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REVIEW

Drug Carriers With Star Polymer Structures

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Summary

In this review we summarize several synthetic approaches to the advanced synthesis of star-like polymer-based drug carriers. Moreover, their application as nanomedicines for therapy or the diagnosis of neoplastic diseases and their biodistribution are reviewed in detail. From a broad spectrum of star-like systems, we focus only on fully water-soluble systems, mainly based on poly(ethylene glycol) or *N*-(2-hydroxypropyl)methacrylamide polymer and copolymer arms and polyamidoamine dendrimers serving as the core of the star-like systems.

Key words

Water-soluble polymers • Drug carriers • Star polymers • Hyperbranched polymers

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Introduction

Generally, conventional treatment with low-molecular drugs presents a number of limits, which include unfavorable body distribution and strong side effects in healthy organs, thus preventing their use in more efficient therapies. The main drawbacks of the vast majority of drugs include their hydrophobicity and, thus, low solubility in physiological environments, non-specific biodistribution, which results in toxicity to healthy organs and cells, and the rapid elimination from the organism. Within the last three decades, significant efforts have been focused on the design and study of systems that enable the limitations of low-molecular-weight drugs to be overcome using so-called drug delivery systems (DDSs). Various DDSs based on micelles, liposomes, polymerosomes or water-soluble polymers were studied extensively and some of them even reached preclinical and clinical stages of development. Water-soluble polymer carriers are one of the most studied systems among DDSs for the controlled delivery of active molecules. The attachment of drugs to the water-soluble polymeric carriers *via* covalent bonds significantly reduces the toxicity of the carried drug during transportation in the body and also prevents undesirable accumulation in healthy organs, thus minimizing the side effects of the carried drug. Another great advantage of water-soluble biocompatible polymers in controlled drug delivery is the significantly prolonged circulation time of the bound drug in the bloodstream from minutes up to several weeks (Etrych *et al.* 2012). The use of water-soluble polymer drug carriers should guarantee the safe transportation of pharmaceutical compounds to the site of interest. In the case of solid tumors, the main driving force for passive accumulation in neoplastic tissue is the enhanced permeability and retention effect (EPR), as described by Maeda and Matsumura (1989). The EPR takes advantage of the highly permeable neo-vasculature of tumor tissue and missing or undeveloped lymphatic drainage. The most widely studied polymers usually used as drug carriers are based on poly(ethylene glycol) (PEG) and the copolymers *N*-(2-hydroxypropyl)methacrylamide (pHPMA), poly(2-oxazolines), etc. (Lidický *et al.* 2016, Pola *et al.* 2016, Chytil *et al.* 2015, Ulbrich *et al.* 2016, Li and Wallace 2008). The effectiveness of polymeric drug conjugates is determined not only by the chemical composition of the

polymer or copolymer (monomers, functional groups), but also by their supramolecular arrangement in the solution. Several polymer structures (Jones *et al.* 2016) have been described in the literature, e.g. linear, diblock, graft, branched, hyperbranched and star-like polymers. (Hrubý *et al.* 2016) Polymers that are to be used as a drug carrier have to contain appropriate, functional groups suitable for active molecule bonding and, after fulfilling the role of a carrier, should be excreted from the organism. The polymer carriers can be eliminated from the body when the molecular weight and hydrodynamic radius (R_h), respectively, are below the renal threshold or their biodegradability ensures that degradation products with an R_h below the renal threshold are obtained. The EPR described above is strongly dependent on the size and $R_{h,}$ of the DDS. It was reported that polymeric systems with higher R_h (star-like polymers or micelles) show a superior EPR when compared to linear polymers (Ulbrich *et al.* 2016). In general, typical R_h for linear polymers is 4-6 nm and R_h of star-like systems or micelles is in range 10-20 nm. Due to the greatly increased EPR and the possibility of incorporation of biodegradable spacers, some of the most studied and promising carrier structures for anticancer therapy are star-like and hyperbranched polymers.

Star-like polymers consist of at least three polymer arms connected to one branching point, called the core. Various types of star-like polymers can be found in the literature. They can vary in the composition of the polymer arms (homopolymer star, block copolymer star or statistical copolymer star), in the type of polymer arm attached to one core (miktoarm star) or in the core structure. Several review papers on the synthesis of star polymers and individual star-shaped polymers have recently been published (Lapienis 2009, Wu *et al.* 2015, Ren *et al.* 2016, Abbina *et al.* 2017). In the present review, we summarize fully water-soluble star-like polymer carriers based on hydrophilic polymers. The main attention is devoted to systems designed for cancer treatment. We split this review into two parts. In the first part, we focus on the synthetic aspects of star-like systems, while the second part is an overview of star-like systems from a biological and application point of view.

Star-like polymer carrier synthesis

Generally, there are two main synthetic approaches that are taken to prepare star-like polymer carriers using synthetic polymers. The first approach, which represents the majority of published works, is called grafting-onto. The second approach, called core-first or grafting-from, can also be found in literature, but represents only a minority of published works. In the core-first strategy, a multifunctional core with functional groups modified by an initiator is used as the multifunctional initiator for polymerization to obtain polymer arms growing directly on the core. In the grafting-onto approach, linear semitelechelic polymers, synthesized in advance, are used for controlled grafting using end groups of these polymers with a functional group on the multifunctional core, thus creating a star-like structure. Nowadays, the synthesis of star-like polymer carriers *via* core-first or grafting-onto strategies is based on commercially available or pre-synthesized multifunctional cores so as to form star-like polymers. Examples of the most common commercially available cores used for star-like carrier synthesis include non-biodegradable poly(amidoamine) dendrimers (PAMAM) (Wang *et al.* 2000, Etrych *et al.* 2011a), multifunctional alcohols such as pentaerytritol and dipentaerytritol, and biodegradable polyesters based on 2,2'-bis(hydroxy-methyl)propionic acid (bis-MPA). Structures of these cores are shown in Figure 1.

The third approach taken to synthesize star-like polymers is the arm-first method. In this case, pre-synthesized linear polymers are cross-linked by a multifunctional coupling agent at the polymer chain end. For example, Learsch *et al.* used a ring-opening metathesis polymerization technique for the cross-linking reaction of pre-synthesized polymeric arms. They used various derivatives of dinorbornenes as cross-linking agents (Learsch and Miyake 2018).

In general, based on the above-described approaches, star-like systems can be sorted into 12 groups, as schematically summarized in Figure 2 (Ren *et al.* 2016).

Star-like polymer systems can be classified according to the composition and sequence distribution of the polymer arms into homopolymer stars, block copolymer stars or statistical copolymer stars. The second division is based on the variability of arm species; here, we can refer to miktoarm stars, which can be subdivided into three groups: compositional miktoarm stars, miktoarm stars with irregular molecular-weight arms or end-functionalized miktoarm stars. A further classification of the star-like system is based on the character of the core used. We can describe the system as comprising small compound core-structured stars, macromolecule core-structured stars or network core-structured stars. Finally, star-like systems can also be classified from the point of view of the location of the functional group in the star-like system. We can therefore refer to core-functionalized, arm-functionalized or end-arm-functionalized stars.

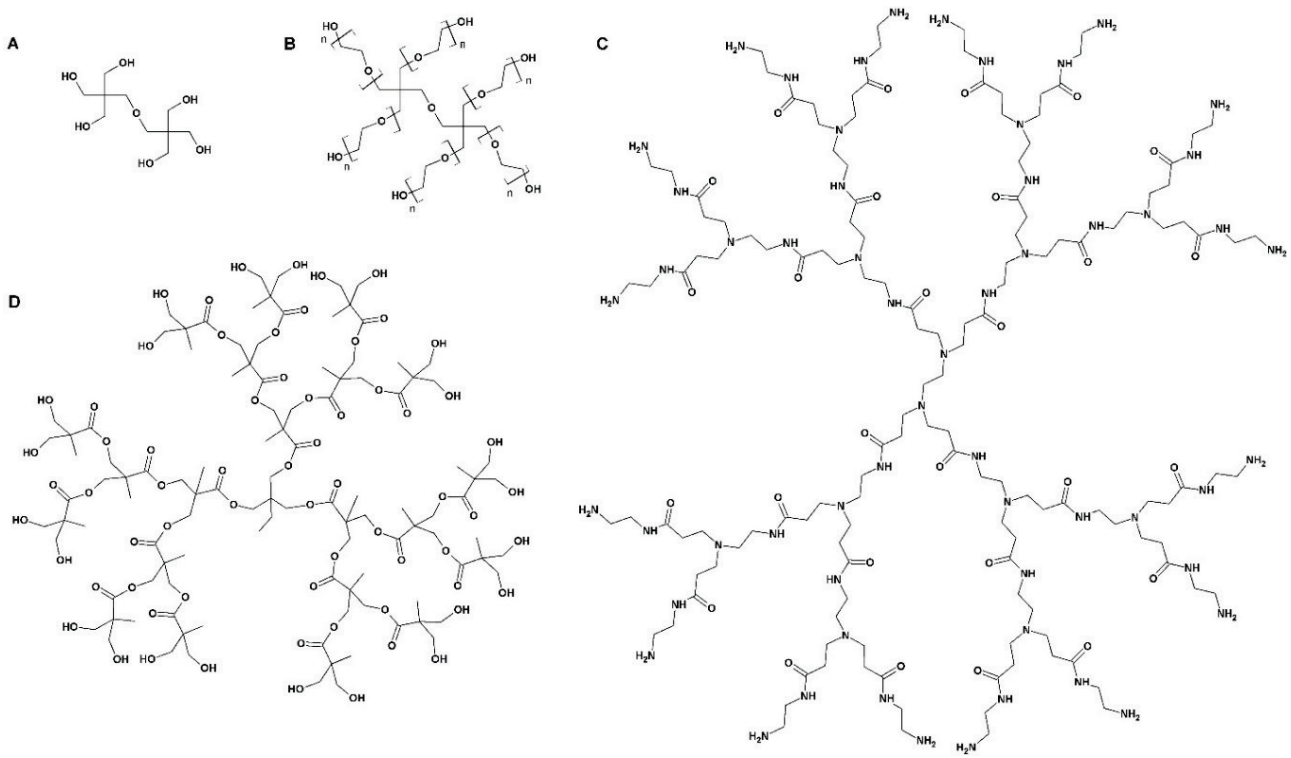

Fig. 1. The most popular cores for star-like polymer carriers: A – dipentaerythritol, B – 6arm-PEG, C – PAMAM (G2), D – bis-MPA (G3).

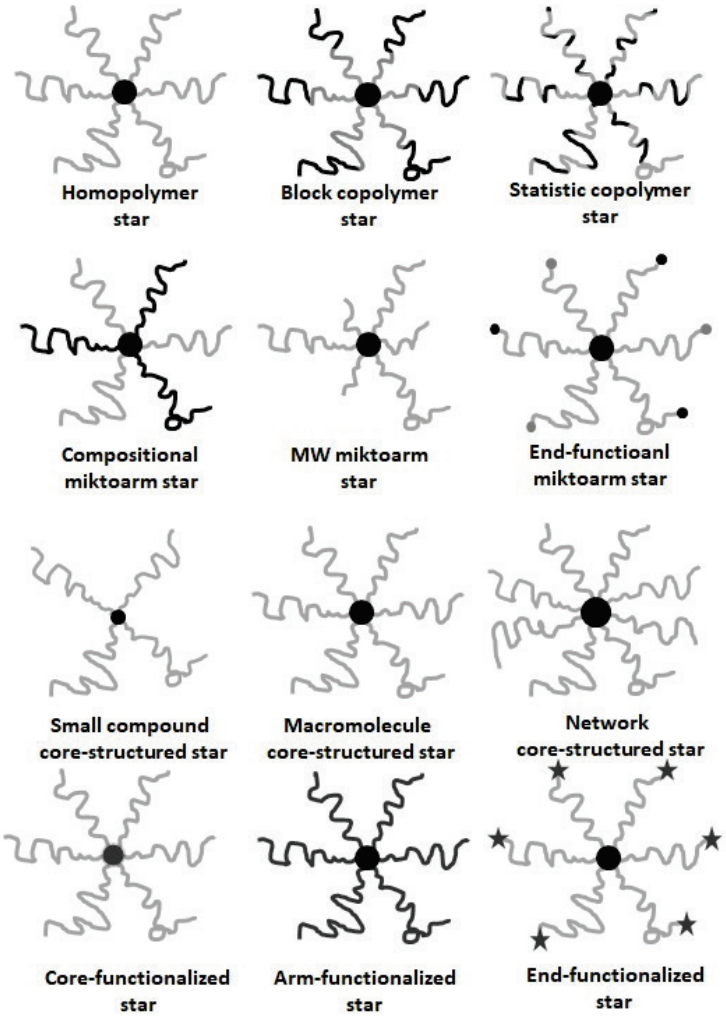

Fig. 2. Classification of star-like polymers based on various points of view (figure adopted from Ren *et al.* 2016).

Star-like polymers based on PEG

PEG is a hydrophilic synthetic polymer widely used as a water-soluble drug carrier or a surface modifier of supramolecular drug carriers such as micelles, liposomes and nanoparticles (Lammers *et al.* 2008, Tran *et al.* 2017). The advantage of PEG carriers is their chemical stability under neutral conditions, non-toxicity, and good biocompatibility, while the main drawback of these polymer carriers is the limited number of functional groups for drug molecule attachment. PEG does not have any functional group along the polymer chain. Therefore, the main-chain end groups are the only option for drug conjugation. The hydroxyl end groups, derived directly from the polymerization process of ethylene glycol, can be used directly for drug coupling or the hydroxyl group can be transformed into various functional groups (e.g. amino groups, propargyls, azides, aldehydes, bromo groups, mesylates, succinimido succinates) (Tekade *et al.* 2009) by a post-polymerization procedure and used for drug conjugation. Another way in which the number of functional groups in the polymer backbone can be increased is through the synthesis of PEG-based multiblocks, e.g. PEG blocks can be connected with oligopeptide "linkages," enabling the attachment of various active molecules (Pechar *et al.* 2000).

One of the most important uses of PEG is as a shielding agent for branched polymers, nanoparticles or proteins. This procedure is called pegylation, a term first used by Davis and Abuchowski in the case of albumin and catalase in the 1977s. The method represented a significant milestone, opening the way for scientists to modify proteins without losing their biological activity (Abuchowski *et al.* 1977a, Abuchowski *et al.* 1977b) Nowadays, pegylation is also a common approach to coating hydrophobic drug carriers in order to achieve solubilization or to reduce their side effects, which determine their toxicity in healthy tissues. One of the mostly used dendritic cores, PAMAM dendrimer, was used for star polymer production with PEG arms (Luong *et al.* 2016). The amino groups on the surface of the dendritic core have positive charges and, as a result, PAMAM shows a degree of cytotoxicity and/or hemolytic toxicity (Malik *et al.* 2000). Pegylation of some of the amino groups on the surface of the PAMAM core helps to prolong the circulation time of such star-like systems in the body due to the decreased reticuloendothelial system uptake. Pegylated PAMAM dendrimers also have higher anticancer drug loading and controlled drug release properties for efficient tumor treatment when compared to unmodified PAMAM dendrimers (Luong *et al.* 2016). The schematic structure of this star-like system is shown in Figure 3.

In order to substitute the PAMAM core, PEG-based cores were synthesized. These dendrons showed almost no toxicity and offered the possibility of carrying biologically active compounds on their surface (Berna *et al.* 2006). These dendrons were prepared with various end-group functionalities, 2-4 amino groups, with molecular weights ranging from 1,300-4,000 $g \cdot mol^{-1}$ and in high purity. To ensure that these systems can enter cells, fluorescence-labeled dendrons were synthesized and cellular uptake was observed. Dendrons with higher molecular weights and higher functionality showed faster uptake than that of smaller ones. Amphotericin B was attached to these dendrons and the conjugate was evaluated *in vitro* for drug release and cytotoxicity (Sedlák *et al.* 2008).

Branched PEG-based systems can also be synthesized by adding a branching linker to the PEG. This approach is represented by aspartic acid-based cores shielded with PEGs (40,000 $g \cdot mol^{-1}$). Cytosine arabinoside (cytarabine, ara-C) was conjugated to these carriers and evaluated for its biological characteristics (Choe *et al.* 2002). The conjugation of ara-C to the described star polymer prolonged the circulation of the carried drugs and showed higher accumulation of ara-C in tumor tissue due to the EPR.

Additional promising polymeric materials for biomedical applications include carriers based on hyperbranched polyglycerols, which are used as a core, and PEG, which is used as the shell of the star-like system. Hyperbranched polyglycerols are biocompatible, can be easily modified and can be designed to be biodegradable by incorporating biodegradable moieties such as ketals, esters and disulfides (Son *et al.* 2015, Hu *et al.* 2012, Shenoi *et al.* 2012).

Nowadays, combination therapy has become a standard in oncology. Moreover, star-like systems with a combination of two or more drugs have been reported. For example, a dendritic scaffold based on polyglycerol, decorated with PEG and equipped with a combination of two cytostatic drugs (doxorubicin (dox) and paclitaxel) has been reported and evaluated by Baabur-Cohen *et al.* (Baabur-Cohen *et al.* 2017).

Star-like polymers based on pHPMA

Copolymers based on HPMA (pHPMA), such as carriers of cytostatics, have been studied for almost the

last four decades. The advantage of pHPMA is the possibility of introducing various functional groups directly into the polymer backbone through the copolymerization of HPMA with suitable comonomers. This enables attachment of the low-molecular-weight drug along the polymer chain by either cleavable or non-cleavable bonds, i.e. hydrazone or amidic bonds. The polymer carriers based on pHPMA show very good biocompatibility, non-immunogenic properties and no toxicity in many biological studies (Ulbrich *et al.* 2016).

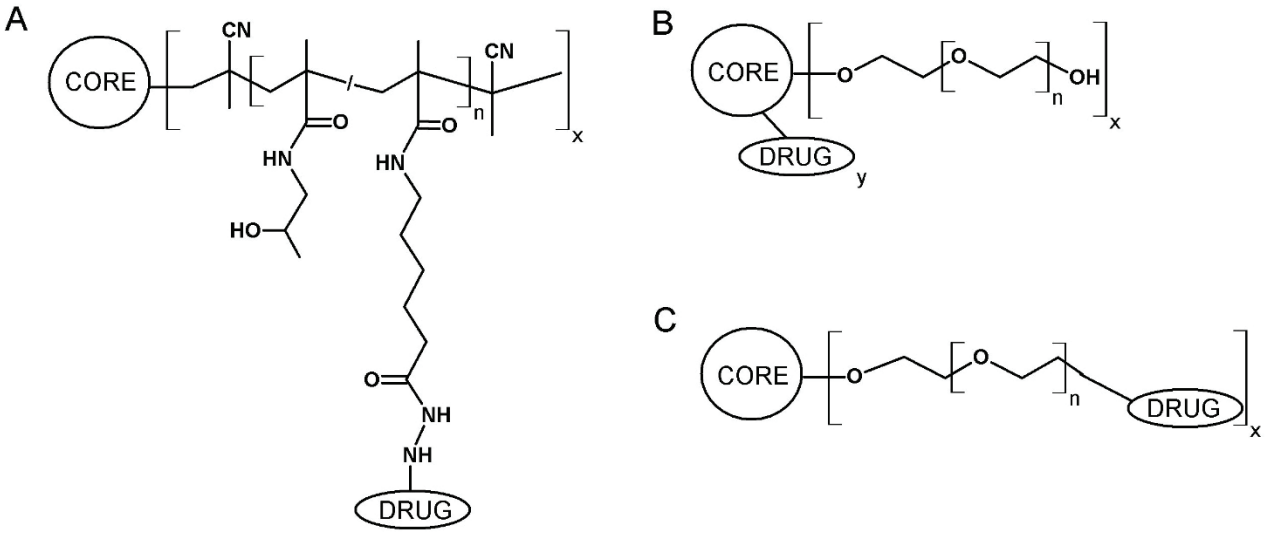

Fig. 3. Schematic structures of representative star-like drug carriers based on pHPMA or PEG: A – star-like pHPMA carrier, B – star-like PEG carrier with drug attached to the core, C – star-like PEG carriers with drug bonded to the polymer arms.

The first star-like pHPMA-based carrier was synthesized by Wang *et al.* (2000) using semitelechelic linear pHPMA-COOH (M_n=5,400), prepared by free-radical polymerization, and subsequent activation of the carboxyl group to NHS. This semitelechelic copolymer was subsequently grafted onto PAMAM dendrimers (G2-G4, 16-64 amino groups). Non-degradable star-like polymer carriers with molecular weights ranging from 20,000-160,000 g·mol^{-1} were synthesized by amidic coupling. For the next 20 years or so, such non-degradable or biodegradable star-like polymer conjugates based on PAMAM cores and pHPMA copolymer arms were synthesized by free-radical polymerization with various biodegradable spacers between the PAMAM core and the pHPMA arms (e.g. oligopeptide sequences, disulfides) (Etrych *et al.* 2011a) and various stimulus-sensitive linkers between the carried drugs (dox, pirarubicin, docetaxel) and the star polymer (oligopeptide sequences, hydrazon bond) (Etrych *et al.* 2011b, Etrych *et al.* 2015, Nakamura *et al.* 2015). These star polymers represented one of the most promising systems in anticancer therapy, especially in the treatment of solid tumors. The schematic structure of the pHPMA-based star-like system is shown in Figure 3. Most attention has been paid to star-like polymer conjugates with polymer arms with an M_w in the range of 20,000-30,000 g·mol^{-1} and with conjugates of an M_w in the range of 100,000-1,000,000 g·mol^{-1} (Etrych *et al.* 2011a, Etrych *et al.* 2011b). Several *in vivo* studies have confirmed that the optimal molecular weight of non-biodegradable HPMA-based star-like systems with the highest antitumor activity and the best drug accumulation in tumors is between 200,000 and 600,000 g·mol^{-1} (Etrych *et al.* 2011b). Star-like carriers based on pHPMA copolymers are much more effective than their linear versions of polymer carriers in terms of therapeutic activity, which corresponds to higher drug accumulation in the solid tumor. Concurrently, degradable star-like pHPMA carriers were studied due to easier and faster elimination from the organism (Etrych *et al.* 2011b). Degradable star-like carriers based on PAMAM cores were synthesized by incorporating a cleavable linker between the PAMAM core and the HPMA polymeric arms. Two different types of biodegradable spacers were incorporated into these systems: either a reductively cleavable spacer (disulfide) or an enzymatically cleavable spacer (tetrapeptide GlyPheLeuGly) (Kostka and Etrych 2016). The cleavability of the disulfide or GlyPheLeuGly linkers and conjugate degradation do not seem to substantially influence the anti-tumor effect of the star polymer

conjugates. The final anti-tumor efficacy, represented as the percentage of long-term survivors, was comparable when a lower dose of 5 mg dox eq./kg was used of both non-degradable and biodegradable disulfide-containing star polymers, while slightly better efficacy was observed with a non-cleavable form of the polymeric drug when a higher dose (10 mg dox eq./kg) was used. Similar experiments conducted with a biodegradable star conjugate containing enzymatically cleavable GlyPheLeuGly sequences showed an impressive treatment efficacy of up to 100 % long-term survivors (LTS) in a group of mice treated with a dose of 10 mg dox eq./kg, and an efficacy of 60 % LTS was observed in a group treated with a dose of 5 mg dox eq./kg (Etrych *et al.* 2011b, Etrych *et al.* 2011c)

Another biodegradable star-like system based on pHPMA copolymers was described by Kostková *et al.* (Kostková *et al.* 2017). Here, the non-degradable PAMAM core was substituted with a biodegradable dendritic core based on polyesters (bis-MPA). For synthesis, semitelechelic linear polymers were prepared by free-radical polymerization and a controlled polymerization technique, radical reversible addition fragmentation chain transfer polymerization (RAFT). Linear arms were grafted onto the bis-MPA core through aminolytic reaction. These systems were well defined, with dispersity below 1.3 (Đ<1.3) compared to the above-described PAMAM-based system, which showed a dispersity of approximately 1.8. These carriers were tested for biodegradability in aqueous buffers and also in human plasma with a half-life of 6 days in buffers (pH 7.4) and 3 days in human plasma. The half-life was estimated to be 50 % of the released linear polymer from the star-like system.

A new generation of pHPMA star-like carriers based on the PAMAM core was introduced with a controlled polymerization technique, mainly RAFT. This step reduced the dispersity of star-like carriers from 1.8 to below 1.2 (Chytil *et al.* 2015).

In summary, PEG is a suitable shielding agent for hydrophobic molecules. It is commercially available in various modifications and is already FDA-approved for human medicine and use in pharmacy. Many various hyperbranched or star-like polymer conjugates with minimized side effects have been synthesized and reported. pHPMA seems to be a promising alternative to PEG. It can also be used as a shielding agent or carrier of diagnostic or drugs attached directly to the pHPMA copolymer backbone.

Biopharmaceutical evaluation of star-like systems

The use of polymers for drug delivery and targeting has shown significant potential, particularly in increasing drug safety and reducing drug-associated toxicity to non-targeted organs and tissues. As we already mentioned above, over the past four decades, biomedical research has focused on developing technologies for various clinical uses. One of the most promising and efficient systems in diagnostic and therapeutic uses is that of carriers based on dendrimers. In this part of the review, we summarize the biological evaluation of star-like systems, especially biodistribution studies.

To follow the biodistribution of star-like carriers in a living organism, a suitable label must be incorporated into such a system. It has been reported that a system can be labeled with radioisotopes (Kojima *et al.* 2010, (Hamilton *et al.* 2016) or fluorescent dye (Chytil *et al.* 2013, Hoffmann *et al.* 2012). Three different systems, based on either hyperbranched polyglycerols, a PAMAM core shielded with PEG or a PAMAM core shielded with pHPMA, were selected for a comparison of their biodistribution.

Hamilton *et al.* (2016) synthesized and evaluated biodegradable systems based on hyperbranched polyglycerols with a molecular mass of 65 or 637 $kg \cdot mol^{-1}$ and a corresponding R_h of 2.7 or 7.7 nm, respectively. These systems were radiolabeled with tritium so as to follow the fate of the carrier in the body of healthy mice. Studies on organ accumulation and biodistribution have indicated a size-dependent accumulation, likely due to phagocytic uptake in the RES system, in the liver and spleen. Animals were injected with a single dose of 43 $mg \cdot kg^{-1}$ and less than 1 % of the injected dose per gram of organ tissue was found in pancreas, brain and femoral muscle over the experiment time (144 h). Between 1 % and 5 % of the injected dose per gram of organ tissue was found for all systems in the spleen, kidney, heart and lung. The highest accumulation was shown by the largest star-like system (7.7 nm) in the liver, wherein after 110 h 13 % of the injected dose per gram of tissue was found.

Similarly, a system based on a PAMAM core and a PEG shell was evaluated for biodistribution by Kojima *et al.* (2010). They synthesized three different systems with various molecular masses. The first two systems were based on the G4 generation of PAMAM dendrimers and the shell of these star-like systems was

generated using PEG with different lengths of the polymer chain (PEG 2,000 or PEG 5,000 $g \cdot mol^{-1}$). The third system was based on the G5 generation of PAMAM dendrimers shielded with PEG 2,000 $g \cdot mol^{-1}$. The schematic structure is shown in Figure 4. The molecular masses of these systems were calculated to be: G4-Peg2k, 162 $kg \cdot mol^{-1}$; G4-Peg5k, 334 $kg \cdot mol^{-1}$; and G5-Peg2k, 284 $kg \cdot mol^{-1}$. The biodistribution of these systems was evaluated by radiolabeling with ^{111}In and the injected dose was 1.5 μCi of ^{111}In (approximately 2 μg in 200 μl of PBS buffer). The authors concluded that PEG coating of the PAMAM core increased the circulation time of the system in the bloodstream approximately fivefold. The distribution over selected organs, such as the liver, spleen, kidney, lung, heart and bone, can be seen in Figure 4, wherein these systems are compared. The biodistribution of pegylated dendrimers was significantly different from that determined for the non-pegylated dendrimers. Pegylation induced prolonged blood circulation and prevented accumulation in normal organs including the kidneys and the liver. After 24 h following injection, more than 25 % of the injected dose remained circulating in the blood independently of the length of PEG used. The organ with the highest accumulation level was the lung in all cases. Here, after 1 h almost 15 % of the injected dose was estimated and after 24 h the level decreased to 8-10 % of the injected dose. In the liver, less than 8 % of the injected dose was observed independently of the time following injection.

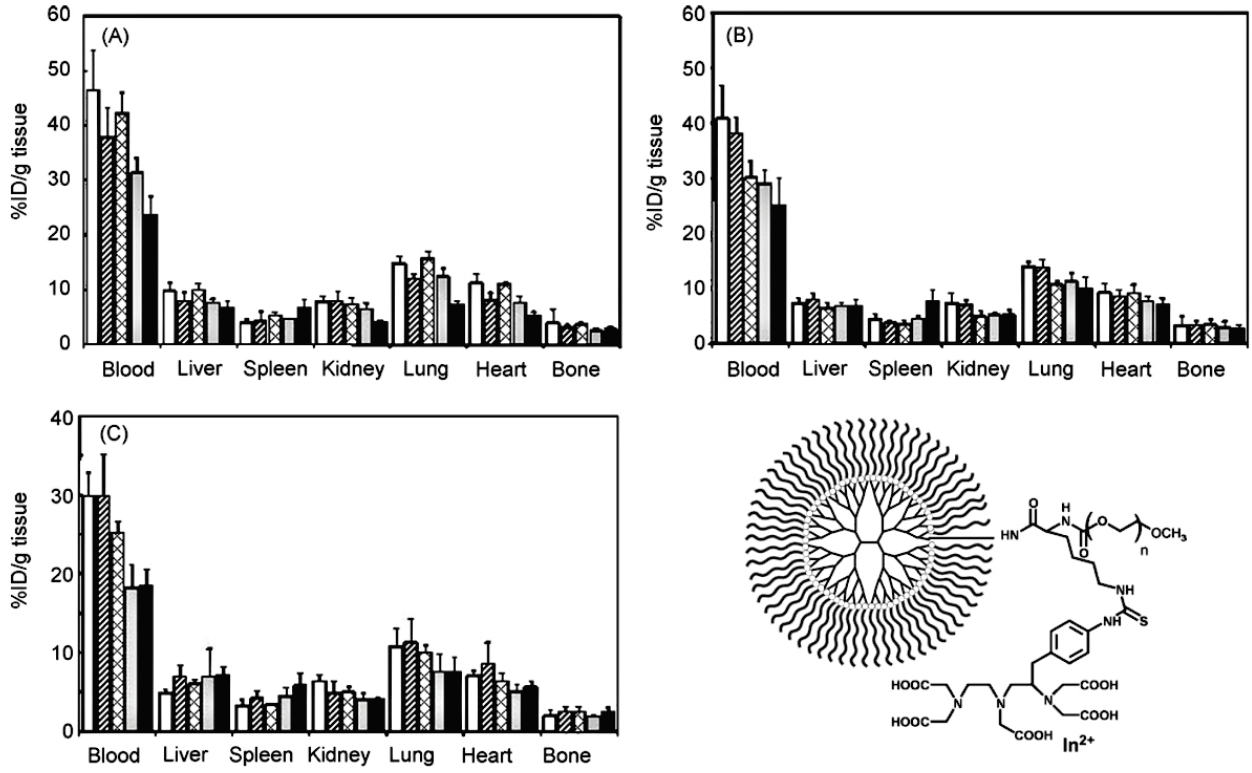

Fig. 4. Biodistributions of (A) PEG2k-Lys-PAMAM (G5), (B) PEG5k-Lys-PAMAM (G4), (C) PEG2k-Lys-PAMAM (G4) and the structure of the studied polymer carrier. The %ID/g tissue after 0.25 h (white bars), 1 h (hatched bars), 3 h (cross-hatched bars), 6 h (gray bars) and 24 h (black bars) is shown. The figure is modified and adapted from (Kojima *et al.* 2010).

Using pegylated PAMAM dendrimers as the drug carrier, Zhu *et al.* (2010) synthesized and characterized a series of dox-bearing polymer conjugates with different pegylation degrees and drug conjugation strategies. Both the pegylation degree and the drug conjugation strategy were found to affect the *in vitro* release, *in vitro* cytotoxicity, and cellular uptake. The acid-sensitive dox release determined the *in vitro* cytotoxicity against SKOV-3 cells. Mechanistic studies revealed that the dox polymer conjugates were internalized by SKOV-3 cells *via* clathrin-mediated endocytosis, which ensured their localization in the acidic cellular compartments, subsequent release of dox from PEG-PAMAM-*cis*-aconityl-dox (PPCD) conjugates, and final drug penetration into the nucleus. *In vivo* fluorescence imaging analysis of the biodistribution

demonstrated that conjugates with 20 molecules of PEG 5 $kg \cdot mol^{-1}$ attached to PAMAM-G4, with a hydrodynamic size of 16 nm, which accumulated at the tumor site the most efficiently. By increasing the degree of pegylation from four to 20 PEG chains per PAMAM core, accumulation at the tumor site almost doubled. Conversely, accumulation in other organs, such as the heart, liver, spleen, lung and kidneys, significantly decreased.

The star-like system based on PAMAM dendrimers decorated with pHPMA copolymers and evaluated for biodistribution has been reported by Pola *et al.* (2016), Hoffmann *et al.* (2012), Chytil *et al.* (2013). In all of these publications, star-like systems were evaluated using fluorescence-labeled carriers in living animals and also in extracted organs. These systems varied in molecular weight from 170 to 200 $kg \cdot mol^{-1}$ and also in different linkers used for the model drug attachment. (Chytil *et al.* (2013) reported the dependence of the accumulation and distribution of the model drug on the pH-sensitive linker used for its attachment to the polymer backbone. It was shown that accumulation at the tumor site can be controlled well by using a linker for the pH trigger release of the model drug. Hoffmann *et al.* (2012) reported a similar star-like system of a PAMAM dendrimer core and a pHPMA copolymer in the shell. The non-degradable star polymer carriers were labeled with the near-infrared fluorescent dye DY-782. The authors reported that the dye showed minimal fluorescence retention in organs and the skin and was therefore suitable for *in vivo* evaluation of biodistribution in mice. Tested animals were inoculated with two tumors, DLD-1 and HT-29, on the left and right sides of the back. Accumulation in both tumors was preferential when compared to the rest of the body. Total accumulation represented by normalized fluorescent intensity (NFI) for both tumors (0.07) was almost double that of kidneys (0.035), four times higher in the case of the liver, lung and testes (NFI=0.015), and seven times higher when compared to the heart and spleen (NFI=0.01).

To summarize this part of the paper, we can state that the biodistribution of all the above-described star-like carriers is quite similar and beneficial compared to low-molecular-weight compounds or even to the linear carriers. In the case of star-like systems based on PAMAM dendritic cores, when we compare the fate of the carriers themselves, the biodistribution is almost independent on the type of hydrophilic copolymer used in the shielding layer, namely PEG or pHPMA. Furthermore, from this point of view, all of the above-mentioned systems showed superior accumulation in tumor tissue compared to the rest of the body.

Conclusions

Several synthetic approaches to the advanced synthesis of star-like polymer-based drug carriers have been described with the aim of discussing the relationship between the structure and physico-chemical and biological characteristics of the star polymer systems. Indeed, the application of these star polymers as nanomedicines in the therapy or diagnosis of neoplastic diseases has been described in detail, showing the potential of these water-soluble polymer systems. We can conclude that star-like polymers are highly interesting polymer carriers suitable for further preclinical development.

Conflict of Interest

There is no conflict of interest.

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