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Medicinal Chemistry

Polymer Therapeutics: Concepts and Applications

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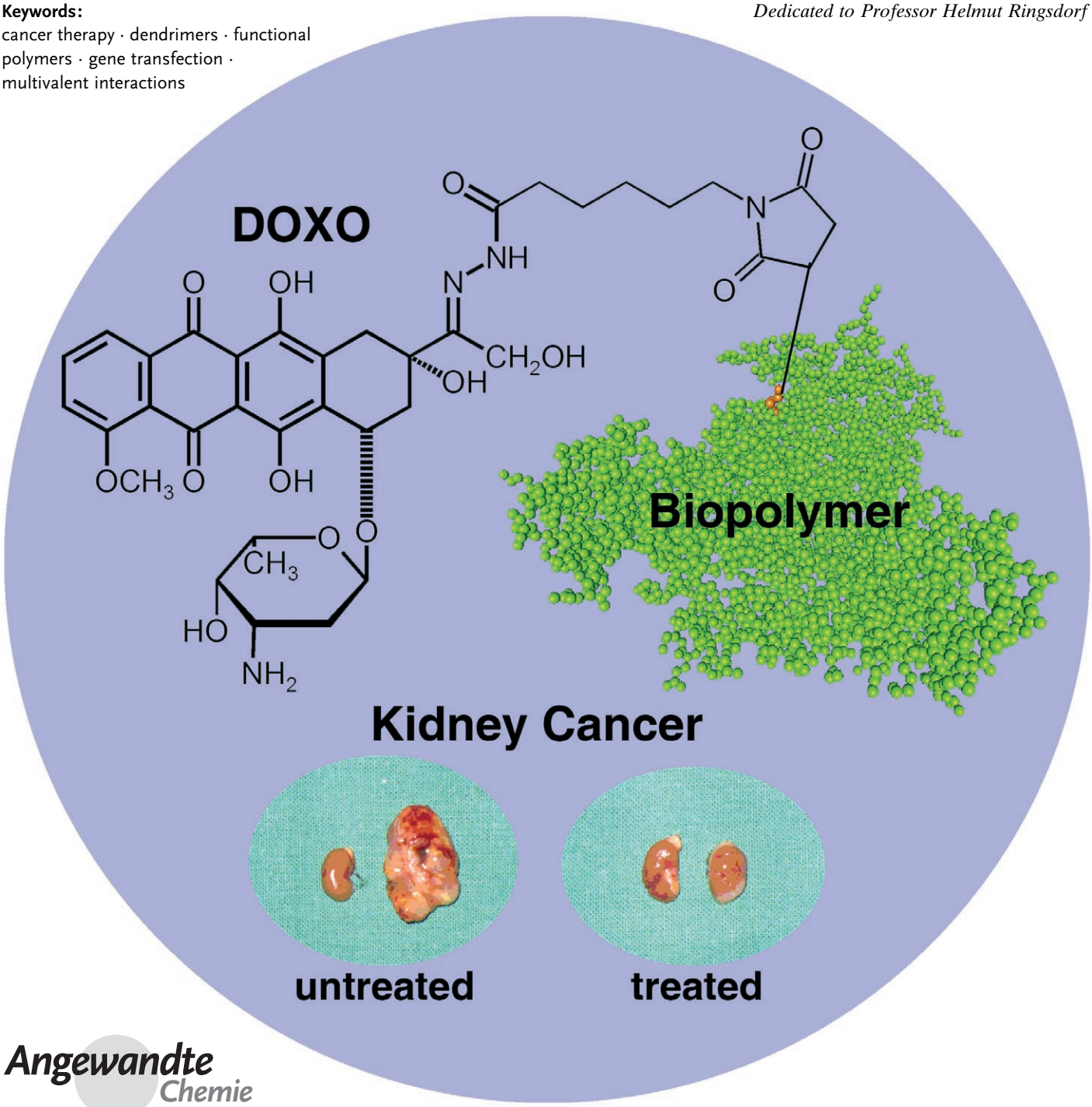
cancer therapy · dendrimers · functional polymers · gene transfection · multivalent interactions

Dedicated to Professor Helmut Ringsdorf



Polymer therapeutics encompass polymer–protein conjugates, drug–polymer conjugates, and supramolecular drug-delivery systems. Numerous polymer–protein conjugates with improved stability and pharmacokinetic properties have been developed, for example, by anchoring enzymes or biologically relevant proteins to polyethylene glycol components (PEGylation). Several polymer–protein conjugates have received market approval, for example the PEGylated form of adenosine deaminase. Coupling low-molecular-weight anticancer drugs to high-molecular-weight polymers through a cleavable linker is an effective method for improving the therapeutic index of clinically established agents, and the first candidates have been evaluated in clinical trials, including, N-(2-hydroxypropyl)methacrylamide conjugates of doxorubicin, camptothecin, paclitaxel, and platinum(II) complexes. Another class of polymer therapeutics are drug-delivery systems based on well-defined multivalent and dendritic polymers. These include polyanionic polymers for the inhibition of virus attachment, polycationic complexes with DNA or RNA (polyplexes), and dendritic core–shell architectures for the encapsulation of drugs. In this Review an overview of polymer therapeutics is presented with a focus on concepts and examples that characterize the salient features of the drug-delivery systems.

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1. Introduction

Improving the therapeutic index[1] of drugs is a major impetus for innovation in many therapeutic areas such as cancer, inflammatory, and infective diseases. The search for new drug-delivery concepts and new modes of action are the major driving force in polymer therapeutics.[2–5]

Today, the vast majority of clinically used drugs are low-molecular-weight compounds (typically under 500 $g\,mol^{-1}$) that exhibit a short half-life in the blood stream and a high overall clearance rate. These small-molecule drugs typically interact through a multiple but monovalent binding with a given receptor. Furthermore, they diffuse rapidly into healthy tissues and are distributed evenly within the body. As a consequence, relatively small amounts of the drug reach the target site, and therapy is associated with side effects. These disadvantages are especially pronounced with drugs that exhibit a narrow therapeutic index,[1] such as anticancer, antirheumatic, and immunosuppressive agents. Frequent side-effects associated with these drugs are nephrotoxicity, bone-marrow toxicity, neurotoxicity, cardiotoxicity, mucositis, and gastrointestinal toxicity, which are dose-limiting and thus prevent effective treatment.

A number of macromolecular delivery systems are under investigation to circumvent these limitations and improve the potential of the respective drug. Generally, these can be classified as nanoparticulate drug-delivery systems or as drug–polymer conjugates. Particulate delivery systems in which the drugs are physically incorporated into nanoparticles include emulsions, liposomes, and noncovalent polymeric carrier systems. In drug–polymer conjugates, however, a drug is covalently linked to polymers such as proteins, polysaccharides, or synthetic polymers.

The coupling of drugs to macromolecular carriers received an important impetus from 1975 onwards with the development of monoclonal antibodies by Milstein and Köhler,[6] and from Ringsdorf's notion of a general drug-delivery system based on synthetic polymers (Figure 1).[3,7] Initially,

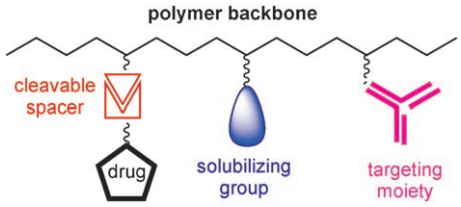

Figure 1. Ringsdorf's model for drug-delivery systems based on synthetic polymers.

research work has focused on realizing drug conjugates with antibodies to selectively target cell-specific antigens or receptors. This propagated the therapeutic concept of drug targeting that was founded on Paul Ehrlich's vision of "the magic bullet" which he proclaimed at the beginning of the last

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century. However, it took many years for the dawning era of "polymer therapeutics" to "kick-off".[4]

In Ringsdorf's original model (Figure 1) a number of drug molecules are bound to a macromolecule through a spacer molecule, which can incorporate a predetermined breaking point to ensure release of the drug at the site of interest. The polymer conjugate can additionally contain moieties, for example, antibodies or sugar moieties, which target disease-related antigens or receptors. In addition, solubilizing groups can be attached to the polymer backbone to modify the bioavailability of the drug–polymer conjugate.

Macromolecules chosen for the preparation of drug–polymer conjugates should ideally be water-soluble, nontoxic, and nonimmunogenic, as well as degraded and/or eliminated from the organism.[8] Finally, the macromolecular carrier should exhibit suitable functional groups for attaching the respective drug or spacer. Initially, *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers were intensively studied as linear polymers for therapeutic applications according to the Ringsdorf model.[9–11] However, a spectrum of other synthetic polymers with structural and architectural variations, including A) monofunctional linear, B) polyfunctional linear, C) starlike, and D) dendritic architectures are being investigated today (Figure 2).

Conjugates of drugs and polymers as well as other polymeric carrier systems have collectively been termed polymer therapeutics,[4,5] which primarily encompass polymer–protein conjugates, drug–polymer conjugates, and more recently supramolecular drug-delivery systems as well as other defined nanosized systems.[12–14] Anchoring of enzymes

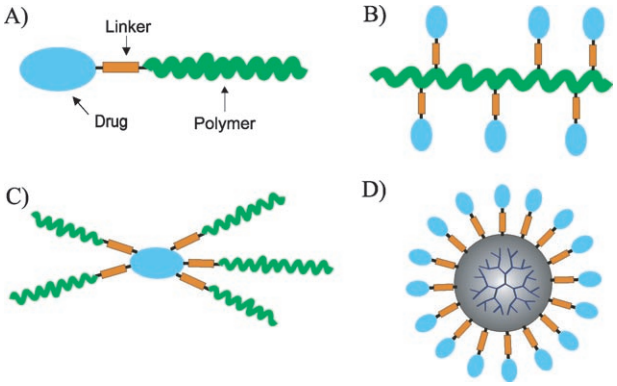

Figure 2. Selected structural and architectural types of drug–polymer conjugates.

or biological response modifiers to polyethylene glycol components (PEGylation) has led to numerous polymer–protein conjugates with improved stability and pharmacokinetic properties. Several polymer–protein conjugates have received market approval (Table 1).[4] The coupling of low-molecular-weight anticancer drugs to polymers through a cleavable linker has been an effective method for improving the therapeutic index of clinically established agents, and the first candidates of anticancer drug–polymer conjugates are being evaluated in clinical trials.

The advance of well-defined polyvalent and dendritic polymers[15] has paved the way for designing tailor-made systems with self-assembling properties which are also classified as polymer therapeutics. These include: a) polyan-

Table 1: Polymer–protein conjugates with market approval.

Trade name	Protein	Polymer	Indication	Marketed
adagen	adenosine deaminase	5 kDa PEG	severe combined immunodeficiency disease	Enzon
oncaspar	asparaginase	5 kDa PEG	acute lymphatic leukemia	Enzon
pegvisomant	GH antagonist	5 kDa PEG	excessive growth (acromegaly)	Pfizer
PEG-intron	interferon α2b	12 kDa PEG	hepatitis C	Schering-Plough
pegasys	interferon α2a	40 kDa PEG	hepatitis C	Roche
neulasta	granulocyte colony stimulating factor	20 kDa PEG	neutropenia	Amgen
SMANCS/ lipiodol	neocarzinostatin	copolymer of styrene maleic acid	hepatocellular cancer	Yamanouchi Pharmaceutical Company

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Felix Kratz graduated in Chemistry from the University of Heidelberg in 1991. He then carried out postdoctoral research at the Bioinorganic Institute of the University of Florence (Italy) and developed tumor-specific carrier systems with ruthenium(III) complexes. Since 1994 he has been Head of Macromolecular Prodrugs at the Tumor Biology Center in Freiburg, Germany, where he is now in charge of organizing and managing translational research from the laboratory to the clinic. His research areas are drug targeting, drug-delivery systems in oncology, prodrugs, receptor targeting, and bioconjugate chemistry.

ionic polymers for the inhibition of virus attachment and as heparin analogues; b) polycationic complexes with DNA or RNA (polyplexes); and c) polymer micelles with covalently bound drugs as well as dendritic core–shell architectures for the encapsulation of drugs. In this Review, we present an overview of polymer therapeutics with a focus on concepts and pertinent examples that characterize the salient features of the respective drug-delivery system. Further examples can be found in review articles that have appeared over the past decade on this topic.[5,8,11,16–26] Not included are polymers for galenic applications and slow-release systems based on bulk degradation of the polymer matrix.[27]

2. Macromolecules as Drug-Delivery Systems: Biological Rationale

2.1. Passive Drug Targeting and Specific Tissue Targeting: The EPR Effect

It has long been known that biopolymers play an essential role as free and membrane-bound "therapeutics". Therefore, it is surprising that synthetic polymers were originally only discussed as plasma expanders, for example, pervirlon or poly(vinyl pyrrolidone) during the Second World War.[28]

Passive accumulation of macromolecules and other nanoparticles in solid tumors is a phenomenon which was probably overlooked for several years as a potential biological target for tumor-selective drug delivery. The rationale for using macromolecules as efficient carriers for the delivery of antitumor agents, even if they are not targeted towards an antigen or receptor on the surface of the tumor cell, is based on the pioneering work of Maeda and co-workers[29,30] as well as Jain et al.[31,32] The results of these studies gave detailed insight into the pathophysiology of tumor tissue that is characteristic of angiogenesis, hypervasculature, a defective vascular architecture, and an impaired lymphatic drainage.

Differences in the biochemical and physiological characteristics of healthy and malignant tissue are responsible for the passive accumulation of macromolecules in tumors. This feature has been termed "enhanced permeability and retention" (EPR effect)[30,33] and is depicted schematically in Figure 3.

In general, low-molecular-weight compounds diffuse into normal and tumor tissue through the endothelia cell layer of blood capillaries. Macromolecules, however, cannot pass through the capillary walls of normal tissue. The entry of macromolecules into tumor tissue takes place in the capillaries where blood flow is diminished and nutrients transfer into the tissue. In contrast to the blood capillaries in most normal tissues, the endothelial layer of the capillaries in the tumor tissue is fenestrated and leaky so that macromolecules and other nanoparticles reach the malignant tissue. Tumor tissue generally has a defective lymphatic drainage system with the result that macromolecules are retained and can subsequently accumulate in solid tumors.

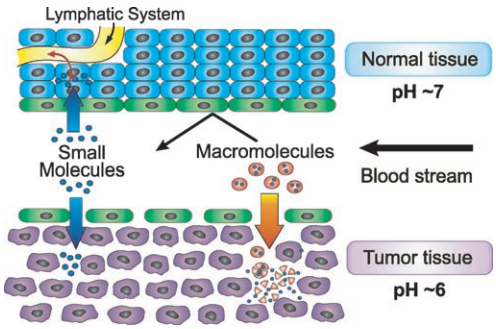


Figure 3. Schematic representation of the anatomical and physiological characteristics of normal and tumor tissue with respect to the vascular permeability and retention of small and large molecules (EPR effect).

The size of the macromolecule is a crucial factor with respect to uptake by the tumor. The EPR effect is observed for macromolecules with molecular weights greater than 20 kDa. Therfore, there is a correlation between the half-life in plasma, the renal clearance, and the accumulation in the tumor of the respective macromolecule. In recent years, most of the research groups involved in the development of drug–polymer conjugates selected macromolecular carriers with molecular weights in the range of 20 to 200 kDa. It is generally assumed that in a healthy organism the renal threshold is in the range of 30–50 kDa to avoid leakage of body proteins into the bladder.[34]

A number of preclinical studies have demonstrated that the physiochemical nature of the biopolymer or synthetic polymer has a strong influence on its pharmacokinetic profile and degree of accumulation in the tumor.[35,36] The biodistribution and uptake by the tumor of the polymer in question is essentially dictated by its molecular weight, charge, conformation, hydrophobicity, and immunogenicity. Preclinical studies have shown that the size of the tumor influences the uptake rate of the polymer in solid tumors: Smaller tumor nodules accumulate larger amounts of the polymer than larger nodules.[37] This observation points to the possibility that polymeric imaging agents could help to detect small tumor nodules.

The influence of the different factors on the EPR-mediated uptake of the polymer in solid tumors is not yet completely understood. As a general rule, a polymer with a molecular weight above the renal threshold (ca. 30 kDa) as well as a neutral charge ensures a long half-life in plasma. This prolonged plasma residence time is an important prerequisite for a significant accumulation of the circulating polymer in the tumor.[35,36] A similar uptake mechanism is also apparent in other leaky tissues, such as inflamed or infected tissue, and can result in an enhanced uptake of macromolecules at the respective sites.[35,36]

In contrast to this simple passive targeting by size, cell-specific targeting using antibodies, oligosaccharides, and peptides has also been addressed by many research groups.[5,38]

2.2. Cellular Uptake of Polymers, Site-Specific Drug Release, and Implications for Drug Design

In general, macromolecules are taken up by the cell through receptor-mediated endocytosis, adsorptive endocytosis, or fluid-phase endocytosis (Figure 4).[39] During endo-

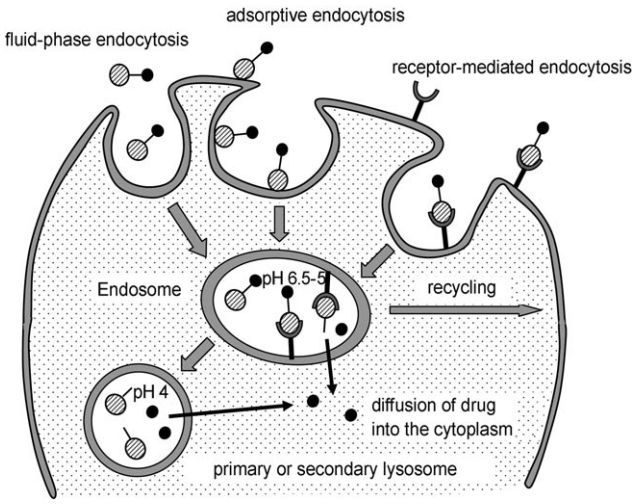


Figure 4. Endocytotic pathway for the cellular uptake of macromolecules and nanocarriers for drug delivery.

cytosis a significant drop in the pH value takes place from the physiological value (7.2–7.4) in the extracellular space to pH 6.5–5.0 in the endosomes and to around pH 4.0 in primary and secondary lysosomes. A great number of lysosomal enzymes become active in the acidic environment of these vesicles, for example, phosphatases, nucleases, proteases, esterases, and lipases.

Drug–polymer conjugates or complexes should be sufficiently stable in the blood stream prior to the drug being liberated at the site of action. In principle, the polymer-bound drug can be released in the body by unspecific hydrolysis by enzymes, by reduction, or in a pH-dependent manner. In an ideal case, cleavage of the drug–polymer conjugate at the tumor site is triggered by a biochemical or physiological property unique for the individual tumor. Although such truly tumor-specific features are rarely encountered, the overexpression of certain enzymes, an acidic and hypoxic environment in solid tumors, as well as the endocytotic pathway of macromolecules offer several options for designing drug–polymer conjugates that are preferentially cleaved within the tumor.

The design of drug–polymer conjugates initially focused on incorporating enzymatically cleavable bonds that allow the prodrug to be cleaved intracellularly after cellular uptake. More recently, a cleavage mechanism involving triggering events that lead to a release cascade have been presented.[40,41] The advantage of this approach is a high local drug concentration with a potential increase in efficacy.[42]

Both the low pH values in endosomes and lysosomes as well as the presence of lysosomal enzymes are therefore intracellular properties which have been exploited for releasing the polymer-bound drug specifically in tumor cells. Furthermore, the microenvironment of tumors has been reported to be slightly acidic in animal models and human patients: Non-invasive techniques have demonstrated that the pH value in tumor tissue is often 0.5–1.0 units lower than in normal tissue (see Figure 3).[43] This difference could contribute to the extracellular release of drugs bound to polymers through acid-sensitive linkers, especially if the drug is trapped by the tumor for longer periods of time.

Finally, drug–polymer conjugates can also be designed to slowly release the polymer-bound drug through hydrolysis under physiological conditions, as exemplified by conjugates of drugs and polyethylene glycol.[44]

2.3. Polymer Conjugates for Protein Stabilization

Coupling polymers to therapeutically relevant proteins imparts several potential advantages: Conjugation can reduce the immunogenicity of the native protein, increase its stability, and prolong its biological half-life, thus resulting in less-frequent administration to the patient. Poly(ethylene glycol) (PEG) has mainly been the polymer of choice for preparing polymer–protein conjugates. In this "PEGylation" technology, linear or branched PEG derivatives are coupled to the surface of the protein.[34,45] The companies Shearwater Polymers and Enzon initiated and refined this technology, which has resulted in the development of clinically as well as commercially successful products such as PEGylated asparaginase, PEGylated adenosine deaminase, PEGylated interferons, and PEGylated granulocyte colony stimulating factor (see Section 3.1).[45–48]

2.4. Multivalent Interactions

In recent years, the development of multivalent drugs which are bridged by polymeric spacers has advanced dramatically (see Section 3.5).[49,50] The great potential of these systems is the high entropic gain in the formation of the multivalent complex (Figure 5). For example, the binding

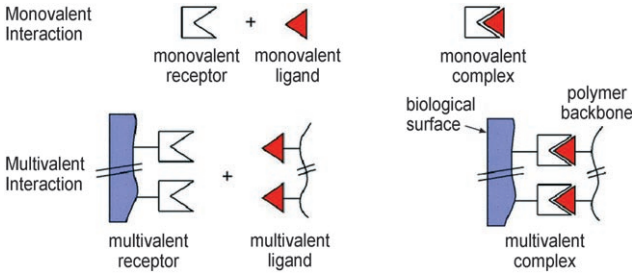


Figure 5. Comparison of monovalent and multivalent interactions.

constants of bivalent interactions can be a factor of 1000 higher than monovalent binding, and for tri- and pentavalent interactions values up to 10^8 have been reported. This possibility allows for completely new ways to develop drugs; however, only a few efforts have been made so far to develop the first candidates for clinical trials.

A challenging approach to the application of multivalent interactions is the mimicry of functional biomacromolecules with therapeutic relevance. Several attempts have been made to mimic specific proteins (e.g., histones) or polysaccharides (e.g., heparin; see Section 3.5). In these cases, mimicry is

mostly based on the surface charge of the polymer molecules (Figure 6). Applications range from DNA-transfection agents (polycationic systems) to anticoagulating, anti-inflammatory, and anti-HIV drugs (polyanionic systems).

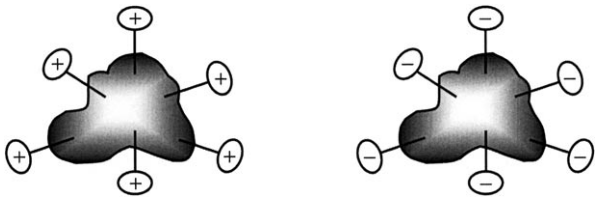


Figure 6. Mimicry of the surface charge of polyionic biomacromolecules and synthetic polymers as an approach for the development for polymer therapeutics.

3. Approaches and Applications: "In Vivo Veritas"

In this section we describe different polymer therapeutics in greater depth, with a focus on their preclinical and clinical potential.

3.1. Polymer Conjugates of Therapeutically Relevant Proteins

Therapeutically relevant proteins such as antibodies, cytokines, growth factors, and enzymes are playing an increasing role in the treatment of viral, malignant, and autoimmune diseases. The development and successful application of therapeutic proteins, however, is often impeded by several difficulties, for example, insufficient stability and shelf-life, costly production, immunogenic and allergic potential, as well as poor bioavailability and sensitivity towards proteases.

An elegant method to overcome most of these difficulties is the attachment of polyethylene glycol chains onto the surface of the protein. PEGylation of the native protein increases its molecular weight and as a result prolongs the half-life in vivo, which in turn allows less frequent administration of the therapeutic protein. In addition, the PEG chains mask the protein, which renders it more resistant to proteases and less immunogenic.

A consequence of the PEGylation of proteins is generally a loss of the protein's biological activity. This loss, however, is outweighed by a substantial increase in the biological half-life of the PEGylated protein.[34]

In the past few years two PEGylation processes have emerged: In the first method one or more linear PEG chains with a molecular weight between 5 and 12 kDa are bound to the surface of the protein (first-generation PEGylated proteins). In the second method a single branched or a multibranched PEG chain is attached to a specific amino acid on the protein's surface (second-generation PEGylated proteins). In most cases activated PEG-carboxylic acids, for example, activated with *N*-hydroxysuccinimide, are bound to the ε-amino groups of lysine residues or the N-terminal amino group, but other chemical modifications with aldehyde, tresylate, or maleimide derivatives of PEG are also used.

The major drawback of first-generation PEGylated proteins was the heterogeneous nature of the pharmaceutical product, since in most cases multiple linear PEGs were attached to the protein. Despite this, several first-generation candidates received regulatory approval. The most prominent examples are adagen (PEGylated adenosine deaminase) for the treatment of severe combined immunodeficiency disease, oncaspar (PEGylated asparaginase) for the treatment of acute leukemia, and PEG-intron (PEGylated interferon α2b) for treating hepatitis C (Table 1).

Second-generation PEGylated proteins, in which a branched or linear PEG chain is attached to a site-specific amino acid on the protein, have the advantage in that they represent defined products with minimal alteration of the three-dimensional conformation of the protein. In 2002, granulocyte colony stimulating factor (G-CSF) PEGylated with a 20-kDa linear PEG chain (neulasta) was the first second-generation PEGylated system to receive market approval (Table 1). Neulasta stimulates the production of white blood cells following bone-marrow depletion in the course of cancer chemotherapy. This treatment is more convenient than with the native protein, human recombinant G-CSF (neupogen); only one injection of neulasta is required every three weeks compared to daily injections of neupogen over two weeks.[51]

Interferon α2a PEGylated with a 40-kDa branched PEG chain (pegasys) is a second-generation PEGylated system that has received market approval, and is a competitor of the first-generation conjugate PEG-intron (Table 1). Both PEG-intron and pegasys have shown significantly better efficacy in the treatment of hepatitis C than the native interferon when combined with the antiviral agent ribavarin.[46,52]

Other examples of PEGylated proteins on the market or in advanced clinical trials are pegvisomant, a PEGylated form of the human growth hormone,[53] and a PEGylated receptor and antibody fragment directed against tumor necrosis factor-α, a major mediator of inflammation (PEG-TNF-RI and PEG-anti-TNF Fab, respectively).[54,55]

Besides PEGylated proteins, one polymer–protein conjugate consisting of the anticancer protein neocarcinostatin and a synthetic copolymer of styrene and a maleic acid anhydride drug (Table 1) has been approved for the treatment of hepatocellular cancer in Japan.[35]

3.2. Drug–Polymer Conjugates with Cleavable Linkers

The development of drug–polymer conjugates is a promising strategy to improve the therapeutic index[1] of toxic drugs, especially in the field of cancer chemotherapy. Several drug–polymer conjugates are being investigated in phase I–III studies at present (Table 2).

Although great efforts are being made to develop novel polymeric carriers, synthetic polymers that have been used in clinically evaluated drug conjugates have been mainly restricted to HPMA, PEG, and poly(glutamic acid) (PG). In addition, albumin, a biopolymer carrier, is being evaluated as a drug-delivery system in anticancer therapy. The cytostatic agents that have been primarily selected for preparing drug–

Table 2: Drug–polymer conjugates in clinical trials.

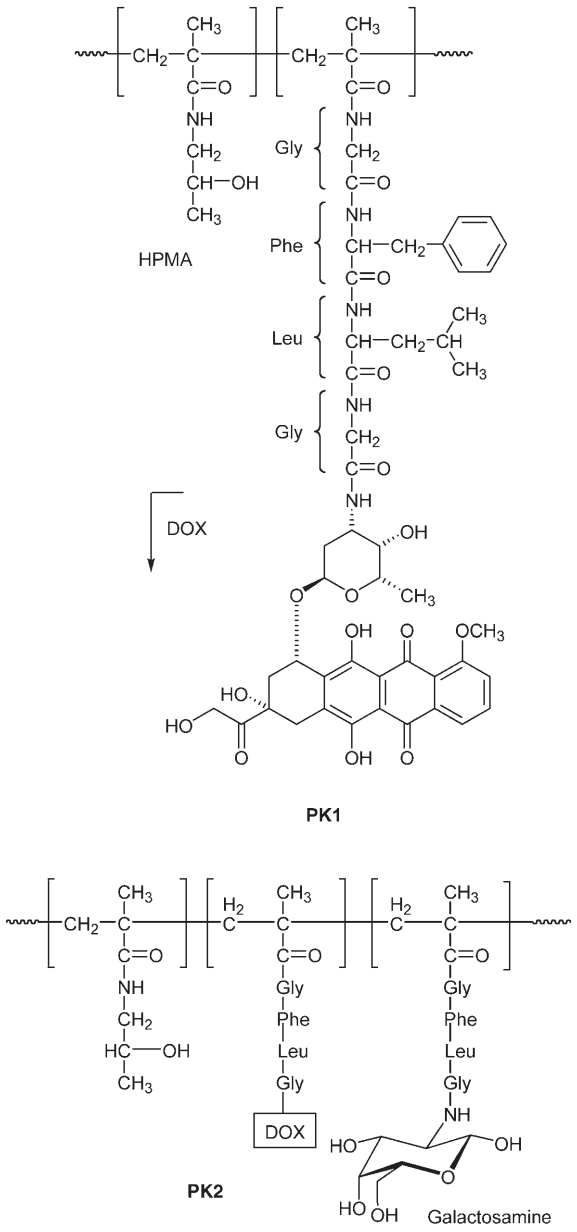
Compound	Spacer	Molecular weight [kDa]	Status of development
PK1, doxorubicin–(HPMA copolymer)	Gly-Phe-Leu-Gly	30	phase II
PK2, galactosaminated doxorubicin–(HPMA-copolymer)	Gly-Phe-Leu-Gly	30	phase I discontinued
PNU-166945, taxol–(HPMA copolymer)	ester	40	phase I completed
MAG-CPT, camptothecin–(HPMA copolymer)	Gly-6-aminohexanoyl-Gly	30	phase I completed
AP5280, diammineplatinum(II)–(HPMA copolymer)	Gly-Phe-Leu-Gly	25	phase I completed
AP5286, diaminocyclohexaneplatinum(II)–(HPMA copolymer)	Gly-Phe-Leu-Gly	25	phase I
prothecan, camptothecin–PEG conjugate	alanine ester	40	phase II
CT-2103, taxol–polyglutamate conjugate	ester	40	phase II/III
CT-2106, camptothecin–polyglutamate conjugate	Gly-ester	50	phase I
MTX-HSA, methotrexate–albumin conjugate	–	67	phase II
DOXO-EMCH, 6-maleinimodcaproyl hydrazone derivative of doxorubicin	acid-sensitive hydrazone	67 (albumin-bound prodrug)	phase I completed

polymer conjugates are doxorubicin, camptothecin, taxol, methotrexate, and platinum complexes.

Several drug–polymer conjugates with HPMA copolymers have been studied clinically. A doxorubicin–(HPMA copolymer) conjugate PK1 was the first drug–polymer conjugate to enter clinical trials.[56] PK1 has a molecular weight of approximately 28 kDa and contains doxorubicin (about 8.5 wt%) linked through its amino sugar to the HPMA copolymer by a tetrapeptide spacer, Gly-Phe-Leu-Gly (Scheme 1). This peptide sequence is cleaved by lysosomal enzymes of tumor cells. Preclinical studies showed that the level of lysosomal enzyme expression in solid tumors, as well as their vascular permeability for macromolecules, correlated with the activity of this conjugate in vivo.[57]

A phase I study revealed that the maximum tolerated dose (MTD) was 320 mg m^{-2} doxorubicin equivalents, which is a fivefold increase relative to the standard dose for doxorubicin.[56] The dose-limiting factors observed in this study were bone-marrow toxicity and mucositis. Other side effects, for example, nausea and diarrhea, were moderate (CTC Grade 1; CTC = common toxicity criteria). A noteworthy finding of this study was that no acute cardiotoxicity was observed even at these high doses. Two partial remissions and two minor responses were seen in four patients with lung, breast, and colorectal cancer. The recommended dose for phase II studies was 280 mg m^{-2} every three weeks. Phase II trials in breast, non-small-cell lung and colon cancer were initiated at the end of 1999; an interim report indicated positive responses in a few cases.[58]

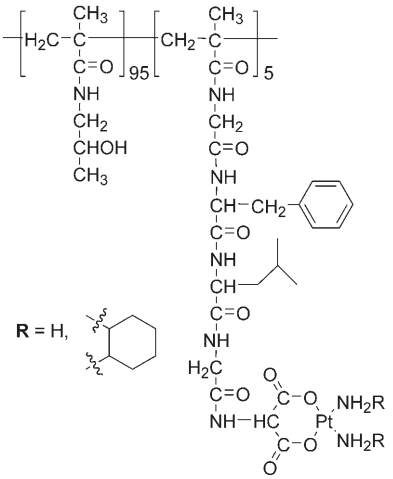
PK2 is a related compound to PK1, but incorporates an additional targeting ligand, namely, a galactosamine group that was designed to be taken up by the asialoglycoprotein receptor of liver tumor cells (Scheme 1). In a phase I study, 31 patients with primary or metastatic liver cancer were evaluated.[59] The MTD of PK2 was 160 mg m^{-2} doxorubicin equivalents which is approximately half the MTD value of PK1, although the molecular weight and the loading ratio are very similar in both conjugates. Dose-limiting toxicity was associated with severe fatigue, neutropenia, and mucositis; a dose of 120 mg m^{-2} doxorubicin equivalents was recommended for phase II studies. Two partial remissions and one minor response were achieved in this study.



Scheme 1. Chemical structure of the first clinically tested polymeric antitumor therapeutics: PK1 (top) and PK2 (bottom).

Two other HPMA conjugates with either taxol or camptothecin, respectively, entered phase I trials (Table 2). PNU-166945 is a water-soluble conjugate in which taxol at its 2-OH position is bound through a Gly-Phe-Leu-Gly linker to the polymer backbone. The camptothecin–(HPMA copolymer) conjugate consists of camptothecin linked at its 20-OH group to the HPMA copolymer through a Gly-6-aminohexanoyl-Gly spacer. Although preclinical results in tumor-bearing mice have been promising, both conjugates have had limited success in early clinical trails because of their toxicity profile.[60,61]

Two drug–HPMA conjugates that have only recently entered phase I studies are AP5280 and AP5286, in which a diamine- or a diaminocyclohexaneplatinum(II) moiety is bound to a dicarboxylate ligand that is coupled to the polymer through the tetrapeptide spacer Gly-Phe-Leu-Gly. This cathepsin B sensitive linker is also present in PK1, PK2, and PNU-166945 (Scheme 2).[62,63] Interestingly, during the

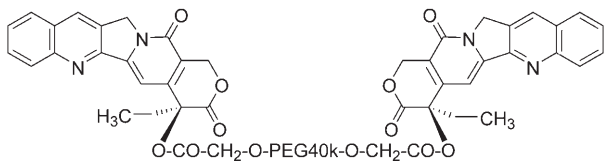


Scheme 2. HPMA–drug conjugates AP5280 and AP5286 with a diamine- or a diaminocyclohexaneplatinum(II) group.

synthesis the platinum(II) group initially forms an O,O chelate which rearranges to the more stable N,O chelate. Preclinical assessment showed a high antitumor efficacy and a significantly increased MTD value for AP5280 compared to the clinical standards (cis- and carboplatin). In a phase I study the dose-limiting toxicity for AP5280 was vomiting (grade 3) at 4500 mg Pt m^{-2} (platinum equivalents); the dose recommendation for a phase II study was 3300 mg(Pt) m^{-2}. Five patients had a stabilization of their disease.[64] Detailed reviews on the clinical studies of drug–polymer conjugates with HPMA copolymer have recently been published by Duncan and Rihova et al.[9,11]

Another approach to doxorubicin–polylactide conjugates was recently reported by Sengupta et al.[65] These conjugates have been embedded into a biodegradable polylactide nanoparticle (ca. 150 nm) to achieve a better tumor selectivity through the EPR effect.

Prothecan, a camptothecin conjugate, is the first drug conjugate with polyethylene glycol that has been clinically assessed (Scheme 3). Conjugating the 20-OH position of

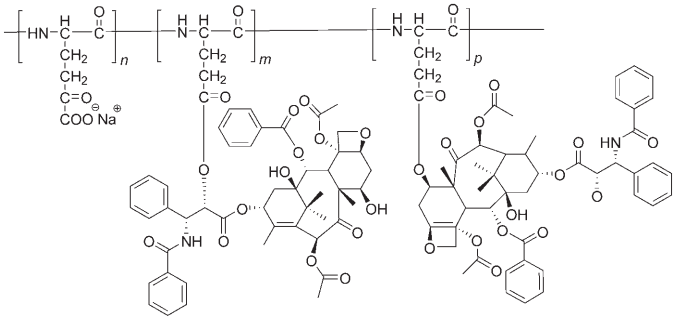


Scheme 3. Prothecan, a camptothecin derivative with 40 kDa PEG.

camptothecin with PEG through a glycine spacer[66–68] proved to have several advantages: a) the EPR effect results in a drug-targeting effect, b) esterifying the 20-hydroxy group of CPT stabilizes the drug in its active lactone form (closed E ring) which otherwise tends to hydrolyze under physiological conditions and lead to the inactive hydroxycarboxylic acid form, c) incorporation of a glycine spacer ensured a controlled release of the drug; and d) use of hydrophilic PEG leads to a highly water-soluble formulation of camptothecin.

Preclinical results with prothecan showed it had better efficacy in animal models of human cancers than free camptothecin.[66–68] Prothecan is currently being assessed in phase II studies for the treatment of gastric and gastro-esophageal tumors after a phase I study showed moderate nonhematologic toxicities at its MTD of 200 mg m^{-2} camptothecin equivalents.[69]

PG-TXL (CT-2103), a poly(L-glutamic acid) conjugate of taxol (Scheme 4), is probably the most successful drug–polymer conjugate to date and is currently undergoing



Scheme 4. PG-TXL (CT-2103), a poly(L-glutamic acid) conjugate of taxol (paclitaxel).

phase III trials in combination with standard chemotherapy against ovarian cancer and non-small-cell lung cancer.[70] PG-TXL has a higher loading ratio (ca. 37 wt% taxol) than other drug–polymer conjugates, and the taxol is linked through its 2′-OH group to the poly(glutamic) acid backbone. Phase I and II studies of various cancers showed promising response rates, even for patients who were resistant to taxane therapy.[71,72] The recommended dose of PG-TXL ranged from 175 to 235 mg m^{-2} (taxol equivalents) which is approximately twice as high as for free taxol. The dose-limiting toxicities of the conjugate are neurotoxicity and neutropenia. A noteworthy feature of PG-TXL is the biodegradability of the polyglutamic acid backbone and the liberation of taxol and taxol glutamic acid derivatives in vitro and in vivo, which, in part, appear to be mediated by cathepsin B.[73] A phase I

study with an analogously constructed PG conjugate with camptothecin has recently been completed successfully.[74]

Besides synthetic polymers, albumin is also being investigated as a drug carrier in clinical trials. A methotrexate–albumin conjugate (MTX–HSA) was synthesized by directly coupling methotrexate to human serum albumin (HSA). This conjugate showed significant accumulation in rat tumors and high antitumor activity in selected nude mice models.[75,76] Stomatitis proved to be dose-limiting above 50 mg m^{-2} MTX–HSA (MTX equivalents) in a phase I study.[77] Two patients with renal cell carcinoma and one patient with mesothelioma responded to MTX–HSA therapy (one partial remission, two minor responses). Renal cell cancer is a malignancy with low response rates to conventional chemotherapy. Phase II studies are ongoing.

New approaches have concentrated on forming a drug–albumin conjugate in vivo by binding prodrugs selectively to circulating albumin after intravenous administration.[78–81] This prodrug concept is based on two features: a) rapid and selective binding of a maleimide prodrug to the cysteine 34 position of endogenous albumin after intravenous administration, and b) release of the albumin-bound drug at the target site as a result of the incorporation of an acid-sensitive or an enzymatically cleavable bond between the drug and the carrier.

A first clinical candidate is the (6-maleimidocaproyl)hydrazone derivative of doxorubicin (DOXO-EMCH; Figure 7) which incorporates an acid-sensitive carboxylic hydrazone bond as a predetermined breaking point. DOXO-EMCH entered a phase I study in 2003 after demonstrating superior efficacy and an improved toxicity profile relative to free doxorubicin, the clinical standard.[79]

As an example, the therapeutic effects of doxorubicin and DOXO-EMCH against renal cell carcinoma (RENCA) are shown in Figure 8. Mice treated with doxorubicin at its MTD value (4×6 mg kg^{-1}) showed distinct kidney tumors (body weight loss of $-10\,\%$), while the group treated with DOXO-EMCH at 4×12 mg kg^{-1} doxorubicin equivalents showed no body weight loss and complete remission was achieved in nearly all the animals.

In a phase I study with DOXO-EMCH, 37 patients with advanced cancer were treated with an intravenous infusion of DOXO-EMCH once every 3 weeks at a dose of 20–340 mg m^{-2} doxorubicin equivalents. Treatment with DOXO-EMCH was well tolerated up to 200 mg m^{-2}, without manifestation of drug-related side effects. Myleosuppression (grade 1–2), mucositis (grade 1–2), alopecia (grade 1–2), nausea and vomiting (grade 1), mouth dryness (grade 1), and fatigue (grade 1) have been noted at dose levels of 260, and myleosuppression (grade 2–3) as well as mucositis (grade 2–3) were dose-limiting at 340 mg m^{-2}. Of 29/37 evaluable patients, 13 had progressive disease, 13 had disease stabilization, a breast cancer and a liposarcoma patient had partial remission, and a patient with small-cell lung cancer had a complete remission. The recommended dose for phase II studies is 260 mg m^{-2}.

Although the clinical data for drug–polymer conjugates is limited to a few hundred patients, some general trends are apparent. The increase in the maximum tolerated dose (MTD) of the drug–polymer conjugates compared to the parent drug noted in preclinical studies is also manifested in clinical trials. Furthermore, no particular toxicity can be attributed to the polymer, and dose-limiting toxicities are comparable to the free drug. The significance of the molecular weight and of the cleavable linker of the drug–polymer conjugate remains unclear. Although the majority of non-biodegradable polymers have molecular weights close to the renal threshold (30–50 kDa, see Section 2.1), which allows enhanced permeation and retention in solid tumors, as well as a certain degree of renal clearance, a few recent examples of conjugates with albumin, polyglutamic acid, and PEG have molecular weights of 40–80 kDa. Whether the differences in the pharmacokinetic profile as a result of the different molecular weights influence the toxicity and tumor response needs to be evaluated in a larger population of patients.

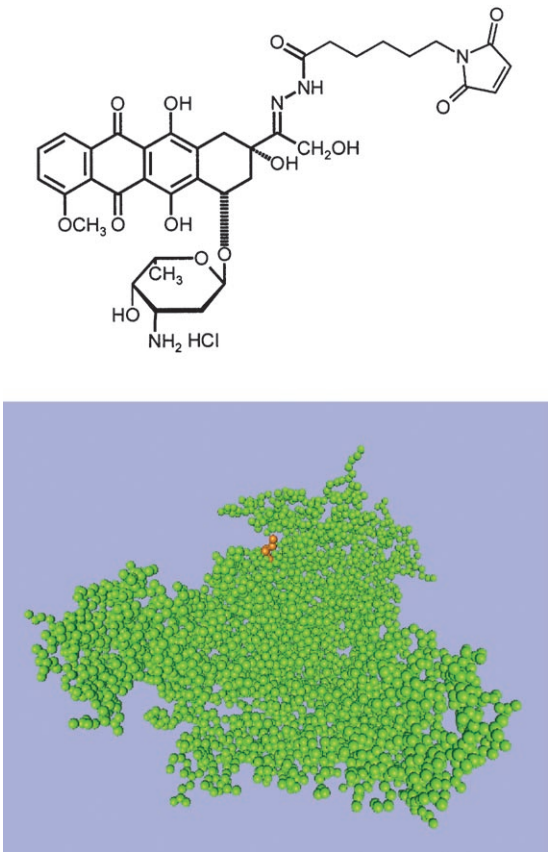


Figure 7. Structure of the prodrug DOXO-EMCH, which is undergoing clinical phase I trials (top), and the structure of human serum albumin (bottom); the prodrug binding position Cys 34 is highlighted in orange.

The effectiveness of the predetermined breaking point incorporated in the drug–polymer conjugate also remains a matter of debate. The majority of drug–HPMA conjugates have made use of the tetrapeptide Gly-Phe-Leu-Gly, which is cleaved by lysosomal enzymes such as cathepsin B. However, preclinical data indicate that antitumor efficacy of such designed conjugates correlates with the expression of cathepsin B in the tumor,[57] a fact that has not been adequately addressed in clinical trials. Detailed knowledge of the

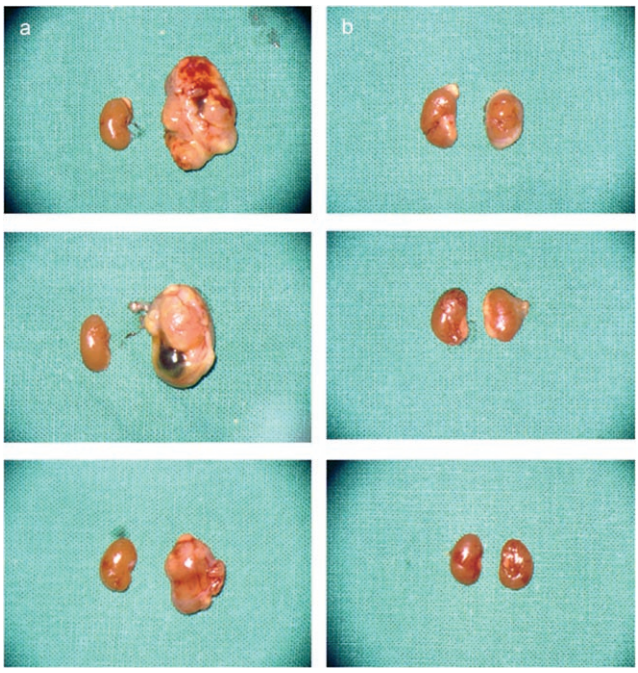


Figure 8. Representative photographic images of the healthy kidneys (left) as well as treated tumor-cell kidneys (right). The three mice from group **A** were treated with 4×12 mg kg^{-1} doxorubicin (body weight change: −10%) and the three mice from group **B** with 4×12 mg kg^{-1} doxorubicin equivalents of DOXO-EMCH (body weight change: +1%) for 24 days.

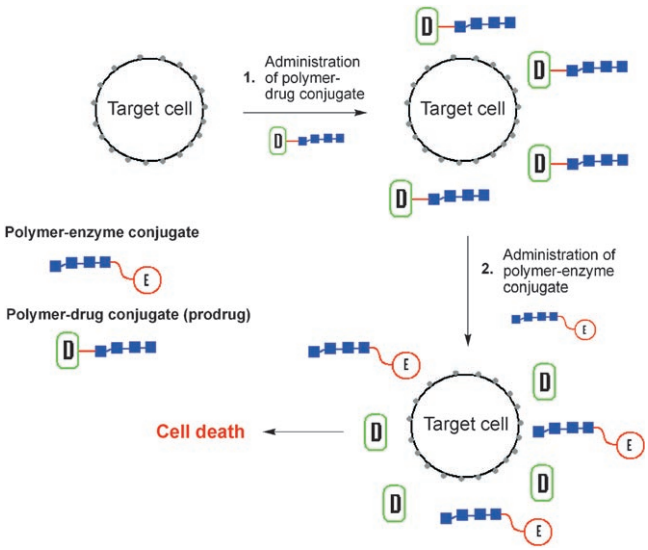


Figure 9. The PDEPT concept: After administration of the polymer–drug conjugate and uptake in the tissue by EPR, the polymer–enzyme conjugate is added to release the drug and induce cell death.

expression of tumor-related proteases in individual tumor entities would certainly be helpful for the future development of cleavable drug–polymer conjugates. Whether drug–polymer conjugates that are cleaved by unspecific hydrolysis or at acidic pH values are more universally applicable needs to be addressed in clinical studies. Preliminary preclinical studies with doxorubicin–HPMA conjugates have indicated that an acid-sensitive linker is more effective than a cathepsin B sensitive one.[82]

3.3. A Combined Approach: The PDEPT Concept

Polymer-directed enzyme–produg therapy (PDEPT) is a novel two-step antitumor approach that combines a polymeric prodrug and a polymer–enzyme conjugate to generate a cytotoxic drug at the tumor site.[83] PDEPT involves initial administration of the polymeric drug to promote tumor targeting before the activating polymer–enzyme conjugate is administrated (Figure 9). PDEPT has certain advantages compared to antibody-directed enzyme–produg therapy (ADEPT): the relatively short residence time of the polymeric prodrug in the plasma allows subsequent administration of the polymer–enzyme conjugate without fear of activation of the prodrug in the blood stream, and also the polymer–enzyme conjugates could have reduced immunogenicity.

Two PDEPT approaches have been investigated with doxorubicin: In the first case, the polymeric prodrug PK1 (FCE 28068; see Scheme 7 in Section 3.6.1), which is currently under phase II clinical evaluation, was selected as a model prodrug in combination with an (HPMA copolymer)–(cathepsin B) conjugate. In the polymer-bound form, the (HPMA copolymer)–(cathepsin B) conjugate retained approximately 20–25% of the cathepsin B activity in vitro. After intravenous administration of the conjugate to tumor-bearing B16F10 mice there was a 4.2-fold increase in its accumulation in tumors relative to the free enzyme. When PK1 and the PDEPT combination were used to treat established B16F10 melanoma tumors, the antitumor activity (% *T*/*C*, the survival time of treated versus control animals) for the PDEPT combination was 168% compared to 152% for PK1 alone, and 144% for free doxorubicin.[84]

Another more successful PDEPT combination consisting of (HPMA-copolymer)–(methacryloyl-gly-gly-cephalosporin)–doxorubicin (HPMA-*co*-MA-GG-C-Dox) as the macromolecular prodrug and an HPMA copolymer conjugate containing the nonmammalian enzyme β-lactamase (HPMA-*co*-MA-GG-β-L) as the activating component has been reported.[85] HPMA-*co*-MA-GG-C-Dox had a molecular weight of about 31600 Da and a doxorubicin–cephalosporin content of 5.85 wt%. Whereas free β-lactamase has a molecular weight of 45 kDa, the HPMA-*co*-MA-GG-β-L conjugate had a molecular weight in the range of 75–150 kDa. The HPMA-*co*-MA-GG-β-L conjugate retained 70% and 80% of its activity against the cephalosporin C and HPMA-*co*-MA-GG-C-Dox substrates, respectively. Intraveneous administration of HPMA-*co*-MA-GG-C-Dox to mice bearing subcutaneously implanted B16F10 melanoma, followed after five hours by HPMA-*co*-MA-GG-β-L induced the release of free doxorubicin in the tumor. Whereas the PDEPT combination caused a significant decrease in the size of the tumor ($T/C = 132\%$), neither free doxorubicin nor HPMA-*co*-MA-GG-C-Dox alone displayed activity. Furthermore the PDEPT combination showed no toxicity at the doses used.[85]

3.4. Polymeric Angiogenesis Factors

Another therapeutic approach, instead of direct tumor targeting with polymer-bound cytostatic drugs, is the targeting of angiogenesis with an HPMA–polymer conjugate of the angiogenesis inhibitor TNP-470.[86,87] This approach showed very promising results in a mouse model, and no drug-related toxicities were observed.

3.5. Multivalent Therapeutics

A fundamentally different approach to polymer therapeutics is based on the multiple interactions of ligands conjugated with a polymer which interact simultaneously with multiple receptor sites in protein complexes or multiple receptors on the cell surface. This concept is a close mimicry of biological interactions such as cellular recognition and signal transduction where multivalent processes play an important role. Although many interesting approaches have been reported, only a few clinical developments have so far been pursued.

3.5.1. Multivalent Drug Concepts (Toxins and Bacteria)

A number of multivalent inhibitors have been designed that are based on low-molecular-weight drugs and target dimeric or multimeric proteins that contain multiple identical receptor sites.[49,50] For example, a pentavalent starlike carbohydrate ligand has been reported that fitted precisely into the binding pocket of the five subunits of the Shiga-like bacteria toxin, a close analogue of the cholera toxin (Figure 10).[88] An increase in the binding affinity by a factor of 10^7 was observed for this pentavalent interaction relative to the monovalent ligand. This example clearly demonstrates that dendritic and starlike molecules are perfect scaffolds for presenting ligands for multivalent interactions.

Another example is the binding of vancomycin derivatives or oligomers to the D-Ala-D-Ala motive of the bacteria cell wall. Whitesides and co-workers have reported on divalent and trivalent vancomycin derivatives which showed extremely high binding affinities. The trivalent model complex of vancomycin-D-Ala-D-Ala, with a binding constant of 4×10^{-17} M, has a higher affinity than the avidin–biotin complex.[89–91] This concept of multivalent interactions with vancomycin has been taken up in the pharmaceutical industry for in vivo and clinical studies. For example, telavancin, a highly bactericidal injectable antibiotic based on a vancomycin derivative with multiple modes of action, was reported by Theravance (South San Francisco).[92] Part of their research program is dedicated to finding new antibiotics for serious infections arising from *Staphylococcus aureus* (including multidrug-resistant strains) and other Gram-positive pathogens. Telavancin is currently in phase III clinical trials.

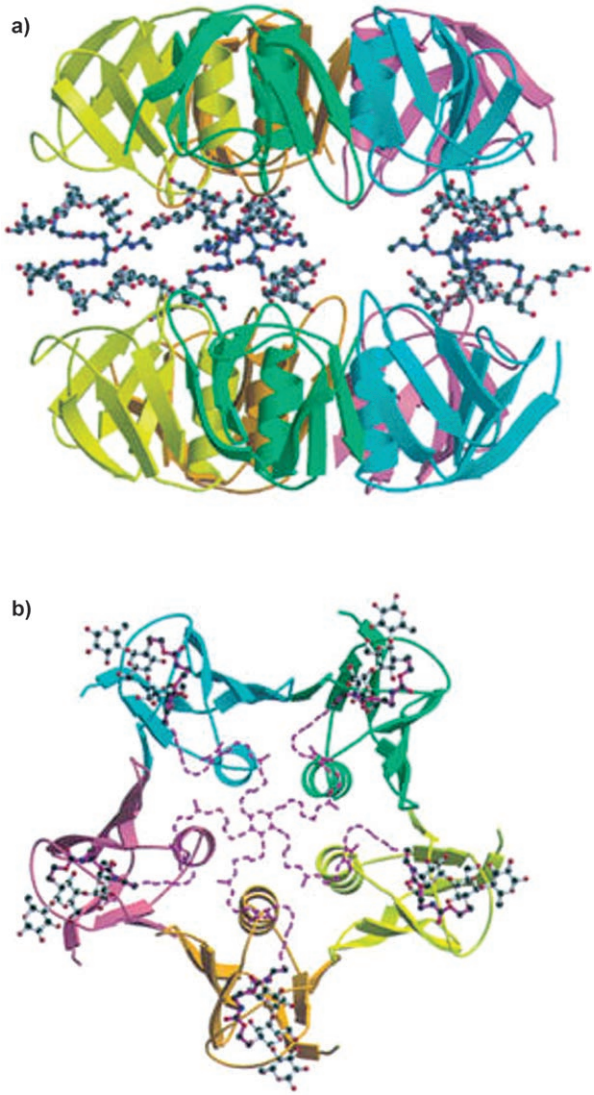


Figure 10. Pentavalent binding of the multivalent polysaccharide inhibitor to the Shiga-like toxin dimer: a) side view, b) top view (adapted from ref. [88]).

3.5.2. Multivalent Interactions at Surfaces—Inhibition of Virus Attachment

The inhibition of virus attachment to cell surfaces is a fundamental problem for the prevention of viral infections, such as influenza and HIV. As depicted in Figure 11, traditional monovalent drugs cannot prevent the multiple adhesion of the virus to the cell surface. Therefore, the development of multivalent ligands (Figure 5) that bind to membrane proteins of viruses is an important goal.

Several polymer architectures, including linear, starlike, and dendritic structures (Figure 2), have been considered as scaffolds for multivalent drugs.[49,50,93–95] Besides linear glycopolymers, various dendrimer structures have been investigated as multivalent ligands for sugar-binding proteins (for example, lectins), with multiple carbohydrate moieties attached at the exterior to form a so-called "sugar-coating". For example, L-lysine dendrimers with 2 to 16 sialic acid units

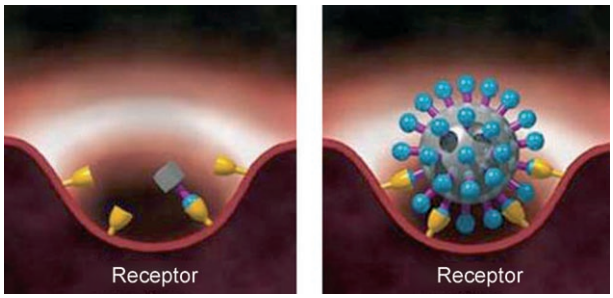


Figure 11. Monovalent binding of a drug (left) versus polyvalent binding of a virus (right) on a cell surface. (Printed with kind permission from Starpharma.)

show enhanced binding affinities in the *Limax flavus* lectin precipitation assay and the hemaglutination assay of erythrocytes.[96] In these systems, four to six sialic acid residues appeared to be an optimal number of functional groups for antiviral activity against the influenza A virus. An approximately 200-fold increase in the binding affinity to the trivalent hemaglutinin as compared to the monovalent ligand was observed. The small size of dendrimers (3–5 nm) relative to the spacing of receptor sites on the virus surface is a major limitation of this approach; hence they can only bind to 1-2 trivalent hemaglutinin receptors (Figure 12). In comparison, a high-molecular weight (10^6 Da) linear acrylamide polymer has shown in vitro an up to 10^8-fold increase in binding affinity, and hence is much more effective in blocking the attack of the influenza virus at the cell surface.[97,98] However, the molecular weight of the polymer is too high to be cleared from the body by the kidneys, and rapid biodegradation is unlikely. In addition to its extremely high binding constant, the polymer can also sterically shield the virus particle when applied in combination with other monovalent ligands.[99]

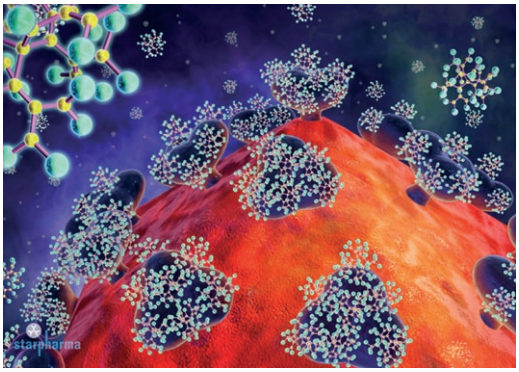


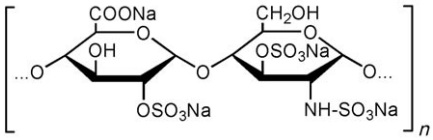
Figure 12. Size relationship between a virus particle with its multivalent cell surface receptors and dendritic drug molecules. (Printed with kind permission from Starpharma.)

Starpharma (Melbourne) is also concentrating on the development of polyvalent drugs. One example is the microbicide VivaGel, a topical vaginal gel that can potentially prevent or reduce transmission of HIV. VivaGel is a dendritic polyanion based on a polylysine core and is currently being evaluated in clinical phase II studies. Many of the approaches used by Starpharmas are based on polyvalent dendrimers which enhance the binding affinity to multivalent receptors or receptors on cell surfaces.[100]

Another approach towards HIV prevention based on polyvalent interactions was reported by Shaunak et al.[101] Dextrin 2-sulfate efficiently blocks HIV infection by binding to cell surfaces. The efficiency of this multivalent interaction has been demonstrated in phase II clinical trials.

3.5.3. *Polyanionic Polymers: Heparin Analogues*

Heparin, a glycosaminoglycan (Scheme 5), has been the drug of choice in the prevention and treatment of thromboembolic disorders for nearly 70 years. There is great interest in finding alternatives to both unfractionated heparin (UFH) and low-molecular-weight heparins (LMWH) because heparin has several disadvantages: First, it has to be isolated from mammalian organs, which implies a potential risk of contamination with pathogens such as viruses or prions, second, the increased use of heparin, especially of LMWH, means there is a growing shortage of the raw material, and third, heparin is a polydisperse mixture of molecules with different chain lengths and chemical structures.[102] Numerous parameters, such as the animal species used for providing heparin, the method of isolation, and the purification step of the product, influence its respective composition and results in wide chemical and subsequent pharmacological variations between different heparin preparations.

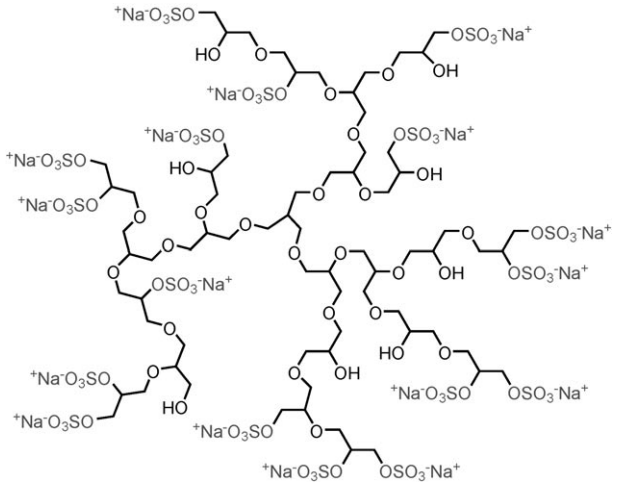


Scheme 5. Structure of a heparin subunit.

In addition to their antithrombic activity, the characteristic feature of heparin and other natural sulfated polysaccharides are complement inhibition,[103] anti-inflammatory,[104,105] antiangiogenic,[106] antimetastatic,[107] antiatherosclerotic,[108] antiproliferative,[109] antiadhesive,[110] and antiviral effects.[111] These additional modes of action can contribute to the overall therapeutic benefit of heparin in some cases.[107]

Consequently, heparin analogues with a similar or even improved pharmacological profile, but lacking the disadvantages of this animal product, are of interest. Besides partially synthetic sulfated linear polysaccharides,[112,113] fully synthetic sulfated linear polymers,[114] which are produced without a starting carbohydrate, may represent promising heparin mimetics.[115] Recently, a new type of polysulfated heparin analogue based on branched polysaccharides was described that possesses a much higher anticoagulant activity than its linear counterparts.[116] However, the accessibility of branched

polysaccharides is problematic because of limited natural sources. Thus, a simple and efficient approach to highly branched polysulfated heparin analogues based on dendritic polyglycerols has been developed (Scheme 6).[117] These



Scheme 6. Dendritic polyglycerol sulfate as an anti-inflammatory heparin analogue.

polyglycerol sulfates prolong the time of activated partial thromboplastin as well as thrombin and inhibit both the classical and alternative complement activation more effectively than heparin itself. In contrast to sulfated polysaccharides, their activities are not directly dependent on the molecular weight, which might be a result of the globular 3D structure of the dendritic polyglycerol sulfates. Since coagulation, complement activation, and inflammation are often present in the pathophysiology of numerous diseases, polyglycerol sulfates with both anticoagulant and anticomplementary activities represent promising candidates for the development of future drugs.

Recently, immunomodulatory and antiangiogenic properties of glucoseamine-modified polyamidoamine (PAMAM) dendrimers have been described. The use of dendrimeric glucosamine and dendrimeric glucosamine 6-sulfate together in a validated and clinically relevant rabbit model of scar-tissue formation after glaucoma filtration surgery resulted in the long-term success of the surgery increasing from 30% to 80%.[118]

3.5.4. Polycationic Polymers as DNA/RNA Transfection Agents

The search for nonviral alternatives remains a challenge because of problems associated with viral gene transfection, such as immune response and limited selectivity,.[119] In the past decade several approaches were pursued in which cationic amphiphiles, polymers, or block copolymers and other pH-responsive polymers were used.[120–125] The colloidal surface and chemical properties of DNA and RNA complexes with polycations are responsible for controlling the extent and rate of delivery of genes to cells. However, additional hurdles on the cellular level have to be overcome on the surface of the cells (Figure 13): Complexes have to enter the cells through the cell membranes, escape degradation in endosomal/lysosomal compartment, traffic through the cytoplasm, and enter the nucleus. The physicochemical characteristics of poly-

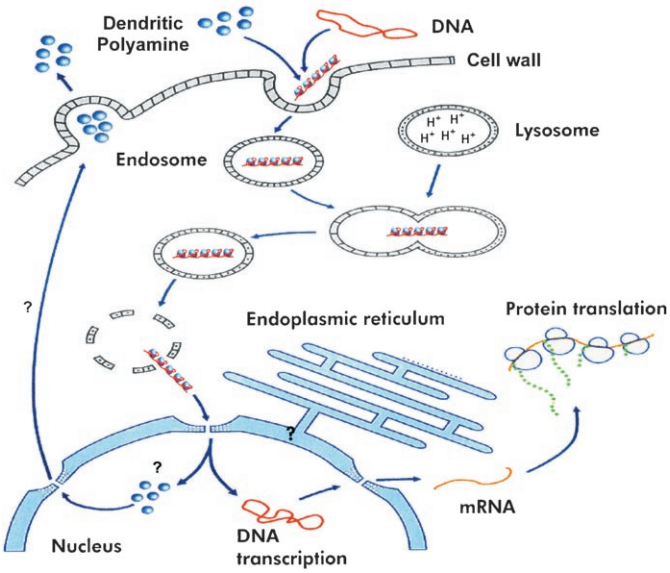


Figure 13. Intracellular uptake of therapeutic DNA or RNA with polycationic polymers, that is, dendritic polyamines.

plexes, such as size, charge, hydrophobicity, and buffering capacity, play a major role in the efficient transport and biological activity of the gene-based drugs.[126]

The "proton-sponge hypothesis" postulates enhanced transgene delivery by cationic polymer–DNA complexes (polyplexes) containing proton-buffering polyamines through enhanced endosomal accumulation of chloride, which leads to osmotic swelling and lysis of the endosome (Figure 13).[127] For therapeutic applications, however, an early endosomal escape mechanism, rather than lysosomal fusion, would be preferable to avoid the release of lysosomal enzymes into the cytosol.[128]

The most frequently used cationic polymers for in vitro gene delivery are poly(ethylene imine) (PEI), poly(L-lysine), and chitosans. Another approach is the use of perfect polyamine-dendrimers[120,129,130] to mimic the globular shape of the natural protein complex. However, the synthetic workload to obtain dendritic structures in the size-range of the natural histone complex (ca. 8 nm) [131] is tremendous (12–18 steps).[132] Also, the observation that a partially destroyed (hydrolyzed) dendritic backbone showed even higher transfection efficiencies[129,133] underlines the significance of readily available alternatives.

A simple approach to dendritic polyamines with different molecular weights and adjustable flexibility (degrees of branching) has been described recently.[134] Both parameters influence transfection efficiency and cytotoxicity. By using a two-step functionalization of hyperbranched PEI, it was possible to generate partially or fully branched pseudodendrimers (poly(propylene imine) (PPI) and poly(amidoamine)

(PAMAM) dendrimer analogues). The highest DNA transfection efficiencies have been observed for molecular weights in the range $M_n = 5000–10000\ g\,mol^{-1}$ for the nonfunctionalized PEI cores, which is comparable in size to the natural histones (8 nm). A maximum transfection efficiency in the β-gal assay for various cell lines was observed when the degree of branching of the PPI analogue was 58 % and the PEI core had a molecular weight of of $M_n = 10000\ g\,mol^{-1}$.

PEGylated polyethylene imines[135] were recently used for the delivery of siRNA to tumor-bearing mice,[136] thus demonstrating the potential of such polycationic carriers for therapeutic application in vivo. However, the toxicity of these systems have to be further reduced.

3.6. *Supramolecular Drug–Polymer Complexes*

One of the major problems in drug development is the poor solubility of many existing and new drugs. Very often the therapeutic effectiveness of these drugs is diminished by their inability to gain access to the site of action at an appropriate dose. Therefore, these drugs are either not clinically used, delivered in large volumes of aqueous or ethanolic solutions, delivered in conjunction with surfactants, or chemically derivatized to soluble prodrugs. Unfortunately, all of these modifications can result in reduced efficacy or adverse effects.

Many approaches for delivering hydrophobic compounds using polymeric carriers, such as block copolymers and dendritic polymers, have been explored.[4,13]

3.6.1. *Block Copolymer Micelles*

Polymeric micelles (Figure 14) are generally more stable than micelles of small surfactant molecules and can retain the loaded drug for a longer period of time.[137,138] The block-copolymer micelles form spontaneously by self-assembly in water when the concentration of the amphiphilic block copolymer is above the critical micellar concentration (CMC).[139] The driving force can be the hydrophobic interactions of the inner block, for example, a nonpolar poly(caprolactone) block (PCL), or ionic interactions, for example, a poly(aspartate) block (PAsp), complexed to a negatively charged polymer such as DNA that forms a polyion micelle.[140] The outer block often consists of a polar poly(ethylene oxide) (PEO) block which forms the shell of the nanocarrier and protects its core. It has been demonstrated that PEO prevents the adsorption of proteins[141,142] and hence forms a biocompatible polymeric nanocarrier shell.

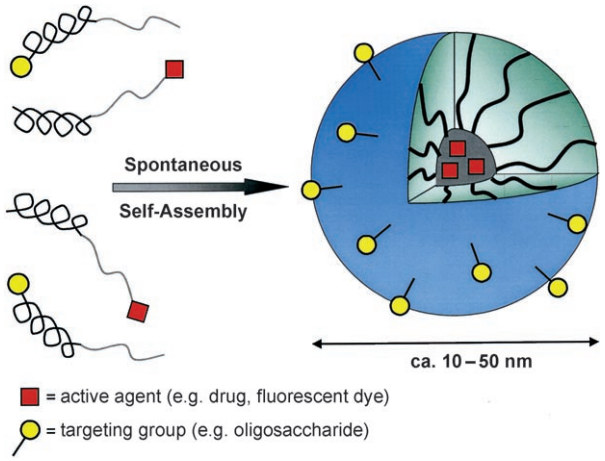


Figure 14. Formation and architecture of block-copolymer micelles which spontaneously form by self-assembly in water. The characteristic features are a pronounced core–shell architecture which can be controlled by the individual polymer blocks. Typical examples of block copolymers are PEO-*b*-PPO, PEO-*b*-PCl, and PEO-*b*-PAsp.

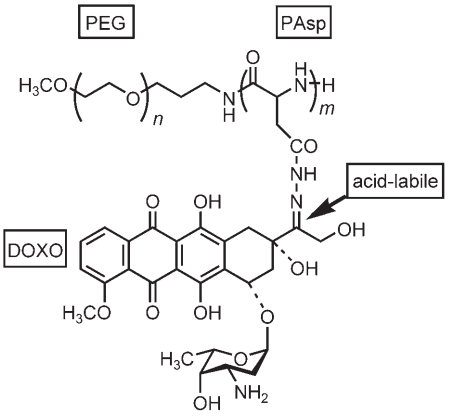
The size of these block-copolymer micelles is determined by thermodynamic parameters, but partial control over the size is possible by variation of the block length of the polymer.[143] Typically, these block-copolymer micelles are 20–50 nm in diameter with a relatively narrow distribution and are therefore similar in size to viruses, lipoproteins, and other naturally occurring transport systems.[137] A major obstacle for these nanocarrier systems is their nonspecific uptake by the reticuloendothelial systems (RES). The size and the surface properties of the nanocarriers based on block copolymers require careful design to achieve long circulation times in the blood and site-specific drug delivery.[144]

The polarity and functionality of each block allow control over the spontaneously formed core–shell architecture. While terminal functionalities on the outer block (the shell) control biocompatibility and may incorporate potential targeting properties, the inner block of such nanocarriers can be used to complex or covalently couple active drug molecules (Figure 14). This core–shell concept is frequently used to dissolve nonpolar drugs. Examples of block copolymers that have poor solubility in water are the pluronics PEO-*b*-PPO or PEO-*b*-PPO-*b*-PEO.[26]

Supramolecular constructs have also been generated by using block copolymers as shells for dendritic porphyrins.[145] These "blown up" micelles (ca. 100 nm) may have a much higher targeting specificity for tumor tissue as a result of an enhanced EPR effect.

Kataoka and co-workers have recently reported a pH-sensitive supramolecular nanocarrier for doxorubicin based on biocompatible block-copolymer micelles.[146] In contrast to drug–polymer conjugates, in which antitumor agents are covalently attached to a single macromolecule chain, doxorubicin was coupled through an acid-labile hydrazone linker to a PEO-*b*-PAsp copolymer (Scheme 7). After spontaneous self-assembly of the drug-loaded supramolecular nanocarrier (Figure 14), kinetic analysis clearly demonstrated the effective cleavage of the hydrazone bonds at $pH \leq 5$, with concomitant release of doxorubicin. Release of doxorubicin was negligible under physiological conditions in cell culture medium ($pH \approx 7$).

The doxorubicin nanocarrier demonstrated in vitro cytotoxicity against a human small-cell lung cancer cell line (SBC-3) in a time-dependent manner, thus suggesting cellular uptake by endocytosis. The first candidates of antitumor drugs based on polymer micelles have entered clinical trials in Japan.[147]



Scheme 7. A doxorubicin block-copolymer conjugate which self-assembles to form block-copolymer micelles in water. The acid-labile hydrazone bond is cleaved at pH < 6 and doxorubicin is released.

3.6.2. *Nanocarriers Based on Dendritic Polymers*

Although physical aggregates such as liposomes and micelles are frequently used as drug-delivery systems,[5] they can be unstable under shear force and other environmental effects, such as high dilution,[143] temperature, and pressure required, for example, for sterilization. An alternative approach is the covalent modification of dendritic macromolecules with an appropriate shell that results in stable micelle-type structures suitable for noncovalent encapsulation of guest molecules (Figure 15).[148,149,162] The size of these dendritic nanocarriers can be defined precisely between 5 and 20 nm. The encapsulation of guest molecules is driven by noncovalent interactions (ionic, H bonding, and van der Waals interactions) and can be simultaneously tailored for various drugs, while a drug–polymer conjugate has to be synthesized individually.

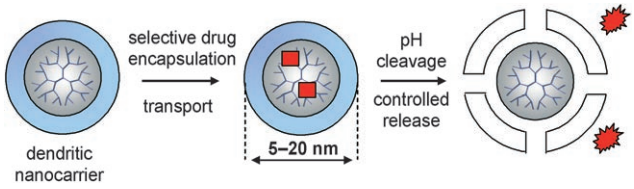


Figure 15. Unimolecular dendritic nanocarriers for encapsulation of biologically active compounds, for example, drugs and oligonucleotides. The drug load can be released selectively in acidic media (such as in tumor tissue) when the acid-labile linkers connecting the shell to the core are cleaved.

Dendritic polymers with their regular and well-defined unimolecular architecture, which can be further chemically modified at either the core (to increase hydrophobicity) or the shell (to increase hydrophilicity), is currently attracting interest as so-called dendritic nanocarriers for applications in drug solubilization and delivery.[15] In previous studies the poorly water-soluble anticancer drug taxol was solubilized in water using polyglycerol dendrimers[150] of the third to the fifth generations.[151] PEGylation of dendritic PEI, PPI, and PAMAM architectures led to water-soluble nanocontainers which were able to solubilize small organic molecules, including anticancer drugs.[152–156]

The encapsulation and the transport of guest molecules in these dendritic architectures have been studied by several research groups.[13,148] However, relatively little is known about the release of the encapsulated guest molecules by pH-triggered cleavage of the shell in the physiological range (Figure 15). In many cases the pH-dependent release from dendritic core–shell architectures has only been achieved under drastic conditions[157] or by protonation of poly(propylene amine) dendrimers[158] and their derivatives.[159]

A simple and general concept for the generation of core–shell-type architectures from readily accessible hyperbranched polymers was recently reported.[160] Several pH-sensitive nanocarriers have been prepared by attaching pH-sensitive shells through acetal or imine bonds to commercially available dendritic core structures (polyglycerol and polyethylene imine; Figure 16). In some cases the pH-responsive nanocarriers showed a very high transport capacity which is an important criterion for efficient drug delivery. Various guest molecules, such as polar dyes, oligonucleotides, and anticancer drugs have been encapsulated inside these dendritic core–shell architectures.

Furthermore, the dendritic polyamine core structure with an imine-linked shell (Figure 16) shows the release profile that is needed for liberating the encapsulated drug in tumor tissue: fast release at pH 5–6 and slow release at pH 7.4.[156] These supramolecular drug-delivery systems are currently being evaluated by us for the transport of cytostatic compounds.

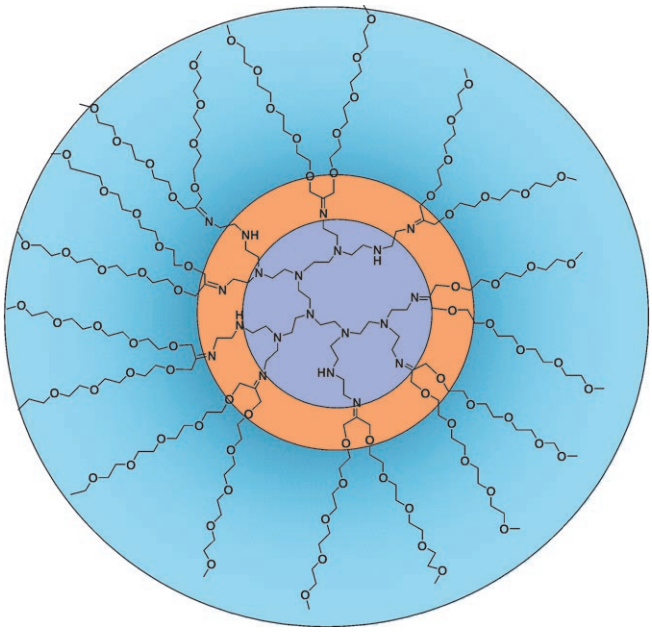


Figure 16. Dendritic core–shell architectures based on commercially available poly(ethylene imine) (PEI) with an acid-labile linker (orange) and PEG shells (blue). Stable supramolecular complexes are formed with various polar guest molecules (dyes, drugs, oligonucleotides). Imine cleavage readily occurs at pH 6 to release the encapsulated guest molecules. The depicted structure shows only an idealized fragment of the much bigger dendritic polyamine core.

4. Summary and Conclusions

The development of polymer therapeutics has emerged as an exciting field of research for improving the therapeutic potential of low-molecular-weight drugs and proteins. PEGylation of therapeutically relevant proteins is an established technology, and it is likely that new PEGylated proteins will attain market approval in the next few years, considering that several hundreds of protein-based therapeutics are under preclinical or clinical development.

The rationale for the development of anticancer drug–polymer conjugates relies on the EPR effect, and various macromolecular prodrugs have shown superior efficacy in preclinical models relative to their low-molecular-weight parent compounds. Several candidates have advanced into clinical studies and have, in most cases, shown a favorable toxicity profile. Comparative studies with established clinical protocols as well as research into the EPR effect in humans and the role of tumor-associated proteases are necessary to select appropriate tumor entities in order to validate the concept of drug–polymer conjugates clinically.

Other concepts, such as multivalent interactions, including the mimicry of functional biomacromolecules by synthetic analogues, have great potential, although the in vivo efficacy data is limited to date.

Finally, bio-nanotechnology has added a new dimension to the development of polymer therapeutics. If nanocarriers based on supramolecular assemblies can be intelligently designed to exploit physiological or biochemical features of infectious or malignant diseases, it should be possible to carry large payloads of the respective drug to the pathogenic site.

In the future more biodegradable polymers with high molecular weights and high precision ($M_n > 30000\ g\,mol^{-1}$, polydispersity < 1.5) as well as new modular approaches to "intelligent" polymeric nanotransporters will be needed. Toxicity and pharmacokinetic issues should be addressed at an early stage when selecting promising new polymer therapeutics, since in vivo studies will primarily decide the fate of a new polymeric drug.

Helmut Ringsdorf's statement on the future perspectives of macromolecular chemistry might serve as a stimulus for the scientists active in the field as well as those of the future:[161]

"It is certainly only a matter of time before pharmaceuticals are required that not only affect cells and tissue specifically, but must also exhibit specific behavior in the cytoplasm of the cell."

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[162] Note added in proof: A recent special issue deals with biomedical applications of dendrimers (*Adv. Drug Delivery Rev.* **2005**, *57*, 2101–2286).

