatures around 40-42°C, the delivery of the drug to the targeted site is considered to be very precised when the TSLs are merged with hyperthermia. Thermosensitive liposomes play vital role in cancer therapy largely due to enhancement of vascular permeability of the drugs to targeted site while the normal tissues are left intact and also boosts the permeability of the drugs into the bilayer lipids. The major upper hand of thermosensitive over the other drug delivery system is faster onset of action. Phospholipids go through a gel to liquid phase transition temperature because of increased permeability of the lipoid bilayer due to the use of high intensity focused ultrasound (HIFU) and therefore contents are released faster. The frame of thermosensitive liposomal preparation comprises of surfactants like polyoxyethylene (20) stearyl ether and poloxamer 188. Similarly, lysolipids like Mono Palmitoyl Phosphotidyl Choline (MPPC), platelet activating factor (PAF) are used that enhance the release of the drug. Another component used in the preparation is polypeptides such as collagen like polypeptides they act as heat triggering agents in the formulation. Lastly and largely the most important compound is PEG lipids which lowers the size of the vesicle and boosts release of thermosensitive liposomes, the example used in this is DSPE-mPEG2000. The advances seen in the thermosensitive liposome carrier system for controlled delivery has previously been discussed with respect to the subject such as

polymer modified thermosensitive liposomes, traditional thermosensitive liposomes, multi-functional thermosensitive liposomes, impact of lysolipids on thermosensitive liposomes, thermosensitive polymersomes. Spotlight is added on the method of preparation of thermosensitive liposomes like incubation of blank liposomal method and reverse phase evaporation method. From the above content it is conclusive that the combination thermosensitive liposomes and hyperthermia are used in the treatment of cancer. Online monitoring of the heating focus, calculating locally released drug concentrations, and externally directing drug release by steering the heating focus and power are all options with MRI-guided drug administration. The unique feature of this nanotechnology technique in medicine will be the combination of external targeting with TSL and MRI-guided medication administration. Overall, this review article on thermosensitive liposomes for tumour targeting should help as a core reference material to better understand the role of TSL in the treatment of cancer and also help in upcoming advances.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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