Available online at www.sciencedirect.com
ScienceDirect
Polymer 48 (2007) 7431–7443
polymer
www.elsevier.com/locate/polymer

Feature Article

Functional amphiphilic and biodegradable copolymers for intravenous vectorisation

K. Van Butsele, R. Jérôme, C. Jérôme*

Center for Education and Research on Macromolecules, University of Liege, B6 Sart-Tilman, B-4000 Liege, Belgium

Received 10 May 2007; received in revised form 21 September 2007; accepted 30 September 2007
Available online 5 October 2007

Abstract

This paper aims at reporting on the design of polymeric drug nanocarriers used in cancer therapy, with a special emphasis on the control of their biodistribution. First, the prominent role of poly(ethylene oxide) in the lifetime of nanocarriers circulating in the blood stream is highlighted, and the origin of a passive targeting based on a difference in the anatomy of tumors and normal tissues is discussed. The main body of the review is devoted to the targeting of nanocarriers towards tumors and the underlying concepts. As a rule, either the constitutive polymer is stimuli-responsive and the locus of drug release is where the stimulation occurs, or a ligand endowed with specific recognition is grafted onto the nanocarrier. Finally, the fate of the nanocarrier after drug delivery and the bioelimination of the polymer(s) involved are briefly considered.

Keywords: Nanoparticles; Vectorisation; Functional colloids

1. Introduction

Performances of drug delivery systems are continuously improved with the purpose to maximize therapeutic activity and to minimize undesirable side-effects. Indeed, the shortcomings of the conventional administration of drugs (tablets, injections…) are well-known. Lack of selectivity in drug delivery is a major limitation that may cause profound damage to healthy tissues. Nowadays, nanocarriers based on amphiphilic copolymers are able to target specific tissues and to control the drug biodistribution, particularly in tumoral tissues. Although this topic will be the focus of this review, it is only a part of the requirements that a drug delivery system must satisfy for being of practical interest. Indeed, the pharmacokinetics of the drug may not be ignored for the system to be biofunctional. For being effective, the administrated drug must be released at a constant predetermined rate that maintains the drug level in the therapeutic zone. The ideal release profile is schematized in Fig. 1. Although crucial, this aspect of the drug release and targeting will not be discussed further.

Various types of carriers with a size of several tens of nanometers have been developed [1–12]. Let us mention polymeric micelles, polymer-based nanoparticles and liposomes. Polymeric micelles are supramolecular assemblies of amphiphilic block copolymers with a core–shell structure (Fig. 2A). Nanoparticles or nanospheres designate solid cores of biodegradable hydrophobic polymers protected by an amphiphilic block copolymer that stabilize their dispersion in aqueous media (Fig. 2B). Liposomes are vesicles consisting of one or more phospholipidic bilayer(s), with an aqueous core (Fig. 2C). Ideally, these nanocarriers should be able to travel safely throughout the vascular system, to reach the intended target at full drug content, where they should act selectively on diseased cells and tissues, without creating undesired side-effects.

Nevertheless, the natural defences of the body trigger a sequence of formidable obstacles on the drug's pathway to the

* Corresponding author.
E-mail address: c.jerome@ulg.ac.be (C. Jérôme).

0032-3861/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2007.09.048

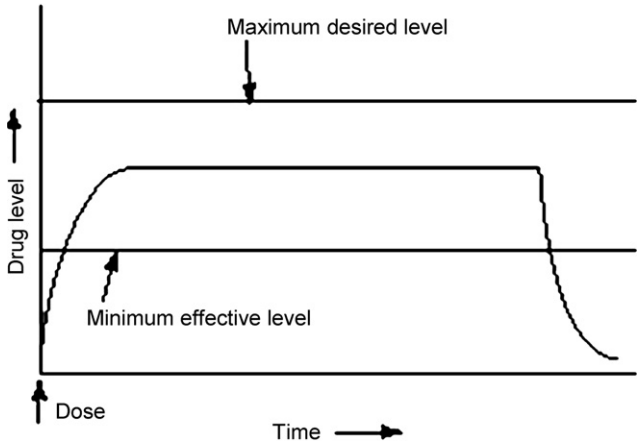

Fig. 1. Drug level in the blood with a controlled delivery dosing.

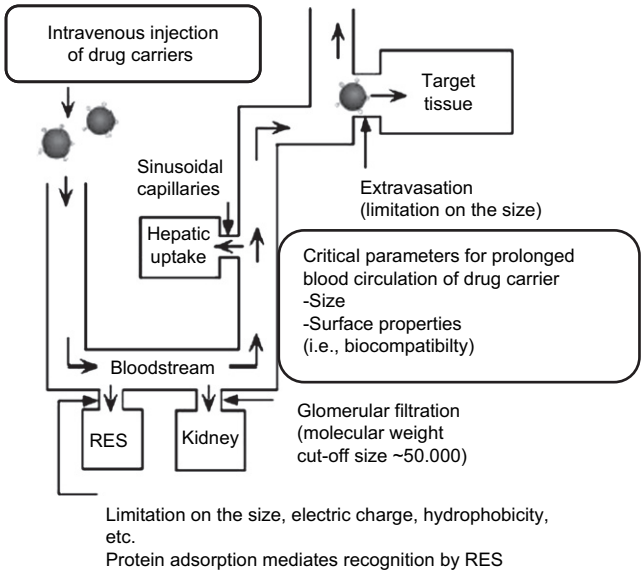

Fig. 3. Itinerary of a drug carrier after intravenous injection. (Reproduced from Ref. [9] with permission.)

intended lesion [13,14]. Drug carriers with a low biocompatibility are therefore recognized by the reticulo-endothelium system (RES) located in the liver, spleen and lung, and eliminated from the blood circulation (Fig. 3). One strong incentive to use macromolecular carriers is their preferential accumulation in solid tumors. The accumulation of macromolecules in tumors is currently explained by the microvascular hyperpermeability of tumors to circulating macromolecules and the impaired lymphatic drainage of macromolecules in these tissues. This phenomenon, known as the ''enhanced permeability and retention'' (EPR) effect [15–18], is very beneficial because it results in the selective uptake of the polymer-encapsulated drug by the tumor. In sharp contrast, healthy tissues are exposed and damaged by non-encapsulated drugs (Fig. 4).

For being used as a biomaterial, a polymer must be biocompatible. Biocompatibility was defined by Williams [19] as the ability of a material to act with an appropriate host response in a specific application. Moreover, biocompatible polymers used in drug delivery are often biodegradable with formation of non-harmful byproducts, such as non-toxic alcohols, acids and other easily eliminated low molecular weight products. Synthetic and natural biodegradable polymers [20–22] are steadily more involved in pharmaceutical, medical and biomedical engineering. They can indeed contribute to the drug release as a result of their erosion/degradation, in addition to drug diffusion through the polymeric material.

Block copolymers are at the root of many drug delivery systems, because their physico-chemical properties, such an amphiphilicity and degradation rate, can be tuned by the choice, content and molecular weight of the constitutive blocks.

2. Block copolymers in drug delivery systems

Distribution control is a key issue when the effectiveness of a drug delivery system is concerned. It is indeed essential that the drug delivery system is directed as precisely as possible to the desired site of activity, and even better that the drug release is triggered once this specific location is achieved.

The control of the drug distribution will be emphasized hereafter for block copolymers, bioeliminable if not biodegradable.

As aforementioned, once injected, drug delivery carriers are rapidly removed from the bloodstream as a result of interaction with the mononuclear phagocyte system (MPS) or with the complement system [13,14]. In this respect, the properties of the nanocarrier are of utmost importance because they decide for or against interaction with plasmatic proteins and cell membranes, and they can also impart some selectivity to the drug distribution.

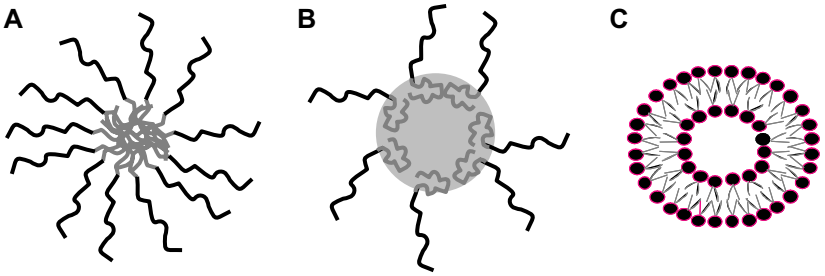

Fig. 2. Schematized polymeric nanocarriers: (A) micelle, (B) nanoparticle and (C) liposome.

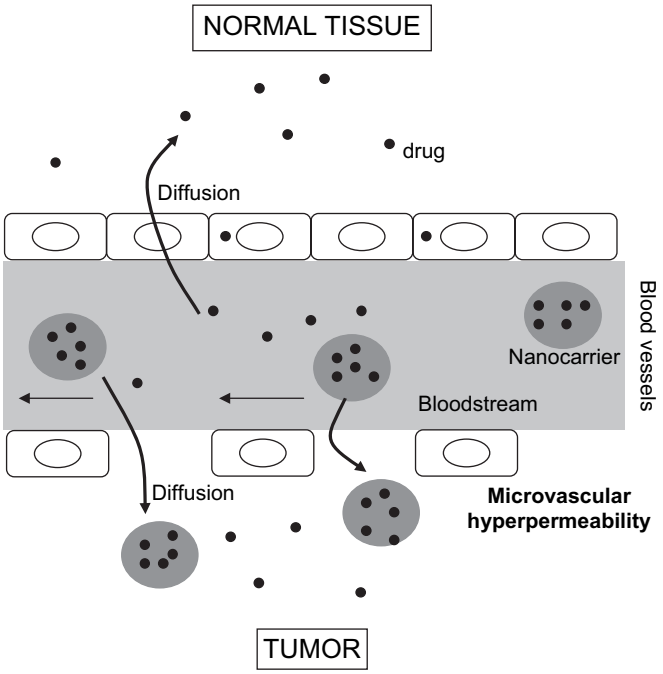

Fig. 4. Anatomical differences between normal tissues and solid tumors.

The carriers of the "first generation" are typically covered by surfactants, such as polyvinylic alcohol (PVA) or charged polymers. They are recognized by the plasmatic proteins and oriented towards the macrophages of the RES. They are applicable to hepatic pathology [23–25].

Whenever the plasma proteins are not deposited on their surface, the nanocarriers are not recognized by the RES and they belong to the "second generation" of the sterically stabilized nanocarriers. Their lifetime in the bloodstream may be long, and they finally accumulate in tumors by the EPR effect, thus as result of a "passive targeting". Poly(ethylene oxide) (PEO), designated as poly(ethylene glycol) (PEG) when capped by a hydroxyl group at both ends, is most commonly used to modify the surface of carriers for making them "stealthy".

3. Long-circulating carriers: the remarkable behaviour of poly(ethylene oxide)

Polycondensation of ethylene glycol leads to poly(ethylene glycol) (PEG), whereas poly(ethylene oxide) (PEO) is prepared by ring-opening polyaddition of ethylene oxide. Because the polymerization mechanisms are not the same, the molecular characteristics of PEG and PEO are different. PEG is indeed an α,ω-dihydroxyl polyether, with a molecular weight which does not exceed 20,000 and a broad molecular weight distribution. Being prepared by "living" anionic polymerization, the molecular weight of PEO is basically controlled by the monomer/initiator molar ratio and the monomer conversion, the polydispersity is low, and the α end-group is the initiator fragment and the ω end-group is a hydroxyl group. In spite of these structural differences, PEO and PEG are often used indiscriminately in the scientific literature.

In addition to linearity, the polyether chains are non-ionic and water-soluble. The water solubility is unlimited whatever the chain length, at least up to temperatures slightly below 100 °C. Then an inverse solubility-temperature dependence is noted. Not only the polyether–water interactions are strong, but also the polymer can fit the tetrahedral water lattice, such that all the lattice points are occupied either by water or by the ether oxygen of PEO. The ethylene segments thus fill out voids in the spacious water structure and minimally perturb the structure of water itself.

Chains of poly(ethylene oxide) being uncharged and linear without bulky side groups are very flexible compared to polymers with bulky pendant groups (steric hindrance) or to polyelectrolytes (steric and electrostatic hindrances). The flexibility explains why a brush of this polymer is protein repellent [26–28]. A volume restriction effect that results in a configurational entropy loss can contribute, at least partly, to this remarkable property (Fig. 5A). When a protein is approaching, the PEO layer is compressed and less configurations are possible for the PEO segments in this interaction region. This reduction in entropy increases the free energy, which accounts for a net repulsion of the proteins. In the case where the protein penetrates the polyether brush, an excluded volume effect can also be effective, which triggers repulsion by an osmotic pressure effect. Although these repulsion phenomena are

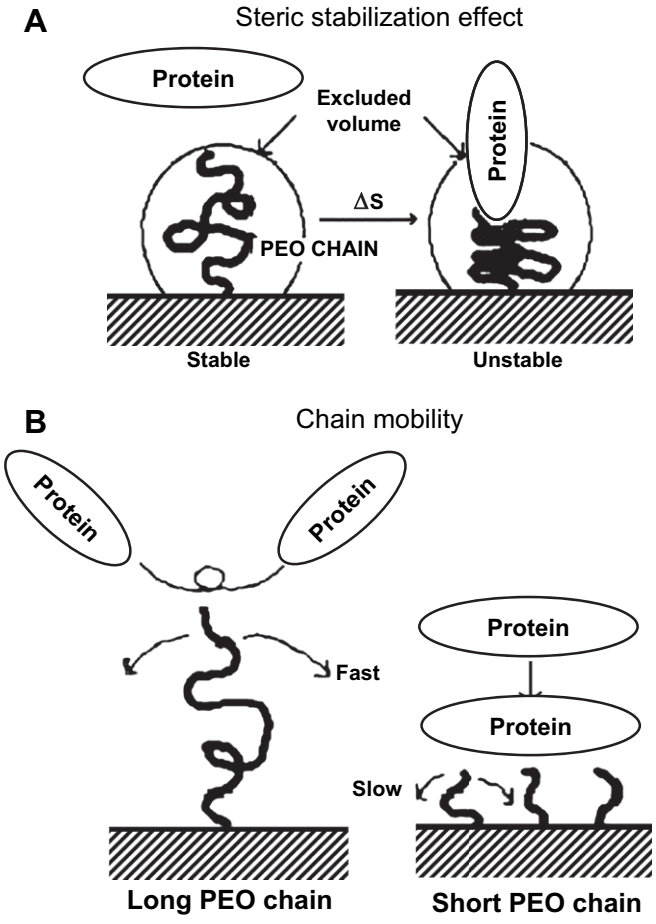

Fig. 5. Basic mechanisms involved in the protein resistance of PEO surfaces. (Reproduced from Ref. [26] with permission.)

well-known for neutral and hydrophilic polymers in water, PEO is the only water-soluble non-ionic polymer that exhibits a highly efficient protein resistance. It is now accepted that the high flexibility, and thus the high mobility, of PEO in water is determinant for its ability to be protein repellent. Indeed, rapidly moving hydrated PEO chains on a surface create locally a large excluded volume that prevents protein molecules from approaching the surface and from being in contact with it for a period of time long enough for irreversible adsorption to occur (Fig. 5B). This explanation was qualitatively consistent with the predictions by a mathematical modelling that a high density of long PEO chains was the best condition for an underlying hydrophobic surface to be protein resistant [29,30].

An alternative explanation of the protein repulsion by PEO chains was proposed by Vert and Domurado [31]. It is well-known that albumin and PEG are compatible in phosphate-buffered saline at room temperature and at concentrations comparable to those measured on the surface of PEO segment-bearing species. In contrast, protein and PEG phase-separate eventhough the protein concentration is much lower [32,33]. Therefore, Vert and Domurado propose that for PEO segments to generate the stealth effect, they must be compatible with albumin, such that PEO-bearing macromolecules or surfaces look like native albumin. This hospitality offered by PEG macromolecules or PEO segments to albumin, which is the dominant plasma protein, results in a 'chameleon' effect that prevents the activation of other PEG-compatible or -incompatible plasma proteins or cells involved in foreign body recognition and elimination.

Gref et al. [34] optimized the thickness and density of a PEO coating at the surface of biodegradable poly(lactic acid) (PLA) nanoparticles, in order to reduce simultaneously surface charge, plasma protein adsorption, and interaction with phagocytic cells. They observed a sharp decrease in the protein adsorption upon increasing the molecular weight of the polyether chains from 2000 to 5000 g/mol. Compared to PEO(2K)–PLA(45K), the amount of adsorbed proteins onto PEO(5K)–PLA(45K) particles was decreased by more than 50% . The plasma protein adsorption did not change significantly with further increase in the PEO length (Fig. 6A). A PEO content lying between 2 and 5% was the threshold value for optimal protein resistance (Fig. 6B). On the assumption that all the PEO chains form a brush (Fig. 7), the distance between near neighbour chains on the PLA surface would be approximately 1.4 nm. Consistently, Lee et al. [35,36] observed that the adsorption of blood proteins (albumin, γ-globulin, fibrinogen) at the surface of poly(MMA-*co*-MPEOMA) copolymers decreased with increasing PEO molecular weight and MPEOMA content in the copolymers.

Peracchia et al. [37] showed that the protein adsorption was also affected by the conformation of the PEO chains, particularly by the immobilization of one or the two chain-ends of PEO to the solid surface. The adsorption of plasma proteins on a known amount of poly(ethylene oxide)-*b*-poly(isobutylcyanoacrylate) nanoparticles was indeed more effectively prevented by loops of PEO than by dangling chains [38].

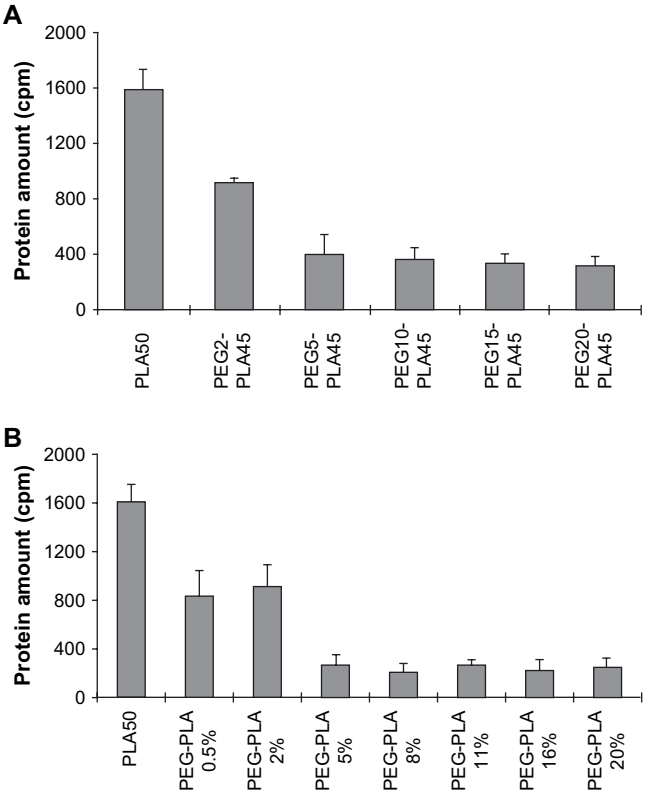

Fig. 6. Total amount of adsorbed proteins at the surface of (A) PEO-*b*-PLA(45K) nanoparticles with different PEO molecular weights and (B) PEO-*b*-PLA nanoparticles with different PEO contents made by blending of PEO(5K)-*b*-PLA(20K) with PLA(40K).The protein amount is expressed in arbitrary units. Data are the average of two experiments. (Reproduced from Ref. [34] with permission.)

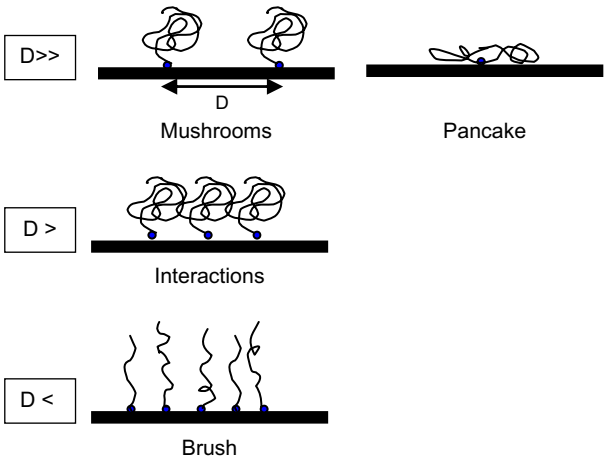

Fig. 7. Different conformations of the PEO chains as a function of the distance (*D*) between the anchoring points [27].

Fig. 8a tentatively schematizes the better protection of the surface by the folding back of the protective PEO chains.

The effect of the architecture of the copolymer precursor of the nanoparticles on their stealthiness was investigated by Rieger et al., who compared PEO-*b*-PCL block copolymers and PCL-*g*-PEO graft copolymers with a gradient structure [39]. In a gradient-type graft copolymer, the PEO grafts are

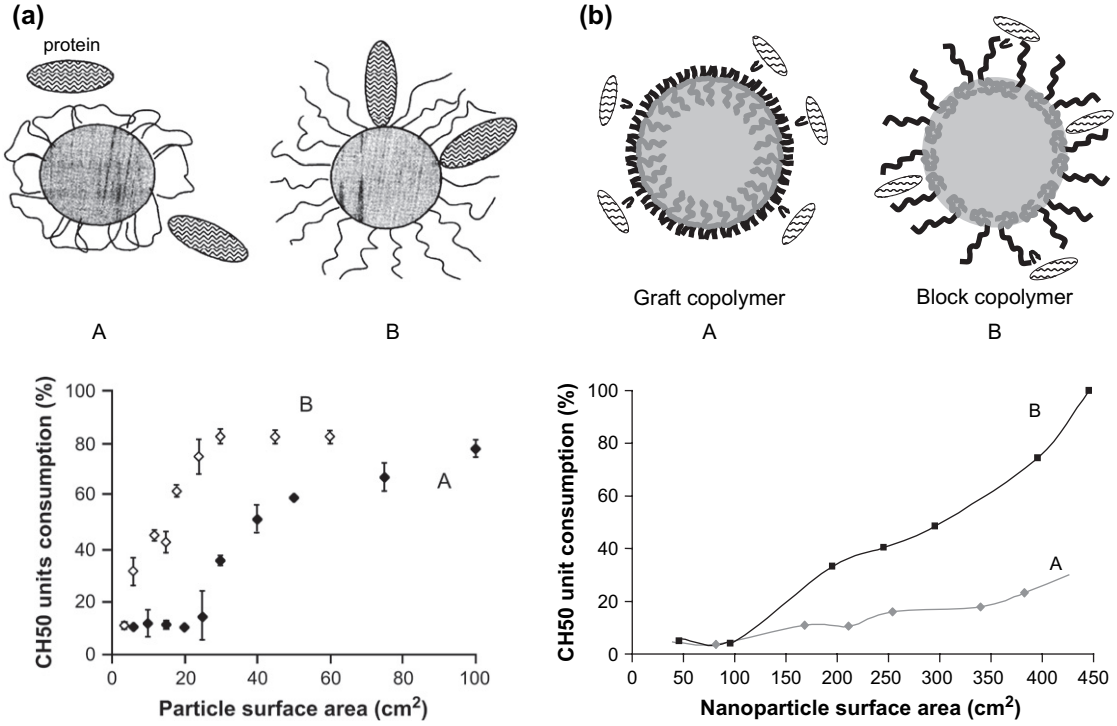

Fig. 8. Complement consumption (CH50) by (a) PEO-*b*-PIBCA (A, mean diameter = 300 nm) and MePEO-*b*-PIBCA (B, mean diameter = 400 nm) (reproduced from Ref. [37] with permission) and (b) PLA nanoparticles coated by PEO-g-PCL (A, mean diameter = 152 nm) and PEO-*b*-PCL (B, mean diameter = 158 nm) as a function of the surface area.

unevenly distributed along the PCL backbone. The grafting density goes actually increasing from one PCL chain-end to the other one. These gradient copolymers were superior to di-blocks of comparable hydrophilic–lipophilic balance (HLB) in stabilizing PLA nanoparticles prepared by nanoprecipitation and in repelling proteins (Fig. 8b).

All these examples show that nanometric polymeric particles covered by a layer of PEO chains can prevent the physiological defence processes stimulated by intravenous injections from being triggered, which accounts for a longer residence time observed in the systemic circulation [40–42]. In this respect, the lifetime in the blood stream was increased and the accumulation in liver/spleen was decreased when poly(lactide-*co*-glycolide) (PLGA) nanoparticles were surface modified by polylactide-*b*-poly(ethylene oxide) copolymers [43,44]. Rolland et al. [45] studied the distribution of poly(ethylene oxide)-*b*-poly(isoprene)-*b*-poly(ethylene oxide), PEO-*b*-PI-*b*-PEO, micelles intravenously injected in mice by measuring the radioactivity of the blood samples. Fig. 9 shows that the percentage of the triblock in the blood remains high after 2 h and even after 24 h, in agreement with a low uptake by the liver and spleen.

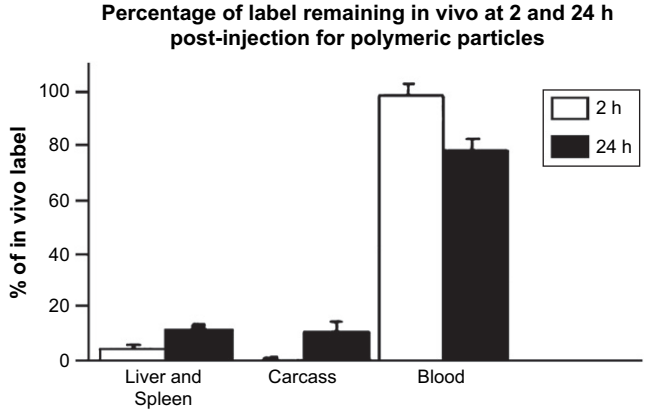

Fig. 9. Distribution of PEO-*b*-PI-*b*-PEO at the liver and spleen, carcass and blood at 2 and 24 h, after injection. (Reproduced from Ref. [45] with permission.)

4. Long-circulating carriers for passive targeting

As aforementioned, high molecular weight compounds, such as polymer carriers, preferentially accumulate in tumor tissues rather than in healthy ones. The higher porosity of tumor vessels (pores from 10 to 500 nm) (Fig. 4) can account for the perivascular accumulation of macromolecules. This ‘‘passive targeting’’ associated to the EPR effect [15–18] was illustrated by Kwon et al. [46], who injected doxorubicin (DOX) in mice and measured the drug content in tumors. DOX is a DNA intercalating agent that inhibits the RNA and DNA syntheses and is commonly administered intravenously for treating tumors. Cardiotoxicity of DOX is, however, a problem. Fig. 10 shows that DOX does not spontaneously accumulate in tumors, in contrast to DOX attached to poly(ethylene oxide)-*b*-poly(aspartate) micelles. The PEO-PAsp-DOX conjugates not only circulated for prolonged periods of time but their selectivity for tumors compared to heart was significantly

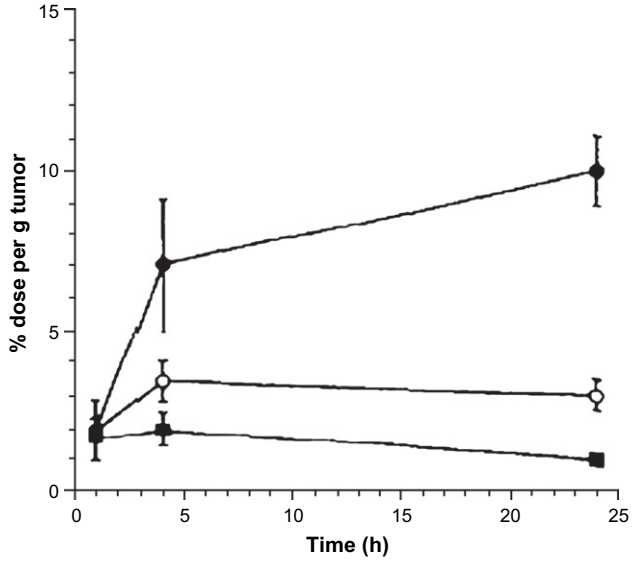

Fig. 10. Time dependence of the level of PEO-*b*-PAsp-DOX conjugates in solid tumors: (●) PEO(12K)-*b*-PAsp(2K); (○) PEO(5K)-*b*-PAsp(2K); (■) DOX. (Reproduced from Ref. [46] with permission.)

improved from 0.9 (% dose per gram of tumor) for DOX to 12 for the PEO-PAsp-Dox conjugate with a PEO molecular weight of 12000 g/mol and a PAsp molecular weight of 2000 g/mol (Fig. 10).

The major benefit of protection of drug nanocarriers by poly(ethylene oxide) is to increase the circulation time in the blood flow. As a result, a certain required concentration of drug carrier is maintained longer in the blood after a single injection, which is beneficial to accumulation in areas with affected (leaky) vasculature and targeting of areas with diminished blood supply and/or low concentration of a target ligand. The effectiveness of long-circulating nanocarriers strongly depends on the tailoring of PEO containing block or graft copolymers, in relation to the ability of PEO to protect the nanoparticles against the RES. As discussed in the next paragraph, performances of polymer-based drug delivery systems can be improved further by functional copolymers of a more complex structure.

5. Functional copolymers for active targeting

Binding pilot molecules [47–50] to nanocarriers is a straightforward way for improving the drug targeting. Polymers have thus to be modified by ligands specific to tumor sites, which may result in a higher selectivity compared to the EPR effect. Several parameters influence the efficacy of drug targeting, such as size of the target, blood flow through the target, number of sites on the target that can bind the drug carrier, number and affinity of targeting ligands on the drug carrier, and multipoint interaction of the drug carrier with the target. Therefore, there is a need for high yield synthesis of biocompatible and biodegradable amphiphilic block copolymers, whose end-group of the hydrophilic block is reactive and easily converted into conjugates of pilot molecules. As previously explained, PEO is the typical component of the hydrophilic shell of nanocarriers, such that the synthetic efforts have been oriented towards well-defined α,ω-heterotelechelic PEO.

Because the anionic polyaddition of ethylene oxide is living, initiation by an alkaline metal alkoxide that contains a functional group, protected or not, is the best strategy to prepare heterotelechelic PEO. This functional group will be the α end-group of the chains to be used further for the anchoring of the pilot molecules. A hydroxyl group is released upon hydrolysis of the propagating chains, and used, as such or after conversion, to initiate the polymerization of a second hydrophobic block. The initiation of the ring-opening polymerization of lactones and lactides is, for instance, direct in the presence of tin octoate.

The pilot molecule can be attached to the α-end of PEO either directly by initiation [51,52] or in a post-polymerization step. As an example of the first approach, Nakamura et al. [52] initiated the anionic polymerization of ethylene oxide by one hydroxyl group of properly protected sugar molecules (four hydroxyl groups out of five were protected by an acetal), such as 1,2,5,6-di-*O*-isopropylidene-D-glucofuranose (DIGL), 1,2,3,4-di-*O*-isopropylidene-D-galactopyranose (DIGA), and 1,2-*O*-isopropylidene-3,5-*O*-benzylidene-D-glucofuranose (IBGL). PEO was accordingly capped quantitatively by one saccharide on a sugar position that was dictated by the protection step of the hydroxyl groups. The regioselectivity of the sugar bonding is actually of great importance, because the cell-involved biorecognition via glyco-receptors on the cellular plasma membrane and the saccharide receptor is often a regioselective process.

In the second approach, the initiator contains a protected reactive group suitable for further grafting of the targeting unit. Kataoka et al. [53–55] synthesized poly(ethylene oxide) with an α-acetal end-group and an ω-hydroxyl end-group by initiating the anionic polymerization of ethylene oxide by potassium 3,3-diethoxypropyl alkoxide. The acetal end-group was then converted into aldehyde, followed by conjugation with an amino derivative in aqueous media. The Schiff's base that was accordingly formed was easily converted to a secondary amine by reductive amination. This reaction pathway is thus well-suited to the surface modification of nanocarriers by amine containing targeting molecules, such as proteins and peptides. Initiators with a protected amine were also used to prepare poly(ethylene oxide) with a primary amine end-group [56–58].

A variety of specific ligands can be attached to PEO, e.g., saccharides, peptides, antibodies, folic acid and transferrin. The functionality and solubility of the ligand obviously dictate the coupling reaction and the solvent to be used. Because most ligands of interest are selectively soluble in water, water-tolerant reactions impose themselves, the most common ones being reductive amination, thiol–maleimide coupling and peptidic coupling. It must be noted that amphiphilic block copolymers form micellar solutions in water, such that the coupling of the pilot molecule to the PEO block is conducted at the surface of preformed nanocarriers. It is then often a problem to separate the modified nanocarrier from the reaction side-products. The

choice of clean and quantitative coupling reaction is therefore a concern. In the same vein, when the hydrophobic block is a hydrolyzable polyester (PCL, PLA), the reaction conditions must be mild enough for avoiding premature chain degradation.

Unprotected sugars (lactose, galactose and mannose) were grafted onto the surface of polymeric carriers through a Schiff's base followed by reductive amination in water. Nagasaki et al. [59] synthesized a PEO-*b*-PLA copolymer with the PEO block capped by an aldehyde end-group. Aldehyde containing micelles were prepared by the dialysis method and reacted with *p*-aminophenyl-β-D-lactopyranoside, the reductive amination being carried out with $NaBH_3CN$. The coupling yield of lactose was 76%, and no side-reaction was observed. Although this strategy did not require protection/deprotection steps of the saccharide, it did not promote regioselectivity, the lactose being 1-0 substituted. Coupling of lactose through other positions, such as C-6 and C-2, was also reported in the scientific literature [60,61]. Peptidyl ligand [62,63] was also conjugated to micelles by the same strategy. In this respect, the impact of the targeting ligand on the general behaviour of the nanocarrier must be pointed out. Indeed, the distribution of the same type of nanoparticles can be strongly influenced by the peptidyl ligand, e.g., negatively charge ligands [phenylalanine (Phe) and tyrosyl–glutamic acid (Tyr–Glu)] vs neutral ligand [tyrosine (Tyr)]. Although the lifetime in the blood compartment was long, whatever the peptidyl ligand, the uptake of the nanoparticles by the liver and spleen was importantly decreased when they were piloted by the anionic Tyr–Glu ligand rather than by the neutral one. Clearly, the ligand can affect the surface properties of the micelles with consequences on the non-specific organ uptake.

Nasongkla et al. [64] successfully attached a cyclic pentapeptide, *c*(Arg-Gly-Asp-D-Phe-Lys) (*c*RGD–SH), to the surface of micelles formed by PEO-*b*-PCL diblocks, whose PEO block was end-capped by a maleimide. *c*RGD–SH was selected as the targeting ligand because of a high affinity for the $\alpha_v\beta_3$ integrin, which is a cell-tumor surface molecule that plays a key role in the endothelial cell survival during angiogenesis.

The covalent attachment of antibodies to polymeric micelles was also reported with the purpose to prepare immunomicelles [65–68]. Roby et al. [66] modified PEO–phosphatidylethanolamine (PEO–PE) containing micelles by an anticancer antibody that was attached by peptidic coupling reaction [69]. The amino containing antibody was reacted with the *p*-nitrophenylcarbonyl derivative of PEO–PE, with a yield lying between 75 and 100%.

Two methods were reported for the surface modification of polymeric micelles by folic acid (folate) based on the solubility of this compound, not only in water but also in various organic solvents: (i) preparation of surface-activated micelles followed by reaction with folate molecules in water [70]; (ii) end-capping of block copolymer by folate molecules in an organic solvent (dimethylsulfoxide (DMSO), or dimethylformamide (DMF)) followed by micellization [71–76]. Yoo and Park [71] synthesized folate-conjugated PEO-*b*-PLGA block copolymers by a peptidic reaction between folic acid and the amino end-capped diblock, NH_2-PEO-PLGA, in water.

Folic acid is a good candidate for tumor targeting because of a high affinity for the folate binding protein (FBP) ($K_d < 1$ nM), which is overexpressed on the surface of cancer cells [77–80]. Therefore, folate-conjugates, which are not transported to lysosomes like most ligands [81], can be directed to cancer cells and internalized by receptor-mediated endocytosis. They remain in recycling endosomes or escape in the cytoplasm. Their intracellular behaviour after ligand-mediated endocytosis thus distinguishes them from other types of ligands, such as antibodies, hormones and peptides.

The obvious advantages of targeted carriers (active targeting) over non-targeted ones (passive targeting) are a shift of the carrier distribution in favour of the tumor cell compartment, a prolonged carrier retention in tumors and delivery of the carrier content in an intracellular compartment. Oyewumi et al. [78] compared the cell uptake and tumor retention of gadolinium nanoparticles coated by folate–PEO and PEO, respectively. At a nanoparticle concentration of 180 μg/ml,

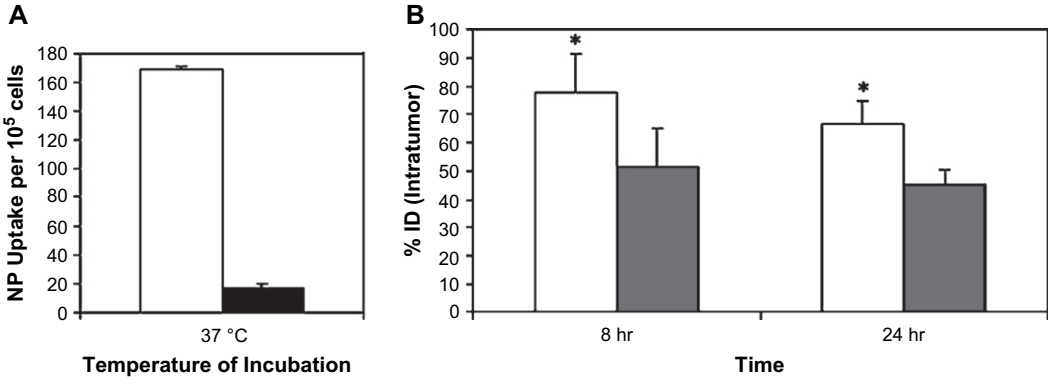

Fig. 11. (A) In vitro uptake of folate–PEO-coated nanoparticles (□) and PEO-coated nanoparticles (■) after incubation (180 μg/ml, 37 °C, 30 min) with KB cells. Each value is an average of three data ± standard deviation. (B) Retention of folate–PEO-coated (□) and PEO-coated (■) nanoparticles after injection into KB tumors developed in athymic mice. After 8 and 24 h, the mice were sacrificed, and the amount of Gd NPs in the tumor was measured by a gamma counter. Each value is an average of 6–7 data ± standard deviation. (Reproduced from Ref. [78] with permission.)

the KB cell uptake of folate–PEO-coated nanoparticles was 20-times higher than that of PEO-coated nanoparticles after 30 min of incubation (Fig. 11A). In parallel, the retention of folate–PEO-coated nanoparticles and PEO-coated ones was analyzed in vivo, thus in tumor tissue, 8 and 24 h after an intratumor injection. Statistically larger amounts of folate–PEO-coated nanoparticles were retained in tumor tissues compared to PEO-coated nanoparticles (Fig. 11B).

6. Responsive copolymers for smart targeting

According to the aforementioned examples, the intracellular delivery of anticancer drugs is improved and thus drug is delivered into the cytoplasm, whenever low molecular weight endogenous ligands and cell penetrating peptides are immobilized at the periphery of drug nanocarriers. This internalization process is a prerequisite for the killing of cells, because most cytotoxic drugs act intracellularly. As a rule, the non-specificity of ligands is responsible for death of a non-negligible amount of normal cells, which is a major concern in tumor targeting. An answer to this problem is in the use of pH-responsive polymers. Indeed, the extracellular pH of tumors is a consistently distinguishing phenotype of most solid tumors compared to surrounding normal tissues [82]. The experimental pH of most solid tumors in patients ranges from 5.7 to 7.8, with a mean value of 7.0. More than 80% of the experimental data are below pH 7.2, while blood pH remains constant at 7.4. Moreover, after cellular uptake, the drug carrier reaches the lysosomes with an even more acidic environment (lysosomial pH of 4.5–5.0).

Although they are typical pH-responsive polymers, polycations can be toxic [83]. As a rule, neutral polymers and polyanions are less cytotoxic than polycations, merely because most of the proteins are negatively charged, which restricts their adsorption. Expectedly, polycations with higher molecular weight and higher cationic charge density interact more importantly with cell membranes and cause cell damage. Surface electrical charge may also have an impact on the biocompatibility. Cationic macromolecules and their drug conjugates are indeed rapidly eliminated from plasma, in contrast to weakly anionic macromolecules that have a long circulation life [84].

6.1. pH-triggered ligand exposure

pH-sensitive multifunctional polymeric micelles with non-specific ligands which are exposed only under slightly acidic conditions ($6.5 < pH < 7.0$) were recently prepared. This specific exposure to tumor cells has the advantage that the ligands may be non-specific and it may be chosen for internalization of the nanocarrier.

As an example, Sawant et al. [85] prepared and tested in vitro PEGylated drug delivery systems (liposomes and micelles) that contained a non-specific internalization function (biotin or TAT peptide). This function was shielded by PEO under normal conditions, which strongly restricts internalization in normal cells, but it was exposed upon brief incubation at lower pH which speeds up the internalization in tumor cells (Fig. 12). This system consists of mixed micelles prepared with at least two surfactants: (i) a PEO-hydrazone-phosphatidylethanolamine (PEO-Hz-PE) surfactant, whose hydrazone junction between the hydrophilic tail and the hydrophobic head is cleaved by acidic hydrolysis, (ii) a biotin–PE shorter amphiphile which is part of the PEO shell.

In a similar approach, Sethuraman and Bae [86] prepared smart micellar nanocarriers in which the non-specific TAT peptide was not detected in normal tissues as result of the inter-polyelectrolyte complex association of PLA-*b*-PEO-TAT micelles with an ultra pH-sensitive smart block copolymer, polysulfonamide-*b*-PEO (PSD-*b*-PEO). Indeed, the positively-charged TAT exposed at the surface of the micelles was shielded by complexation with the polyanionic sulfonamide block (PSD) of the copolymer. This nanocarrier was merely prepared by the physical mixing of the two components. The PSD component is negatively charged at pH 7.4 and neutral below pH 7.0 (extracellular tumor pH), so that the TAT micelles are deshielded at the lower pH of the tumor environment. Once again, the TAT peptide contributes to the targeting of the drug loaded micelles into the cells and nuclei where the cytotoxic effect takes place (Fig. 13). The fate of TAT micelles and TAT micelles complexed by PSD-*b*-PEO at pH 6.6 and 7.4 was comparatively analyzed in vitro by flow cytometry. After 30 min of incubation, the TAT micelles were taken up by the cells in contrast to the complexed ones that remained uncaptured. After 1 h, the micelles complexed

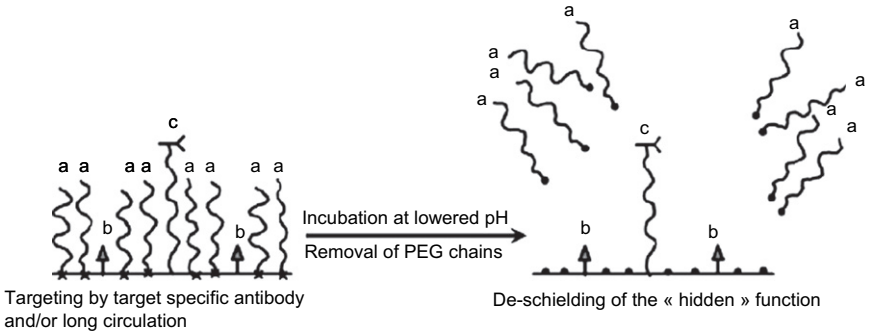

Fig. 12. Schematized multifunctional nanocarriers including: (a) pH-cleavable PEO-Hz-PE, (b) temporarily "shielded" biotin or TAT peptide, and (c) monoclonal antibody attached to the surface of the system through a pH-uncleavable spacer. (Reproduced from Ref. [85] with permission.)

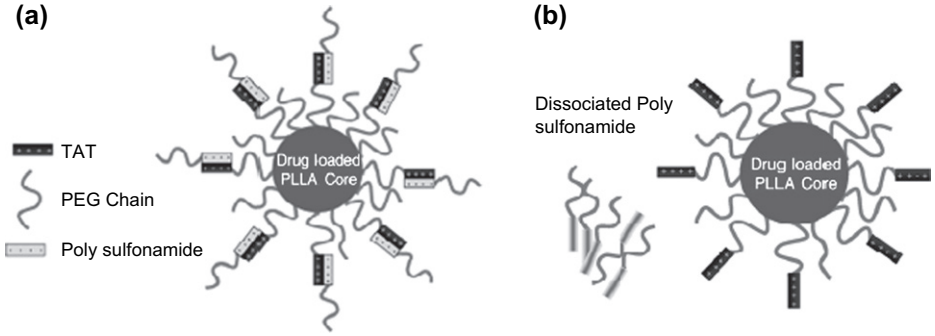

Fig. 13. (a) At normal blood pH, the sulfonamide is negatively charged, and complexes the positively-charged TAT exposed at the surface of the micelles. Only PEO is exposed to the outside which makes the carrier long circulating. (b) When the system experiences a decrease in pH (near tumor), sulfonamide loses charge and is released, thus exposing TAT to interaction with tumor cells. (Reproduced from Ref. [86] with permission.)

by PSD-*b*-PEO at pH 6.6 were internalized, while the micelles complexed by PSD-*b*-PEO at pH 7.4 had not entered the cells. The PSD-*b*-PEO chains are thus able to shield effectively the TAT peptide at pH 7.4.

6.2. pH-triggered micelles destabilization

The drug release from polymeric micelles can also be triggered by a change in pH [87,88]. The local drug delivery by the micellar carriers can indeed be improved by the destabilization of the micelles in pathological tissues of a lower pH, thus by combining the EPR effect with stimulus-responsiveness.

pH-sensitive block copolymer micelles [82,89,90] and nanoparticles [91–93] prone to dissociation when accumulated at the tumor sites and/or entered the cytoplasm were designed as schematized in Fig. 14a. Lee et al. [89] prepared pH-sensitive micelles of PEO-*b*-poly(L-histidine) (PEO-*b*-PHis). The hydrophobic imidazole of the histidine repeat units is protonated at the tumor extracellular pH ($pH \leq 7.2$), so making the PHis block hydrophilic and destabilizing the long-circulating polymeric micelles with release of the drug. The adriamycin release by the PHis–PEO micelles was indeed accelerated by a pH decrease from 8 to 6.8 (Fig. 15, circles). The sensitivity of the polymeric micelles to the more acidic extracellular pH of tumors was modulated, and the micelle stability at pH 7.4 was improved by preparing mixed micelles consisting of PHis-*b*-PEO and PLLA-*b*-PEO block copolymers, with or without a folate ligand [82,90]. This hybridization of the micelles shifted the triggering pH to lower values (7.2–6.6) (Fig. 15).

An even more elaborated systems combined the strategies discussed in the subsections 6.1 and above [94]. Mixed micelles of two block copolymers, i.e., poly(L-histidine)-*b*-poly(ethylene oxide) (PHis-*b*-PEO) and poly(L-lactic acid)-*b*-PEO-*b*-PHis-biotin, were prepared. Both the PHis and the PLLA blocks formed the core of the micelles, and PEO was the shell. Because of the high water solubility of PEO and biotin, the short PHis block in the PLLA-*b*-PEO-*b*-PHis-biotin copolymer was located at the core–shell interface, which caused the bending of the PEO block and the preferential location of biotin in the PEO shell built up by the PHis-*b*-PEO block copolymer (Fig. 14b). The micelles were stable above pH 7.2 and hided the conjugated biotins. At pH below 7.2, PHis was ionized, which was detrimental to the hydrophobic interaction of PHis with the micellar core. As a result, the PEO-*b*-PHis-biotin expanded, and biotin was exposed out of the PEO shell. The micelles degradation being also pH dependent, the release of doxorubicin was enhanced at the early endosomal pH (Fig. 14c).

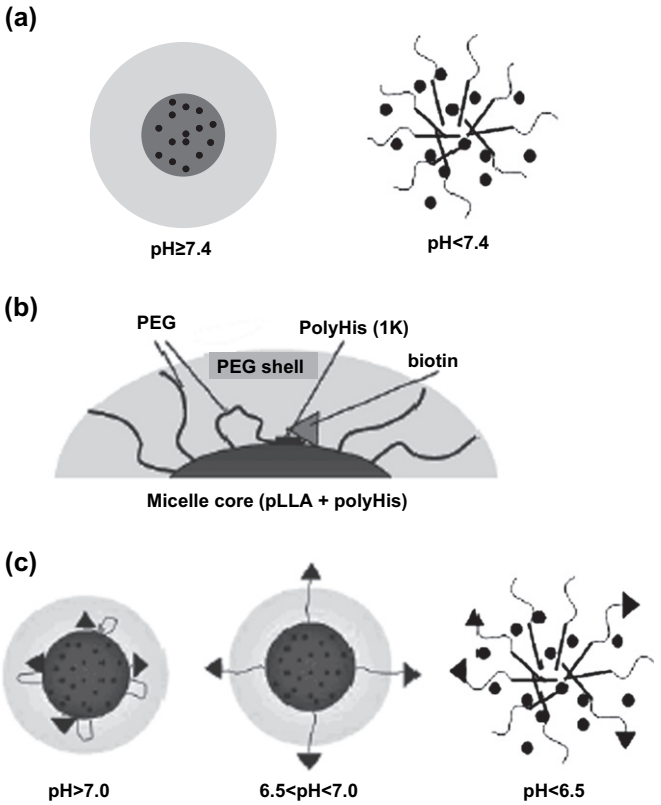

Fig. 14. Schematic illustration of (a) pH-triggered destabilization of micelles and (b and c) pH-triggered ligand exposure. Above pH 7.0, biotin (which is anchored to the micellar core through a pH-sensitive chain actuator (polyHis)) is shielded by a PEO shell. At $6.5 < pH < 7.0$, biotin is exposed at the micellar surface and can interact with cells, which facilitates biotin receptor-mediated endocytosis. Upon a further decrease in pH ($pH < 6.5$), the micelles are destabilized, which results in enhanced drug release and disruption of cell membranes, such as endosomal membranes. (Reproduced from Ref. [94] with permission.)

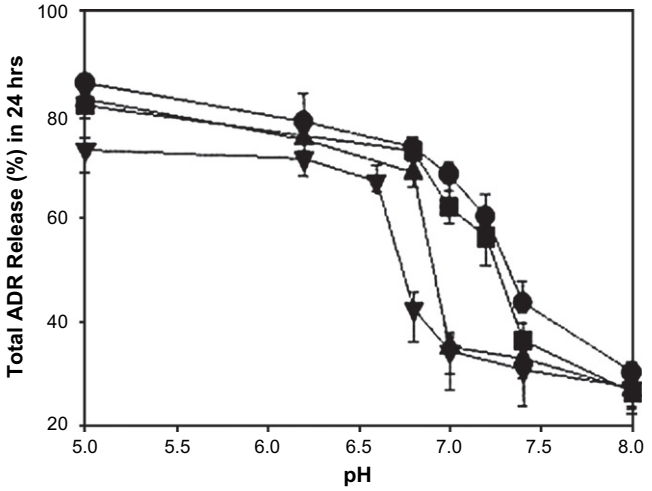

Fig. 15. pH-dependent cumulative ADR release from mixed micelles of poly-His-*b*-PEO and PLLA-*b*-PEO (PLLA-*b*-PEO content in the mixed micelles): (●) 0 wt.%, (■) 10 wt.%, (▲) 25 wt.% and (▼) 40 wt.% after 24 h. (Reproduced from [82] with permission.)

Wang et al. [95] prepared environmental-sensitive micelles made of poly(L-lactide)-*b*-poly(2-ethyl-2-oxazoline)-*b*-poly(L-lactide) (PLLA-PEOz-PLLA) triblock copolymer. In a similar approach, Hsiue et al. [96] used poly(2-ethyl-2-oxazoline)-*b*-poly(L-lactide) (PEOz-PLLA) diblock copolymer. PEOz is a pH-sensitive polymer with a low cytotoxicity and a favourable p*K*a close to neutral pH. The release profiles of DOX-loaded micelles were different in neutral and acidic buffer solutions. Compared to triblock copolymer micelles, micellar diblock copolymers offered several advantages, including easy preparation, smaller DOX-loaded micelles, improved micellar structure and sharper pH-response and drug release.

6.3. pH-cleavable drug–polymer conjugates

Rather than using a targeting ligand, an alternative approach may be found in the covalent linkage of the drug to the carrier, however, through a hydrolyzable bond. The targeting of tumor tissue relies again on a pH effect, i.e. the cleavage of the drug-carrier bond at low pH, particularly in the lysosomes of tumor cells.

Cis-aconityl acid, Schiff's base derivatives and hydrazones are the most prominent acid-labile linkers which have been used in these pH-triggered release systems [97–99]. Because hydrazone is cleaved within a short period of time in an acidic environment, Bae et al. [100] synthesized an amphiphilic block copolymer PEO-*b*-poly(aspartate-hydrazone-adriamycin) (PEO-*b*-p(Asp-Hyd-ADR) and prepared micelles therefrom. The ADR release by the micelles was dependent on time and pH (in the 7.4–3.0 range). Although the micelles were stable under physiological and early endosomal conditions, the ADR was gradually released considering that pH in late endosomes and/or lysosomes in the cells is ~5.0, so fitting the conditions of effective cleavage of hydrazone.

The superiority of this approach over the previous one (Section 6.2) is that the covalent anchoring of the drug totally prevents its release under physiological conditions. However, attention must be paid to the possible alteration of the therapeutic activity of the drug as a consequence of the chemical grafting.

7. Biodegradation issue

Once injected, there is no choice for the nanocarrier but to accumulate in the body after drug release, which may be a problem sooner or later. This explains why biodegradable and/or bioeliminable polymers are unavoidably used in the design of drug nanocarriers [2,20–22].

Although biodegradation results from a biological activity, particularly from an enzymatic action, all the polymers said "biodegradable" in the literature do not fit this definition.

At the time being, the hydrophobic constitutive component of known medical devices and controlled release formulations is an aliphatic polyester selected for biocompatibility and degradability. They are bioresorbable after hydrolytic degradation followed by bio-assimilation or elimination of degradation-byproducts.

Homo- and copolymers of lactic acid (LA) and glycolic acid (GA) are being extensively used in controlled release carriers because of high degradation rate [21]. They are commonly synthesized by ring-opening polymerization of lactide and glycolide, respectively, at 140–180 °C with a tin catalyst [101–104], particularly tin 2-ethylhexanoate, which is approved by FDA as a food stabilizer.

These aliphatic polyesters are degraded by bulk hydrolysis of the ester bonds on a timescale of weeks. PLA is not only an excellent biomaterial but also safe for in vivo application because it is degraded into lactic acid, which is a natural metabolite of the body. PLA has also an excellent loading capacity and the drug release is mediated by the non-enzymatic hydrolysis, which is autocatalyzed by the carboxylic acid end-groups of the chains.

PCL is also well-suited to controlled drug delivery because of high permeability to many drugs and non-toxicity. Its degradation is, however, much slower (year timescale) than PLA and PGA, which makes it less common in delivery nanocarriers. Nevertheless, the degradation rate can be extensively modulated by copolymerization of LA with glycolide and ε-caprolactone (ε-CL). The degradation kinetics of PLA is indeed increased by glycolide and decreased by ε-CL, to an extension that depends on the comonomer content.

Although not biodegradable, PEO is eliminated from the body by the natural filtration when the molecular weight does not exceed 40,000 g/mol. So, self-assembly copolymers of PEO and aliphatic polyesters do not accumulate in the body even in case of repeated injections, which makes them very attractive as materials for building-up nanocarriers.

8. Conclusion

Nowadays, effective drug delivery systems are emerging as result of a more accurate targeting of pathological tissues.

Indeed, higher doses are released at the desired sites, with less damage for healthy tissues. This substantial progress is the consequence of the steadily improved understanding of the biological and chemical impacts of disease at the molecular level, the new therapeutic concepts accordingly devised, and their implementation by the design of intelligent drug nanocarriers. The engineering of synthetic polymers play a key role in the building-up of these polymeric nanocarriers that basically results from the self-assembly of amphiphilic block copolymers into stealthy micellar drug reservoir guided to tumor tissues, where the drug is selectively released.

This review has emphasized the prominent role of poly(ethylene oxide) in the construction of nanocarriers circulating in the blood stream for a long time and how a passive targeting is effective because of natural anatomic differences between tumors and normal tissues. Performances of cancer treatment have been improved by the more accurate tailoring of PEO containing amphiphiles, which increased the complexity of the nanocarriers at the benefit of the active targeting. The recent advent of pH-sensitive nanocarriers has improved further the control of the drug distribution and bioavailability. Last but not least, the proper choice of the constitutive components of the polymeric amphiphiles takes into account the bioelimination of the carrier after the drug delivery.

Acknowledgment

The authors are grateful to the ''Services Fédéraux des Affaires Scientifiques, Techniques et Culturelles'' in the frame of the ''Pôles d'Attraction Interuniversitaires: Supramolecular Chemistry and Supramolecular Catalysis (PAI 6/27)''. K.V.B. is grateful to the ''Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture'' (FRIA) for a fellowship.

References

[1] Kwon GS, Okano T. Advanced Drug Delivery Reviews 1996;21(2):107–16.
[2] Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Chemical Reviews (Washington, DC) 1999;99(11):3181–98.
[3] Kwon GS, Okano T. Pharmaceutical Research 1999;16(5):597–600.
[4] Jones MC, Leroux JC. European Journal of Pharmaceutics and Biopharmaceutics 1999;48(2):101–11.
[5] Rosler A, Vandermeulen GWM, Klok H-A. Advanced Drug Delivery Reviews 2001;53(1):95–108.
[6] Kataoka K, Harada A, Nagasaki Y. Advanced Drug Delivery Reviews 2001;47(1):113–31.
[7] Barratt G, Couarraze G, Couvreur P, Dubernet C, Fattal E, Gref R, et al. Polymeric Biomaterials 2002:753–81.
[8] Nishiyama N, Bae Y, Miyata K, Fukushima S, Kataoka K. Drug Discovery Today: Technologies 2005;2(1):21–6.
[9] Nishiyama N, Kataoka K. Polymer Therapeutics II. In: Advances in Polymer Science, vol. 193; 2006. p. 67–101.
[10] Satchi-Fainaro R, Duncan R, Barnes CM. Polymer Therapeutics II. In: Advances in Polymer Science, vol. 193; 2006. p. 1–65.
[11] Torchilin VP. Pharmaceutical Research 2007;24(1):1–16.
[12] Gaucher G, Dufresne M-H, Sant VP, Kang N, Maysinger D, Leroux J-C. Journal of Controlled Release 2005;109(1–3):169–88.
[13] Owens DE, Peppas NA. International Journal of Pharmaceutics 2006;307(1):93–102.
[14] Belting M, Sandgren S, Wittrup A. Advanced Drug Delivery Reviews 2005;57(4):505–27.
[15] Iyer AK, Khaled G, Fang J, Maeda H. Drug Discovery Today 2006;11(17–18):812–8.
[16] Takakura Y, Hashida M. Critical Reviews in Oncology/Hematology 1995;18(3):207–31.
[17] Maeda H, Seymour LW, Miyamoto Y. Bioconjugate Chemistry 1992;3(5):351–62.
[18] Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Journal of Controlled Release 2000;65(1–2):271–84.
[19] Williams DF. The Williams dictionary of biomaterials. Liverpool: Liverpool University Press; 1999.
[20] Chandra R, Rustgi R. Progress in Polymer Science 1998;23(7):1273–335.
[21] Domb AJ, Kumar N, Sheskin T, Bentolila A, Slager J, Teomim D. Polymeric Biomaterials 2002:91–121.
[22] Kumar N, Ravikumar MNV, Domb AJ. Advanced Drug Delivery Reviews 2001;53(1):23–44.
[23] Flandroy P, Grandfils C, Daenen B, Snaps F, Dondelinger RF, Jerome R, et al. Journal of Controlled Release 1997;44(2–3):153–70.
[24] Seyler I, Appel M, Devissaguet J-P, Legrand P, Barratt G. Journal of Nanoparticle Research 1999;1(1):91–7.
[25] Liu X, Heng PWS, Li Q, Chan LW. Journal of Controlled Release 2006;116(1):35–41.
[26] Lee JH, Lee HB, Andrade JD. Progress in Polymer Science 1995;20(6):1043–79.
[27] Vermette P, Meagher L. Colloids and Surfaces B: Biointerfaces 2003;28:153–98.
[28] Szleifer I. Current Opinion in Solid State and Materials Science 1997;2(3):337–44.
[29] Jeon SI, Lee JH, Andrade JD, De Gennes PG. Journal of Colloid and Interface Science 1991;142(1):149–58.
[30] Jeon SI, Andrade JD. Journal of Colloid and Interface Science 1991;142(1):159–66.
[31] Vert M, Domurado D. Journal of Biomaterials Science, Polymer Edition 2000;11(12):1307–17.
[32] Kavlak S, Güner A. Journal of Applied Polymer Science 2006;100(2):1554–60.
[33] Park JH, Bae YH. Journal of Biomaterials Science, Polymer Edition 2002;13(5):527–42.
[34] Gref R, Luck M, Quellec P, Marchand M, Dellacherie E, Harnisch S, et al. Colloids and Surfaces, B: Biointerfaces 2000;18(3–4):301–13.
[35] Lee JH, Oh JY, Kim DM. Journal of Materials Science: Materials in Medicine 1999;10(10/11):629–34.
[36] Lee JH, Oh SH. Journal of Biomedical Materials Research 2002;60(1):44–52.
[37] Peracchia MT, Vauthier C, Passirani C, Couvreur P, Labarre D. Life Sciences 1997;61(7):749–61.
[38] Passirani C, Benoit J-P. Biomaterials for delivery and targeting of proteins and nucleic acids; 2005. p. 187–230.
[39] Rieger J, Passirani C, Benoit J-P, Van Butsele K, Jerome R, Jerome C. Advanced Functional Materials 2006;16(11):1506–14.
[40] Klibanov AL, Maruyama K, Torchilin VP, Huang L. FEBS Letters 1990;268(1):235–7.
[41] Kwon GS, Kataoka K. Advanced Drug Delivery Reviews 1995;16(2–3):295–309.
[42] Gabizon AA. Advanced Drug Delivery Reviews 1995;16(2–3):285–94.
[43] Stolnik S, Illum L, Davis SS. Advanced Drug Delivery Reviews 1995;16(2–3):195–214.
[44] Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R. Science (Washington, DC) 1994;263:1600–3.
[45] Rolland A, O'Mullane J, Goddard P, Brookman L, Petrak K. Journal of Applied Polymer Science 1992;44(7):1195–203.
[46] Kwon G, Suwa S, Yokoyama M, Okano T, Sakurai Y, Kataoka K. Journal of Controlled Release 1994;29(1–2):17–23.

[47] Mammen M, Chio S-K, Whitesides GM. Angewandte Chemie, International Edition 1998;37(20):2755–94.
[48] Torchilin VP. European Journal of Pharmaceutical Sciences 2000; 11(Suppl. 2):S81–91.
[49] Jaracz S, Chen J, Kuznetsova LV, Ojima I. Bioorganic and Medicinal Chemistry 2005;13(17):5043–54.
[50] Torchilin Vladimir P. Advanced Drug Delivery Reviews 2005;57(1): 95–109.
[51] Yasugi K, Nakamura T, Nagasaki Y, Kato M, Kataoka K. Macromolecules 1999;32(24):8024–32.
[52] Nakamura T, Nagasaki Y, Kataoka K. Bioconjugate Chemistry 1998; 9(2):300–3.
[53] Otsuka H, Nagasaki Y, Kataoka K. Biomacromolecules 2000;1(1): 39–48.
[54] Otsuka H, Nagasaki Y, Kataoka K. Langmuir 2004;20(26): 11285–7.
[55] Nagasaki Y, Kutsuna T, Iijima M, Kato M, Kataoka K, Kitano S, et al. Bioconjugate Chemistry 1995;6(2):231–3.
[56] Yokoyama M, Okano T, Sakurai Y, Kikuchi A, Ohsako N, Nagasaki Y, et al. Bioconjugate Chemistry 1992;3(4):275–6.
[57] Nagasaki Y, Iijima M, Kato M, Kataoka K. Bioconjugate Chemistry 1995;6(6):702–4.
[58] Huang J, Wang H, Tian X. Journal of Polymer Science, Part A: Polymer Chemistry 1996;34(10):1933–40.
[59] Nagasaki Y, Yasugi K, Yamamoto Y, Harada A, Kataoka K. Biomacromolecules 2001;2(4):1067–70.
[60] Kopecek J, Duncan R. Journal of Controlled Release 1987;6: 315–27.
[61] Julyan PJ, Seymour LW, Ferry DR, Daryani S, Boivin CM, Doran J, et al. Journal of Controlled Release 1999;57(3):281–90.
[62] Yamamoto Y, Nagasaki Y, Kato Y, Sugiyama Y, Kataoka K. Journal of Controlled Release 2001;77(1–2):27–38.
[63] Yamamoto Y, Nagasaki Y, Kato M, Kataoka K. Colloids and Surfaces, B: Biointerfaces 1999;16(1–4):135–46.
[64] Nasongkla N, Shuai X, Ai H, Weinberg BD, Pink J, Boothman DA, et al. Angewandte Chemie, International Edition 2004;43(46): 6323–7.
[65] Lukyanov AN, Elbayoumi TA, Chakilam AR, Torchilin VP. Journal of Controlled Release 2004;100(1):135–44.
[66] Roby A, Erdogan S, Torchilin VP. European Journal of Pharmaceutics and Biopharmaceutics 2006;62(3):235–40.
[67] Torchilin VP. Cellular and Molecular Life Sciences 2004;61(19–20): 2549–59.
[68] Kabanov AV, Chekhonin VP, Alakhov VY, Batrakova EV, Lebedev AS, Melik-Nubarov NS, et al. FEBS Letters 1989;258(2):343–5.
[69] Torchilin VP, Levchenko TS, Lukyanov AN, Khaw BA, Klibanov AL, Rammohan R, et al. Biochimica et Biophysica Acta, Biomembranes 2001;1511(2):397–411.
[70] Stella B, Arpicco S, Peracchia MT, Desmaele D, Hoebeke J, Renoir M, et al. Journal of Pharmaceutical Sciences 2000;89(11):1452–64.
[71] Yoo HS, Park TG. Journal of Controlled Release 2004;96(2): 273–83.
[72] Park EK, Kim SY, Lee SB, Lee YM. Journal of Controlled Release 2005;109(1–3):158–68.
[73] Park Eun K, Lee Sang B, Lee Young M. Biomaterials 2005;26(9): 1053–61.
[74] Licciardi M, Giammona G, Du J, Armes SP, Tang Y, Lewis AL. Polymer 2006;47(9):2946–55.
[75] Liu S-Q, Wiradharma N, Gao S-J, Tong YW, Yang Y-Y. Biomaterials 2007;28(7):1423–33.
[76] Bae Y, Jang W-D, Nishiyama N, Fukushima S, Kataoka K. Molecular BioSystems 2005;1(3):242–50.
[77] Lu Y, Low PS. Advanced Drug Delivery Reviews 2002;54(5): 675–93.
[78] Oyewumi MO, Yokel RA, Jay M, Coakley T, Mumper RJ. Journal of Controlled Release 2004;95(3):613–26.
[79] Gabizon A, Shmeeda H, Horowitz AT, Zalipsky S. Advanced Drug Delivery Reviews 2004;56(8):1177–92.
[80] Gabizon A, Horowitz AT, Goren D, Tzemach D, Shmeeda H, Zalipsky S. Clinical Cancer Research 2003;9(17):6551–9.
[81] Wang S, Low PS. Journal of Controlled Release 1998;53(1–3):39–48.
[82] Lee ES, Na K, Bae YH. Journal of Controlled Release 2003;91(1–2): 103–13.
[83] Moreau E, Domurado M, Chapon P, Vert M, Domurado D. Journal of Drug Targeting 2002;10(2):161–73.
[84] Wang Y-X, Robertson JL, Spillman WB, Claus RO. Pharmaceutical Research 2004;21(8):1362–73.
[85] Sawant RM, Hurley JP, Salmaso S, Kale A, Tolcheva E, Levchenko TS, et al. Bioconjugate Chemistry 2006;17(4):943–9.
[86] Sethuraman VA, Bae YH. Journal of Controlled Release 2007;118(2): 216–24.
[87] CdlH Alarcon, Pennadam S, Alexander C. Chemical Society Reviews 2005;34(3):276–85.
[88] Schmaljohann D. Advanced Drug Delivery Reviews 2006;58(15): 1655–70.
[89] Lee ES, Shin HJ, Na K, Bae YH. Journal of Controlled Release 2003; 90(3):363–74.
[90] Lee ES, Na K, Bae YH. Journal of Controlled Release 2005;103(2): 405–18.
[91] Potineni A, Lynn DM, Langer R, Amiji MM. Journal of Controlled Release 2003;86(2–3):223–34.
[92] Shenoy D, Little S, Langer R, Amiji M. Pharmaceutical Research 2005; 22(12):2107–14.
[93] Devalapally H, Shenoy D, Little S, Langer R, Amiji M. Cancer Chemotherapy and Pharmacology 2007;59(4):477–84.
[94] Lee ES, Na K, Bae YH. Nano Letters 2005;5(2):325–9.
[95] Wang C-H, Wang C-H, Hsiue G-H. Journal of Controlled Release 2005; 108(1):140–9.
[96] Hsiue G-H, Wang C-H, Lo C-L, Wang C-H, Li J-P, Yang J-L. International Journal of Pharmaceutics 2006;317(1):69–75.
[97] Yoo HS, Lee EA, Park TG. Journal of Controlled Release 2002;82(1): 17–27.
[98] Etrych T, Jelinkova M, Rihova B, Ulbrich K. Journal of Controlled Release 2001;73(1):89–102.
[99] Hruby M, Konak C, Ulbrich K. Journal of Controlled Release 2005; 103(1):137–48.
[100] Bae Y, Fukushima S, Harada A, Kataoka K. Angewandte Chemie, International Edition 2003;42(38):4640–3.
[101] Penczek S, Duda A, Kowalski A, Libiszowski J, Majerska K, Biela T. Macromolecular Symposia 2000;157:61–70 (International symposium on ionic polymerization, 1999).
[102] Albertsson A-C, Varma IK. Biomacromolecules 2003;4(6):1466–86.
[103] Lecomte P, Stassin F, Jerome R. Macromolecular Symposia 2004;215: 325–38 (Proceedings of the 2003 international symposium on ionic polymerization and related processes).
[104] Kowalski A, Libiszowski J, Biela T, Cypryk M, Duda A, Penczek S. Macromolecules 2005;38(20):8170–6.

Kathy Van Butsele was born in 1982 in Liege, Belgium. She received her Master degree in Chemistry at Liege University in 2005. She is currently working as Ph.D. student in the group of Prof Dr. R. Jérôme at the Center for Education and Research on Macromolecules in Liege University. Her Ph.D. research focuses on the synthesis of new macromolecular architectures for the elaboration of drug delivery system.

Christine Jérôme born in 1971 in Belgium, completed her Ph.D. in 1998 at the University of Liege, Belgium and then worked as a post-dosctoral researcher at the same University. In 2000, she joined the University of Ulm in Germany as a recipient of the Humboldt scholarship. She returned to the University of Liege in 2001 as Research Associate of the National Foundation of the Scientific Research in the group of Prof. R. Jérôme, where she became Professor in 2006. Her research interests include electropolymerization, polymer functionalized nanoparticles and biomaterials.

Robert Jérôme was born in 1942 in Belgium. He received his Ph.D. degree in Chemistry at the University of Liege, Belgium, in 1970. He collaborated for more than three decades with Prof. Ph. Teyssié to the development of the laboratory of ''Macromolecular Chemistry and Organic Catalysis'' in the same university. Since 1994, he is director of the ''Center for Education and Research on Macromolecules'' (CERM), and he is, at present, president of the Department of Chemistry at the University of Liege.

His research effort is devoted to macromolecular engineering anytime oriented towards novel or at least improved multiphase polymeric materials. He is also active in macromolecular chemistry without using organic solvents, thus in the melt and in supercritical CO_2.