REVIEW ARTICLE

Nanoparticulates with drug release based on temperature change

Nanočásticové systémy uvolňující léčivo při změně teploty

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Summary

The stimuli-induced release systems are able to respond to an external stimulus resulting in drug release in a controlled pattern. The origin of the external stimuli may be of physical, chemical or biological nature. Thermo-responsive delivery systems respond to the change in temperature and they were mainly designed in order to be used in the cancer treatment method using elevated temperature, i.e. hyperthermia. The thermo-responsive systems can be divided into several groups, such as thermo-responsive hydrogel polymer systems, liposomes, nano- or microparticles, and polypeptide-drug conjugates. While liposomes are temperature-sensitive by their nature, the other systems are usually based on thermo-sensitive polymers, namely poly-(*N*-isopropyl-acrylamide). This article summarizes recently available items of information regarding thermo-responsive drug delivery.

Keywords: drug delivery system • thermo-responsive system • poly-(*N*-isopropyl-acrylamide) • liposome • nanoparticle • polypeptide-drug conjugate

Souhrn

Lékové transportní systémy reagující na vnější podněty jsou schopné uvolnit léčivou látku požadovaným řízeným způsobem v závislosti na spouštěcím mechanismu. Spouštěcí mechanismus může být fyzikální, chemické nebo biologické povahy. Termoresponzivní lékové transportní systémy odpovídají na změnu teploty a byly navrženy zejména k léčbě rakoviny metodou využívající působení zvýšené teploty, tj. hypertermii. Termoresponzivní systémy lze rozdělit do několika skupin, např. termoresponzivní hydrogelové polymerní systémy, lipozomy, nano- nebo mikročástice a polypeptidové konjugáty s léčivem. Zatímco lipozomy jsou citlivé na zvýšení teploty již svým složením, ostatní systémy jsou obvykle založené na termosenzitivních polymerech, zejména poly-(*N*-izopropyl-akrylamidu). Tento článek shrnuje poslední dostupné informace týkající se cíleného uvolňování léčiv v závislosti na změně teploty.

Klíčová slova: lékový transportní systém • termoresponzivní systém • poly-(*N*-izopropyl-akrylamid) • lipozom • nanočástice • peptidový konjugát s léčivem

Introduction

Controlled-release drug delivery systems (second and third generation dosage forms), which predetermine the drug release profiles and the drug bio-distribution due to the unique properties of the delivery system, have gained great interest in pharmaceutical research and development throughout the world. The drug release from the second generation drug delivery systems may have the profiles of a prolonged release, a delayed release or a pulsatile release. The third generation of the drug delivery systems may influence the drug bio-distribution and target the drug to the specific site (organ, tissue, cells).

Second generation dosage forms have been developed, introduced on the market and described in many articles[1–5]. They already contribute to safe and efficient pharmacotherapy. However, the third generation dosage forms based on a specific drug release in the demanded part of the body are the objective of present international research. These sophisticated systems can respond –

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i.e. release the included drug – to some stimuli. That is why these systems are also called stimuli-responsive systems[6].

The stimuli-induced delayed release systems, also known as "smart" or "intelligent" systems, are able to receive and process an external stimulus, to which they consequently respond, resulting in drug release (Fig. 1). The system response to the stimuli may be based on a change in the system properties such as the volume, the secondary structure, the phase, the solubility, or the permeation. The alteration of the system physical or chemical behaviour leads to the release of the incorporated drug in a controlled pattern. The origin of the external stimuli may be physical, e.g. temperature change, electric or magnetic field, ultrasound; chemical (pH or glucose change), or biological triggered by antigen or enzyme[7, 8].

Temperature-induced release systems

The well-known fact that physiological temperature changes in the presence of pathogens or pyrogens was the main idea of developing the thermo-responsive (TR) systems. Another motive for designing TR systems is the cancer treatment method using the externally applied, induced hyperthermia targeted to the tumor tissue. The adjuvant effect of hyperthermia (heating the tumor tissues to temperatures by 2–8 °C higher than the body temperature) has been clinically proved in the treatment of solid tumors when it is combined with chemotherapy or radiotherapy. It is explained by the fact that the temperatures above 41–42 °C have a direct cytotoxic effect. In addition, hyperthermia usually increases the perfusion of most tumors, which may result in an enhancement of the drug delivery to the tumors[10–12]. The externally applied, induced hyperthermia may also serve as the stimuli to trigger the drug release from the thermo-sensitive systems. The ideal TR system stays intact at the physiological body temperature (T_B = 37 °C), the drug from the system is released after exceeding a certain threshold temperature of the system due to the induced hyperthermia – such a phenomenon is called the active targeting. This implies that after the administration of the drug-loaded TR system to the blood circulation, the drug is released only in the externally heated tumors. This method of the active drug targeting may fundamentally reduce the cytotoxic side-effects of anticancer medicaments and can enhance the efficiency of the tumor treatment[13, 14].

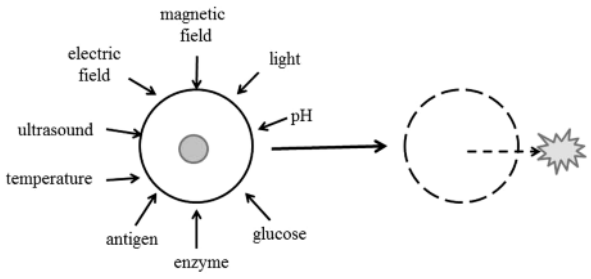

Fig. 1. The illustration of the stimuli-induced release from a drug-loaded particle (modified from[9])

Furthermore, the systems used in the anti-cancer therapy employ passive targeting to the tumors. The passive targeting is dependent on the properties of the drug delivery system and the pathologic conditions of the tumor neovasculature which is disorganized with dead ends, loops and openings into the perivascular space. The presence of the openings enhances the macromolecular or nanoparticulate permeation of the tumor. In addition, the absence of the tumor lymphatic drainage reduces the drug clearance. The occurrence of these two processes is generally known as the enhanced permeability and retention (EPR) effect[15, 16] as shown in Figure 2.

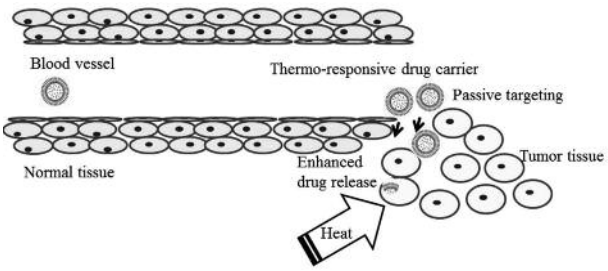

Fig. 2. The scheme of the passive targeting using the enhanced permeability and retention (EPR) effect and the drug release from the thermo-responsive drug carriers in the heated tumor (modified from[21])

However, passive drug targeting itself has failed to achieve increased clinical efficacy in humans when compared with the free drug as a result of several shortcomings. Accumulation of nanoparticles depends on the specific structure of tumor vasculature[17], it competes with uptake in the liver and spleen and less than 10% of the injected dose accumulates in the tumor[18].

To solve this problem, an additional approach for temperature-triggered drug delivery including a pre-hyperthermia treatment was proposed. This pre-hyperthermia method opens up the tumor vasculature for passive accumulation[17] and is followed by a second heat trigger for interstitial drug release[19]. Such formulations might be further surface-modified for active targeting to the tumor vasculature or tumor cells[20].

The thermo-responsive systems can be divided into several groups, such as TR hydrogel polymer systems, TR liposomes and other nano- or microparticles, and TR polypeptide-drug conjugates.

The thermo-sensitive hydrogel polymers are capable of reversible swelling/deswelling dependent on temperature changes. The solubility of these polymers has a non-linear relationship with temperature. Each thermo-responsive polymer is characterized by the specific sharp transition temperature, known as the lower critical solution temperature (LCST), at which the polymer solubility shifts from soluble to insoluble. Various synthetic and natural polymers show the thermo-responsive behaviour, such as synthetic poly(*N*-isopropyl-acrylamide) (pNIPAAm), polyethylene oxide-*co*-polypropylene oxide-*co*-polyethylene oxide, or semi-natural chitosan[22].

The commonly used TR polymer is cross-linked

(pNIPAAm) (Fig. 3) due to the LCST (32 °C) in an aqueous solution near the body temperature. Below the LCST the pNIPAAm segments are hydrated forming expanded structures enabling water to diffuse, the hydrogel absorbs water and swells. Above the LCST polymer chains dehydrate, water is squeezed out and the polymer shrinks, as it is shown in Figure 4. This swelling/deswelling phase transition leading to a volume change is caused by the disruption of the hydrogen bindings between the water molecules and the polymer due to the temperature increase and the consequent interaction within the polymer matrix resulting in the formation of the intra- and inter-molecular hydrogen bonding within the polymer chains. The addition of either hydrophilic or hydrophobic compounds may alter the value of the LCST of the mixture with the polymer[23].

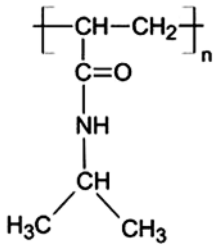

Fig. 3. The chemical structure of pNIPAAm

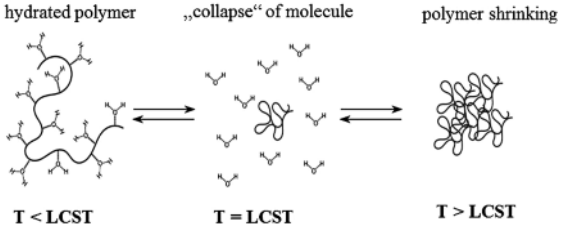

Fig. 4. The thermo-sensitive behaviour of the TR polymer pNIPAAm (modified from[24])

TR ability of pNIPAAm has been applied in numerous temperature-induced drug delivery systems. The transformation process is reversible and repeatable, which enables the formation of the device with an "on-off" model of the drug release. The release mechanism lies in squeezing out the drug during the deswelling phase, or in entrapping the drug within the polymer matrix in the shrunken state, which is followed by amplified drug permeation during the swollen phase[25]. TR drug release profile depends on the system construction (matrix systems, micro- or nanoparticles), as it is described in Figure 5.

Hydrogel systems

Numerous hydrogel systems based on thermo-responsive polymers, especially pNIPAAm, have been synthesized. Thermo-responsive polymeric hydrogel may form polymeric matrices, membranes, or it may be incorporated into micro- or nanoparticle systems. The

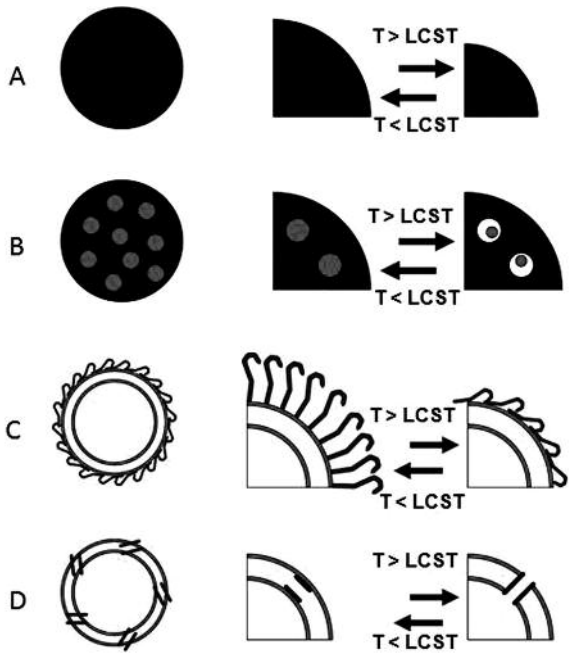

Fig. 5. The TR behaviour of various systems including the TR polymer: A) The hydrogel matrices squeeze out the drug during the hydrogel shrinking above the LCST[26]; B) the grafts of TR polymers within microspheres form pores above the LCST resulting in the drug release[25]; C) the micro- or nano-particles with the surface modified with TR polymer chains[27], below the LCST polymer chains in a form of open coils allow drug permeation, above the LCST the collapsed polymer chains cover the surface and reduce drug permeability; D) the microcapsules with TR gates in the membrane release the drug above the LCST[28–30].

pNIPAAm hydrogel with the LCST = 32 °C has been further modified in order to obtain a system with optimal characteristics such as an accelerated response to temperature[27, 28] or an increased transition temperature of the system[33] in order to employ the induced hyperthermia in cancer-treatment.

Okuyama et al. presented the thermo-responsive indomethacin release from the cross-linked poly(*N*-isopropylacrylamide-*co*-alkyl methacrylate) polymer. The polymer formed a hydrogel matrix with the dispersed drug, which swells below the LCST and the drug is released just via diffusion. Upon a temperature increase, the polymer becomes hydrophobic and shrinks. The shrinking induces the burst drug release due to the "squeezing effect" – the flux of water with the dissolved/dispersed drug is squeezed out from the polymer matrix[33].

The methods that accelerate structural changes of polymer in response to temperature changes have been reported by Kaneko and some other authors[31, 34, 35]. Grafted the cross-linked pNIPAAm hydrogels by the free mobile linear pNIPAAm chains allows a rapid swelling/deswelling transition of the system. The swelling/deswelling kinetics of the grafted gels is dependent on the graft chain lengths, as well as the drug release profiles of the drugs with large molecular weights.

Another method of designing TR drug delivery systems was reported by Oh et al. They used a mixture of

polyethylene oxide-polypropylene oxide-polyethylene oxide triblock copolymer and polyvinyl alcohol, which forms a complex gel via hydrogen bonding in an aqueous solution. These intra/intermolecular interactions are thermo-sensitive. The gel beads of the polymer were stabilized by coating with the poly-(lactide-*co*-glycolide) membrane. The polymer ratio determines the swelling transition properties of the gel. The model drug, acetoaminophen, was released at the temperatures between 35 and 40 °C cit.[35].

The incorporation of TR polymers into the micro- or nano-particulate systems presents another possibility of designing a TR system with suitable properties.

Thermo-responsive liposomes

Liposomes are spherical nanoscale vehicles formed by a membrane bilayer usually composed of phospholipids. The membrane encloses an aqueous core that can be used to encapsulate hydrophilic drugs, whereas lipophilic drugs can be incorporated into the membrane. The liposome phospholipid membranes undergo phase transitions at the particular temperature (transition temperature T_T) from a solid gel phase to a liquid crystalline phase, which increases the hydration of the bilayered membrane leading to a potential drug leakage from the liposome[36, 37], as it is shown in Figure 6.

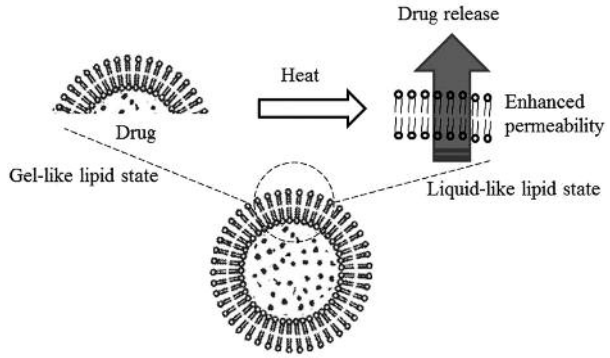

Fig. 6. The scheme of the drug release from thermo-responsive liposomes (modified from[21])

The first TR system designed by Yatvin et al. was based on liposomes of 1,2-dipalmitoylphosphatidylcholine (DPPC). The gel-to-liquid crystalline phase transition of the phospholipid membrane occurred at the temperatures (41 °C) above the body temperature $T_{B.}$ The liposomes became consequently leaky to small water-soluble molecules. Adding a small portion of the co-lipid distearoylphosphatidylcholine (DSPC) to the liposomal membrane helped to adjust the transition temperature to the desired value[38, 39].

At the beginning of the 21st century, Needham et al. prepared the TR liposomes with entrapped doxorubicin. They also used DPPC phospholipid as the structural material of the liposome membrane, but with the addition of 1-palmitoyl-2-hydroxy-*sn*-glycero-3--phosphocholine. Novel liposome formulation improved the delivery properties of the system. The transition temperature was lower (39–40 °C) compared to the transition temperature of the pure DPPC liposome (42 °C); thus the release of the entrapped doxorubicin was exceptionally fast when the system was exposed to the temperature of 42 °C. The ultrafast drug release is regarded to be absolutely essential to enhance the drug permeation of the heated tumor due to the short circulating passage time in the tumor. *In vivo* testing in a human squamous cell carcinoma tumor xenograft model proved the complete response of all tumors and no tumor regrowth was reported during the following 60 days[39]. The TR liposome formulation entered clinical trials, which was a breakthrough in the field visible by approximately 300 citations of the original paper[40].

The incorporation of lysolipids into the membrane bilayer of liposomes introduced a novel approach in the development of clinically usable TR liposome formulations. The surfactant lyso-PC mediates drug release by formation of lysolipid-stabilized membrane pores[41]. The release rate of doxorubicin from lysolipid containing TR liposomes at 41.3 °C was 80% in 20 seconds[42]. This type of liposomes with encapsulated doxorubicin Thermodox® designed by Celsion Corporation is in the Phase III clinical trials to treat hepatocellular carcinoma[42].

In 2004, a new liposomal formulation composed of DPPC, DSPC and 1,2-dipalmitoyl-*sn*-glycero-3--phosphodiglycerol ($DPPG_2$) was reported by Lindner et al.[43]. $DPPG_2$ is a synthetic phospholipid with a molecular weight close to the natural one, differing by one additional glycerol molecule in order to prolong the circulation half-life. The release rate of doxorubicin was very fast at the temperature of 42 °C. Later on, Lindner et al. used a soluble lysolipid to modify the liposome membrane. The phase transition of the designed liposomes occurred at 41–42 °C exhibiting a rapid and efficient drug release in response to a temperature increase, which was proved *in vivo* in rats and hamsters to treat amelanotic melanoma[44].

A sterically stabilized liposomal formulation modified with PEG was developed for improved stability in serum and a better *in vivo* half-life. This formulation had very good stability in serum at 37 °C and the maximum drug release occurred at 42 °C. A two-step treatment, i.e. pre-hyperthermia to open up the tumor vasculature and second hyperthermia after passive accumulation in tumor tissue allowed drug release for precise intratumoral drug delivery[19].

Different approach to improve the characteristics of the drug delivery from the TR liposomes was presented by Tagami et al. TR liposomes with a simplified formulation were prepared by the incorporation of the non-ionic surfactant Brij78 composed of the PEGylated acyl chain (stearyl ether) to the liposome bilayer of DPPC – the molar ratio of DPPC:Brij78 was 96 : 4. The minority component of Brij78 is thought to prolong the liposome circulation and improve the system pharmacokinetics. The drug release from the liposome induced by a mild hyperthermia (40–42 °C) was rapid (100% release in 2–3 minutes). Liposomes had a prolonged circulation time and they were relatively stable in serum at 37 °C temperature. The *in vivo* efficiency and the minimal toxicity of the system were

proved using the mouse mammary carcinoma implanted to mice. Doxorubicin uptake into the cells of the heated tumor was 5.2-fold higher when compared to free doxorubicin[45].

Temperature-induced response of liposomes may also be accomplished by incorporating the TR polymers (e.g. pNIPAAm) into the liposome structure. The fixation of the relatively hydrophilic TR polymers in the liposome membrane is provided via hydrophobic anchors (e.g. octadecyl/pyrene groups), which are incorporated to the polymer backbone. The position of the anchor within the backbone may influence the drug release behaviour. Below the LCST, the hydrophilic polymeric chains surround the liposome membrane improving the liposome stability. Above the LCST, the polymer dehydration and deswelling intensify the hydrophobic character of the liposome membrane leading to the exposure of the uncovered lipid membrane surface and the liposome destabilization and aggregation (Fig. 7). The TR transformation of the liposome surface properties permits the liposomes to interact with the cells. The TR polymers attached to the liposome membrane can also improve the liposome surface properties. Below the LCST, the highly hydrophilic polymer chains surrounding the liposome membrane suppress the interaction between the liposomes and proteins or cells resulting in an improved liposome stability *in vivo*[46].

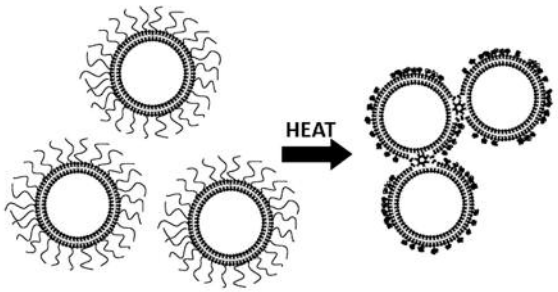

Fig. 7. The scheme of the thermo-responsive behaviour of the liposomes modified with the TR polymers[46]

Yoshino et al. prepared phosphatidylcholine liposomes modified with the TR polymers. The blocks of copolymers of NIPAAm and *N*-isopropylmethacrylamide (NIPMAM) were fixed in the liposome membrane using the anchors bound to the terminal end of the polymer chain in order to obtain the TR system, which would release the drug at the temperatures slightly higher than the body temperature enabling the targeted-specific treatment with the employment of mild hyperthermia. Above the LCST of the copolymer (40 °C), the dehydrated polymeric chains destabilized the liposome membrane leading to the liposome aggregation and the drug release[27].

Other thermo-responsive micro- and nano-particles

Incorporating TR polymer material to the structure of micro- or nanoparticles opens the design opportunities of the drug delivery systems with targeted release. The properties and the size of these systems enable the target-specific delivery of problematic drugs (cytotoxic anti-cancer drugs, macromolecules, insoluble compounds) and genes in order to improve the treatment efficiency and minimize the undesirable side-effects. Microspheres and microcapsules, and various types of nano-particles such as polymeric micelles or dendrimers may be utilized for the temperature-induced drug delivery[14].

The thermo-sensitive microspheres were designed by Curcio et al. The size of microspheres was 10–100 μm, they had a spherical shape and monolithic composition. They were prepared by grafting of NIPAAm with gelatine and parallel polymer cross-linking with *N,N'*-methylenebisacrylamide. Incorporation of hydrophilic gelatine units into the polymeric matrix of pNIPAAm leads to an increase in the LCST of the system (34.6–34.8 °C) compared to the LCST of the pure pNIPAAm (32 °C). Below the LCST the hydrogel swells allowing the enclosed model drug, diclofenac sodium salt, to diffuse slowly to the medium. The burst drug release occurs at 40 °C, when the temperature is higher than the LCST, the pNIPAAm segments deswell and the system collapses. To obtain the delayed release, the system requires further optimization to prevent the diffusion of the drug during the swollen state[25].

Chu et al. presented different designing of the thermo-sensitive microparticles – the core-shell microcapsules with the gating shell. The shell is represented by the outer porous membrane with the gates which are capable of responding to temperature. To obtain the TR gates, the linear polymer of pNIPAAm is grafted to the porous surface of the polyamide microcapsules loaded with the model substance (NaCl or vitamin B_{12}) by the plasma-graft pore-filling polymerization method. At a low graft yield, a positive system response to the temperature was achieved, which means that the release rate increases with temperature (Fig. 8). The linear polymer chains of the gates become hydrophobic and coil up above the LCST allowing the encapsulated drug to diffuse out[28].

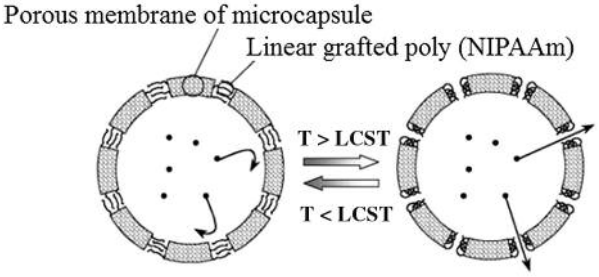

Fig. 8. The thermo-responsive release principle of the micro-capsules with a porous membrane and the TR polymeric gates (modified from[28])

The thermo-sensitive polymeric micelles have recently been subjected to intensive research. The polymeric micelle consists of the amphiphilic block copolymers, surfactants. The surfactants contain hydrophobic and hydrophilic groups within the same molecule. When immersed in an aqueous solution, the interaction between water and the amphiphilic molecules

causes a self-assembly of micelles with a specific structure of a hydrophobic core and a hydrophilic corona. To achieve the micelle self-assembly, the concentration of the surfactants in the solution needs to exceed a certain value – the critical micelle concentration[47]. After incorporating the TR polymer to the amphiphilic block copolymers, the drug may be released in response to the temperature.

The properties and the *in vivo* behaviour of polymeric micelles allow them to act as a drug carrier of the drug delivery systems with targeted release. The exceptional structural properties of the polymer micelles and their nano-size hinder the micelles from being detected by the reticuloendothelial system. The micelles may be passively targeted to cancerous or inflamed tissues through the EPR effect[9, 48, 49].

The thermo-sensitive polymeric micelles may be divided into two categories: polymeric micelles with TR outer coronas and polymeric micelles with TR inner cores. The drug release from each system is based on a different mechanism, as it is shown in Figure 9[16].

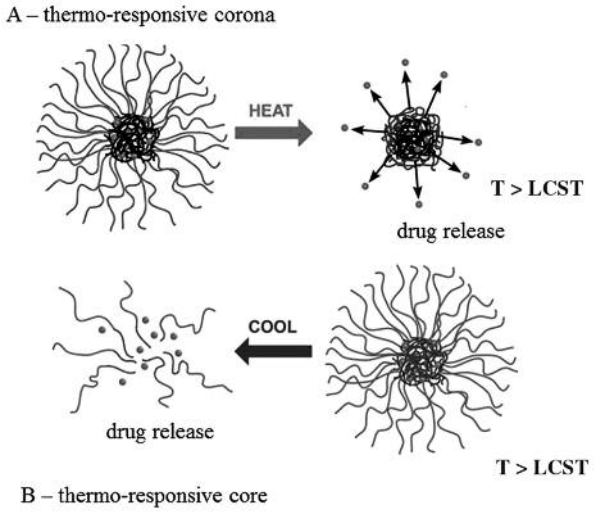

Fig. 9. The drug release from the TR polymeric micelles: A) polymeric micelles with TR outer coronas; B) polymeric micelles with TR inner core (modified from[16])

Polymeric micelles with TR outer coronas are preferentially applied *in vivo*, because a mild hyperthermia is easily attainable. Below the transition temperature the pNIPAAm corona is hydrated and the block copolymers form the micellar structure in an aqueous solution. Above the LCST of pNIPAAm (32 °C) the corona becomes hydrophobic because of the polymer chains dehydration. Thus the micelles aggregate and precipitate leading to the drug release[16].

Polymeric micelles with TR inner cores are composed of block copolymers with hydrophilic segments (e.g. PEG) and TR polymer segments (pNIPAAm). Below the LCST the molecules of copolymers are dispersed in the aqueous solution due to the water-solubility of both copolymer components. Above the LCST the TR polymer segments are dehydrated and hydrophobic resulting in an association of polymer chains. The associated chains form a micelle structure with a core and a corona, as it is displayed in Figure 9B. After the administration of the polymeric micelles to the circulation, the temperature of the certain tissue or organ is decreased in order to obtain the TR drug release with the targeted-specific pattern. The demand of the temperature decrease treatment, hypothermia, limits the application of the system. The benefits of these systems are a fast drug release in response to the temperature, the water-soluble polymer chains are dispersed in the solution and the drug does not need to diffuse from the micelle. Furthermore, the micelle preparation does not require the use of organic solvents and the drug incorporation is facile. The polymeric micelles with TR inner cores composed of polyethylene glycol and poly(*N*-isopropylacrylamide) were designed by Neradovic et al.[50].

Chung et al. prepared TR polymer micelles with outer coronas from the block copolymers of poly (*N*--isopropylacrylamide-*co*-butylmethacrylate (pNIPAAm-pBMA). The hydrophobic core formed by pBMA segments serves as a reservoir of the lipophilic anti-cancer drug – adriamycin, whereas the hydrophilic pNIPAAm chains respond to temperature changes. The efficiency of the system was tested on endothelial cell culture media. The micelles exhibited no cytotoxicity below pNIPAAm's LCST, when the drug is not released. At the temperature of 37 °C, an enhanced interaction between the precipitated micelles and the cells was reported implying that the adriamycin-loaded thermo-responsive micelles possess higher cytotoxic effect than free adriamycin[51]. The authors also studied the intracellular distribution of the doxorubicin released from the drug-lodaed TR micelles within the human breast cancer cell culture under the conditions of mild hyperthermia (42 °C). In comparison with free doxorubicin, the employment of the drug loaded in the TR micelles enhances significantly the cellular uptake of doxorubicin when exposed to a high temperature (42 °C). The improved doxorubicin uptake by cells may be caused by the hydrophobic interaction of the aggregated micelles with the cellular membrane above the LCST. On the other hand, below LSCT the hydrated micelle corona prevents from micelle adhesion to the hydrophobic cellular membrane. The promoted cellular uptake of the aggregated micelles leads to an enhanced intracellular drug release in the heated tumor cells, which maximises the anti-cancer treatment and reduces the toxic effect of the chemotherapy[41, 52].

To be employed in the human body, the system required further adjustment of the value of the LCST and more biocompatible material than the used hydrophobic polymers. Kohori et al. managed to increase the system biocompatibility and the LCST of the system to the value right above the body temperature by utilizing the copolymer of poly-(NIPAAm-co-*N,N*-dimethylacryl-amide) and biodegradable polylactic acid with the LCST of 40 °C to form TR micelle containing adriamycin. The drug molecules were incorporated into the micelle structure during their formation[53, 54]. Instead of polylactic acid, the polymer of poly-ε-caprolactone or the copolymer of poly(lactide-*co*-ε-caprolactone) may also be used[55, 56]. By means of an induced hyperthermia at 42.5 °C, the system may be used for *in vivo* targeting.

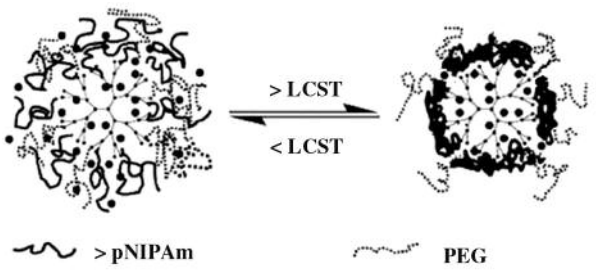

Fig. 10. The formation of the hydrophobic barrier on the surface of the TR dendrimer[57)]

Zhao et al. prepared PEGylated thermo-responsive dendrimers. Dendrimers are synthetic macromolecules, whose polymer chains emanate from a central core. The unique tree-like structure forms internal cavities that enable the encapsulation of water-soluble drugs. The polyamidoamine (PAMAM) polymer forming the dendrimer structure was polymerized with the thermo-responsive polymer of pNIPAAm and the copolymer was subsequently covalently linked with PEG in order to eliminate the dendrimer toxicity and immunogenicity and to prolong the circulation time. The encapsulated model drug, indomethacin, diffuses from the core below the LCST (30 °C), when the pNIPAAm chains are hydrated. Above the LCST (37 °C), the pNIPAAm chains become hydrophobic and shrink creating the water-impermeable barrier around the particle, which prevents the drug from diffusing, as it is shown in the Figure 10. The system response to the increase of the temperature is negative – the release rate decreases[57)].

Thermo-responsive polypeptide-drug conjugates

TR characteristics of certain lipidic and polymeric materials are mentioned above. However, the thermo-responsive systems based on proteins have been designed as well. The biopolymers consisting of a repeated sequence of Val-Pro-Gly-Xaa-Gly (valine-proline-glycine-any amino acid except proline- glycine) pentapeptide, commonly known as elastin-like polypeptides (ELPs), undergo a phase transition at the specific temperature T_T. ELPs are water-soluble below their T_T. When the temperature within the material exceeds the T_T, the ELPs become hydrophobic leading to the protein collapse and aggregation. This effect was first described by Urry et al.[58)].

The T_T of the ELPs is dependent on the ELPs sequence, the chain length, and the concentration. The equation of the three parameters which quantitatively predicts the value of T_T was reported. By varying these three parameters the desired T_T may be obtained[59)].

By employing TR ELPs as a drug delivery vehicle, the targeted release to tumors may be achieved using externally applied hyperthermia. Furthermore, the ELPs are soluble polymers, which tend to accumulate in tumors due to the EPR effect. Raucher et al. conjugated the anti-cancer drug doxorubicin to the macromolecule of ELPs to obtain an anti-tumor drug system with targeted release. Their polypeptide-drug conjugate undergoes the phase transition at mild hyperthermic temperatures (42 °C). The system is composed of four functional domains (Fig. 11). The derivative of doxorubicin is covalently attached to the enzymatically cleavable tetrapeptide, Gly-Phe-Leu-Gly (GPLG, glycine-phenylalanine, leucine, glycine). After the cellular uptake of the system, the GPLP, which is a substrate for the lysosomal protease, is degraded in the lysosomal environment allowing the drug to release. The GPLP is bound to the thermo-responsive ELPs macromolecule. The terminal part of ELPs is modified with the cell penetrating peptide Tat consisting of 11 amino acids which enhances the system binding to the plasmatic membrane. Upon the exposure to a mild hyperthermia (42 °C), the ELPs collapse and aggregate. The protein aggregation and the presence of the Tat significantly enhance the cell uptake of the system. The fact that the liberation of the cytotoxic doxorubicin is intracellular predetermines the system to be highly effective with reduced side-effects[60, 61)]. Furthermore, the authors reported that the Tat peptide fused with ELP prevents the ovarian cancer cells from adhering, spreading, invading and migrating *in vitro* and *in vivo*. It is known that the cancer cell migration and their attachment to the cells play the key role in tumor dissemination and metastasis. Therefore the interaction of the Tat peptide-ELP conjugate with the tumor cells significantly limits the tumor cell migration, which caused the 80% reduction of the overall tumor mass in the *in vivo* mouse model[62)].

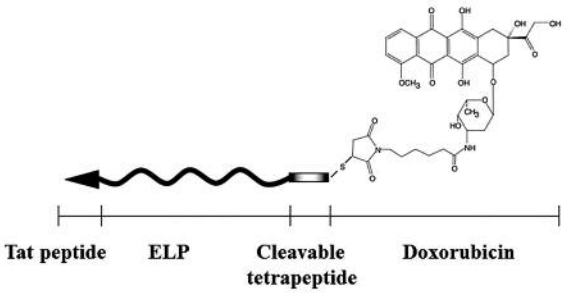

Fig. 11. The scheme of the ELP-based doxorubicin delivery vector (modified from[60)])

In addition, the authors prepared the TR drug-ELP conjugate consisting of the TR ELP fused with a peptide drug and with the cell penetrating Bac peptide that helps the system to be delivered to the nucleus[63)].

Meyer et al. designed a similar ELP-drug conjugate system based on the thermo-responsive ELPs and studied the system biodistribution *in vivo* using the human ovarian carcinoma implanted in mice. The rhodamine-labelled ELPs were applied to mice and the system accumulation in the tumor or the heated tumor was studied with the fluorescence videomicroscopy. The ELP-based system showed a 2-fold higher accumulation in the heated tumor than in the non-heated tumor tissue, which may be explained by the ELPs soluble-to-insoluble transition and their subsequent aggregation above the transition temperature leading to the ELP attachment to the cell membrane[64, 65)]. In comparison with the TR copolymer of p(NIPAAm-co-AAm) the

ELPs showed enhanced accumulation in the heated tumor cells[66, 67].

Conclusion

In conclusion, TR systems may represent a novel approach to drug targeting to a specific site, e.g. the tumor tissue. The drug release from TR dosage forms is induced and controlled via temperature variation. Up to now, numerous designs of TR systems have been reported. Certain systems were loaded with a drug and consequently subjected to the *in vitro* release testing. In order to prove the TR drug dosage form efficiency, the TR systems were added to cell culture models (e.g. of the tumor tissue). Furthermore, the biodistribution and *in vivo* system efficiency were tested using mice and other model animals. When compared with the free anti-cancer drug, the certain TR systems loaded with the same active substance showed a higher concentration in the tumor tissue and lower toxicity resulting in an increase in cancer therapy efficiency. Finally, the TR liposomes Thermodox® are currently undergoing the Phase III clinical trials for the treatment of liver cancer, so the complete clinical studies of the system are expected to be published soon.

Conflicts of interest: none.

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