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PEGylated polymers for medicine: from conjugation to self-assembled systems

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Synthetic polymers have transformed society in many areas of science and technology, including recent breakthroughs in medicine. Synthetic polymers now offer unique and versatile platforms for drug delivery, as they can be “bio-tailored” for applications as implants, medical devices, and injectable polymer-drug conjugates. However, while several currently used therapeutic proteins and small molecule drugs have benefited from synthetic polymers, the full potential of polymer-based drug delivery platforms has not yet been realized. This review examines both general advantages and specific cases of synthetic polymers in drug delivery, focusing on PEGylation in the context of polymer architecture, self-assembly, and conjugation techniques that show considerable effectiveness and/or potential in therapeutics.

Introduction

Improving the efficacy and efficiency of therapeutic molecules by focusing on the delivery platform or vehicle, rather than the drug itself, is an emerging and challenging area of opportunity in modern medicine. In state-of-the-art chemotherapy, a number of organic molecules used clinically effectively kill cancer cells, but are also toxic to healthy cells and induce numerous undesirable side-effects. This indiscriminant action of potent drugs presents a myriad of complex challenges associated with optimizing their clinical utility. Strategies designed to overcome the undesirable characteristics of such drugs must be implemented to both optimize drug performance and minimize side effects. Improved delivery vehicles present an opportunity to maximize the therapeutic benefit of existing drugs.

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Synthetic polymers, long-established as plastics, adhesives, foams, and rubbers, have more recently emerged as key components of drug delivery platforms.^{1,2} For injectable therapeutics, fundamental problems that can be addressed with synthetic polymer delivery platforms include poor drug solubility in aqueous media, short *in vivo* circulation time, fast clearance, and undesirable (even life-threatening) side-effects such as dehydration. While current research activity in the area of polymers for medicine is growing at a distinctly rapid pace, it was a few forward-looking researchers who recognized early on the potential of synthetic polymers in drug delivery. In the 1950s, polymer-drug conjugates composed of poly(vinyl pyrrolidone)-*co*-poly(acrylic acid) random copolymers containing drug-bound oligopeptide pendent groups were seen to prolong drug circulation time relative to the drug alone,³ suggesting that synthetic polymers could markedly impact drug behavior *in vivo*. Major momentum in favor of synthetic polymer-drug conjugates evolved from the reports of

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to understand their assembly behavior in solution and in thin films. Since completing her postdoctoral position, she has been a chemistry instructor at Mount Holyoke College.

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Ringsdorf and coworkers in the 1970s, in which a rational design, or model, for polymer drug delivery was presented.⁴ This model described polymer-based drug delivery systems as having three fundamental components: (1) a water soluble polymer scaffold; (2) a therapeutic moiety bound covalently to the polymer scaffold, and (3) a hydrolytically or enzymatically degradable linkage between the polymer and the drug. These pioneering studies led to the subsequent establishment of polymer therapeutics as an active research field, merging the concepts and techniques of synthetic chemistry, encapsulation/micellar phenomena, cell biology, pharmacokinetics, and human clinical trials into a cohesive program geared towards improved human health.

Despite recent advances, there remain significant barriers associated with the conversion of new polymer–drug conjugates from the research laboratory to clinical implementation. Synthetic strategies chosen for polymer drug delivery systems have encountered regulatory difficulties due in part to the inherent characteristics of conventional polymer synthesis, such as molecular weight distribution of a polymer sample. With few exceptions, a synthetic polymer sample is composed of many different molecules having similar repeat unit composition, but different molecular weights. This leads to heterogeneity of the conjugated therapeutic agent, both in terms of polymer molecular weight, and the number of therapeutic moieties per polymer chain that results from anything less-than-quantitative drug attachment chemistry, for example on the polymer chain-ends. These features naturally lead to questions concerning variable *in vivo* behavior. Nonetheless, significant regulatory obstacles associated with synthetic polymers for drug delivery were overcome in the 1990s, with the Food and Drug Administration (FDA) approval of polymer therapeutic conjugates. In the FDA approval process, polymer–drug conjugates are defined as new chemical entities, thus requiring thorough characterization, validated analytical techniques and toxicological protocols, scaled-up synthetic procedures of appropriate manufacturing quality, and protocols to determine appropriate dosing parameters for clinical trials.³ In 1990, the Enzon product,

Adagen[®] (PEGylated adenosine deaminase) became the first approved therapeutic polymer–protein conjugate in the U.S.⁴ Subsequently, as the first generation of synthetic polymer–drug conjugates was reaching the clinical stage, the field as a whole generated considerable interest, initiating a wave of innovation in polymer therapeutics.

Synthetic polymer compositions and architectures relevant to polymer therapeutics have evolved considerably with the advent of modern synthetic techniques, and now include not only linear polymers, but also branched and dendritic polymers, and polymer micelles, as illustrated in Fig. 1. Alternatives to conventional linear polymers evolved rapidly in the 1980s and 90s, as described by a number of research groups, such as Fréchet and Tomalia for dendritic polymers,^{5–9} Eisenberg, Wooley, Bates, Discher and others for polymer micelles,^{10–21} and Torchilin for polymer modified liposomes.^{22–26} In conjunction with advances in polymer synthesis came novel synthetic strategies for the conjugation, encapsulation, release, and imaging²⁷ of drugs. This review will examine some of the advances in synthetic polymers as drug delivery platforms focusing on PEGylation, and discuss ongoing efforts to improve existing conjugates and diversify the range and targets of drug delivery platforms.

PEGylated drugs: linear PEG conjugates

Conjugating poly(ethylene glycol) (PEG) to both protein and small molecule drugs is an attractive strategy for improving drug delivery, as PEG has compiled a substantial track-record while becoming a workhorse polymer in the drug delivery field. Relative to unmodified drugs, PEGylated therapeutics exhibit the critically important characteristics of increased water solubility and *in vivo* circulation time, with decreased enzymatic degradation and immunogenicity.²⁸ PEGylated platforms also exhibit passive tumor targeting by the enhanced permeability and retention (EPR) effect.²⁹ The EPR effect describes the observed preferential uptake of polymers into the leaky vasculature of cancer tissue relative to the tighter vasculature of normal (healthy) tissue, and subsequent retention of the drug in the tumor tissue due to poor lymphatic drainage. Drugs functionalized with linear PEG have been studied extensively *in vitro* and *in vivo*, and the use of PEG for drug conjugation is now well-established.

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Fig. 1 Synthetic polymer architectures as drug delivery vehicles, including linear, branched, dendritic, and micellar structures.

Most commonly, PEG-to-drug conjugation is performed by coupling a reactive chain-end of PEG to the therapeutic agent. Some of the more widely utilized chemistries for PEG attachment to therapeutic proteins or peptides are shown in Fig. 2. PEG-NHS ester and PEG-aldehyde have proven very useful for conjugation to amines of lysine residues, while PEG-maleimide reacts with thiols of cysteine residues. Such reactions are also applicable to the conjugation of small molecule drugs where appropriate reactive functionality is available. Recent efforts geared towards expanding the scope of conjugation chemistry for proteins and small molecules include “click” chemistry. A “click” reaction is one which has a wide scope of applications, gives high yields, produces by-products which are removed easily, and is regiospecific. In addition, “click” reaction conditions should be mild, and the product should be isolated by facile methods such as recrystallization or distillation. A common example of “click” chemistry being introduced in bioconjugation is the 1,3-dipolar cycloaddition of alkynes and azides.³⁰ For example, Kataoka and coworkers recently reported the synthesis of heterobifunctional PEG derivatives, having for example a primary amine or carboxylic acid group at one chain end, and an azide group at the other chain end.³¹ Such functional PEGs are expected to have significant versatility in click chemistry, due to the mild reaction conditions used (aqueous buffers) and the high degree of chemoselectivity of the reaction.³¹ The scope of click chemistry is not limited to the reaction of azides with terminal alkynes. For example, Bertozzi and coworkers³² introduced Staudinger ligation reactions as a modified version of the classical Staudinger reaction, where an azide and phosphine react to give a primary amine and phosphine oxide. The reaction proceeds through an aza-ylide intermediate, and Bertozzi and coworkers have shown that by using a triarylphosphine with a strategically placed electrophilic trap, such as a methyl ester, the unstable aza-ylide can rearrange to form a more hydrolytically stable intermediate and goes on to produce a stable covalent adduct by amide bond formation. The reaction is “click-like” because it can be carried out in water, and both the azide and the phosphine are generally unreactive towards biomolecules (*i.e.*, such as those found on cell surfaces). Using the modified Staudinger ligation, stable cell-surface adducts were produced, showing the potential applicability of this reaction to the study of intercellular processes.³²

PEGylated proteins

FDA approval of the PEGylated protein Adagen[®] represented the successful application of synthetic polymers in therapeutics, serving as an alternative to bone marrow transplants for patients suffering from severe combined immunodeficiency disease (SCID).⁴ Adagen[®] is an enzyme replacement therapy for the missing adenosine deaminase (ADA) in SCID patients.³³ Administration of the unmodified enzyme is hampered by immunogenicity and very short *in vivo* circulation time.³⁴ Other PEG-protein conjugates have since gained clinical acceptance as part of the vibrant growth of PEGylated therapeutics. A general decrease in side effects with less frequent dosing has enabled PEGylated protein therapeutics

to comprise a significant part of the protein therapeutics platform. PEG Intron[®], a PEGylated version of Intron A[®] (interferon- α -2b) developed by Schering-Plough, is another example of a PEGylated protein drug that exhibits advantages over the unmodified protein. Interferon- α -2b is a cytokine that inhibits tumor growth and angiogenesis, and is important therapeutically for treatment of hepatitis B and C, malignant melanoma, and leukemia.^{35,36} Administration of the unmodified protein results in a range of undesirable side effects (*e.g.*, flu-like symptoms, depression, and anorexia), and due to a short half-life in the blood stream, only a very small window of effective therapeutic level is achieved. The high dosing frequency thus required for unmodified interferon limits the practicality of administration to patients.³⁷ To prepare PEG Intron[®], a 12 000 g mol⁻¹ succinimidyl carbonate functionalized PEG is conjugated to interferon- α -2b at histidine 34 to give the monoPEGylated protein.³⁷ *In vivo* degradation of the carbamate releases the protein during circulation in the bloodstream. In the treatment of hepatitis C, PEG-Intron[®] (often used in combination with ribavirin, an *anti-viral* drug) is found to minimize drawbacks associated with the unmodified protein, exhibiting a 10-fold increase in circulation time at therapeutic levels in plasma, from a half-life of 4 h for the unmodified protein, to 40 h for the PEG-protein conjugate.³⁸ This dramatically increased circulation time allows less frequent dosing, typically once weekly for PEG-Intron[®] compared to three times per week for interferon- α -2b. Moreover, drug availability in plasma increases, while the toxicity does not.³⁸ Additional reports suggest that PEGylated interferon- α -2b may also improve treatment of cancer, multiple sclerosis, and HIV/AIDS.³⁵ Recent reports demonstrate the continued emergence of PEGylation into new products. For example, Cimzia[®] was developed and is now FDA approved for the treatment of Crohn's disease and is under investigation for treating rheumatoid arthritis.³⁹ Cimzia[®] is a PEGylated monoclonal antibody directed against tumor necrosis factor alpha. This and other PEGylated drugs are described on the Nektar website on platform technologies.

Other notable PEG-protein conjugates include PEGylated erythropoietin (PEG-EPO) and PEGylated granulocyte-colony stimulating factor (PEG-G-CSF). EPO, a glycoprotein of ~40 kDa, stimulates proliferation of erythrocytes into mature red blood cells, and is used clinically to treat conditions such as anemia stemming from chemotherapy and AIDS.^{40–42} EPO is also used “non-clinically” as a blood doping agent in competitive athletics.⁴³ The short (<12 h) *in vivo* half-life of recombinant EPO has driven researchers to develop longer-lasting injectable EPO products, and PEGylation has proven successful in this regard. Key elements of current efforts include site-specific PEGylation of “non-essential” amino acid residues to minimize loss of *in vitro* bioactivity associated with random lysine PEGylation, or PEGylation within the enzyme's active site. G-CSF, a 20 kDa four-helix bundle protein, is a cytokine that regulates differentiation of hematopoietic progenitor cells towards mature neutrophils.⁴⁴ The G-CSF protein drug filgrastim, marketed by Amgen under the registered trade name Neupogen[®], is produced by recombinant methods, and used in chemotherapy. Unfortunately, the tendency of G-CSF to aggregate at moderate concentrations

Activated PEG Derivatives for Conjugation

For Amine Conjugation

For Thiol Conjugation

For "Click" Conjugation

Fig. 2 Examples of conventional PEGylation reagents and click approaches to bioconjugation.

and physiological conditions compromises its therapeutic benefit. However, PEGylated G-CSF, prepared by conjugating a 20 kDa PEG to the N-terminus of the protein, gives a new drug composition, Neulasta[®], that exhibits much longer serum half-life, effectively reducing the number of doses per chemotherapy cycle needed to effect the desired reduction in infections experienced by chemotherapy patients.^{45,46} Thus, the success of PEGylated G-CSF appears to be due to a combination of preventing gross protein aggregation, which can cause adverse reactions during therapeutic treatments, and solubilizing the aggregated states that tend to form even after PEGylation.⁴⁴

While the benefits of PEGylation for increasing the efficacy, and reducing immunogenicity, of therapeutic proteins and small molecule drugs is proving fruitful, other polymer structures present intriguing alternatives. Phosphorylcholine (PC)-based polymers represent one interesting potential example of a PEG-replacement. Like PEG, "PC-polymers"

are hydrophilic, due to the close association of water with the zwitterionic moieties. In fact, these zwitterionic polymers differ from PEG in that they are strictly hydrophilic, whereas PEG exhibits amphiphilic character. Most prominent among PC-polymers is poly(methacryloyloxyethyl phosphorylcholine) (polyMPC). PolyMPC is becoming recognized as perhaps the most biocompatible of synthetic polymers, naturally leading to various successful applications including, for example, contact lenses, stents, and various medical devices and implants.^{47–53}

Advances in controlled free radical polymerization, especially copper-catalyzed atom transfer radical polymerization (ATRP), are enabling the preparation of novel polyMPC derivatives appropriate for conjugation to therapeutic proteins (Fig. 3). In 2008, two groups nearly simultaneously reported the synthesis of polyMPC for protein functionalization.^{54,55} One report described the synthesis of N-hydroxysuccinimide- and benzaldehyde-terminated polyMPC (Fig. 3A), and the conjugation of these polymers to lysozyme (as a model enzyme), and to the therapeutic proteins erythropoietin (EPO) and granulocyte colony stimulating factor (G-CSF).⁵⁴ Another report described an elegant synthesis of a bis-thiol specific derivative of polyMPC (Fig. 3B) for conjugation to interferon- α 2a (IFN).⁵⁵ The half-life of elimination of a polyMPC-protein conjugate was markedly extended relative to the native protein, and even longer than the PEGylated version. Taken together, these reports point to an excellent potential for PC-polymers to become important new synthetic polymers in drug delivery.

PEGylated small molecule drugs

Just as protein therapeutics benefits tremendously from covalent PEGylation strategies, small molecule drugs can also be improved by PEG attachment. Camptothecin (Fig. 4A) for

Fig. 3 Poly(methacryloyloxyethyl phosphorylcholine) derivatives for conjugation to therapeutic proteins: (A) N-hydroxysuccinimide- and benzaldehyde-terminated polyMPC for conjugation to amines, and (B) polyMPC designed for bis-thiol specificity.

example, is a potent topoisomerase I inhibitor, and thus an active drug against many types of cancer. However, poor water solubility, and physiological instability, make its clinical implementation problematic. Synthetic derivatives of naturally occurring camptothecin (the extract of *Camptotheca acuminata* trees in southern China), such as the piperidinyl-functionalized Camptosar[®] (Fig. 4B), have been synthesized to provide better aqueous solubility relative to the naturally occurring compound. However, this and other camptothecin derivatives suffer from reduced efficacy and serious side effects (e.g., severe dehydration), leading to its somewhat limited use as a treatment for small-cell lung and colorectal cancers.⁵⁶ A PEGylated camptothecin known as Prothecan[®] (Fig. 4D) was developed by Enzon, Inc., to improve both water solubility and *in vivo* circulation time. PEGylated camptothecin consists of a 40 000 g mol⁻¹ PEG chain with camptothecin at each of the two chain-ends, connected by ester linkages at the C-20-OH position of the drug, as shown in Fig. 4.⁵⁷ Acylation of the camptothecin hydroxyl group inhibits lactone ring-opening under physiological conditions, a critically important feature of the substituted versions, as ring-opening leads to loss of therapeutic potency. Prothecan[®] shows significantly improved circulation time over the unmodified drug, with a half life > 75 h, and therapeutic drug levels maintained for weeks after dosage.⁵⁸ With such improvement in the therapeutic profile, Prothecan[®] entered phase II trials for solid tumors (e.g., in soft tissue and the stomach),⁴ though these trials were later reported as terminated or suspended.⁵⁶

In another example of PEGylated camptothecin prodrugs, Davis and coworkers introduced a linear PEGylated polymer, based on cyclodextrin-containing repeat units, shown in Fig. 4.⁵⁹ This camptothecin-polymer conjugate, known as IT-101 (Fig. 4F), displayed markedly enhanced pharmacokinetics and biodistribution compared to unconjugated CPT. IT-101 showed plasma half-lives between 17–19 h on average, compared to ~1 h for CPT alone. Additionally, one intravenous dose of IT-101 to tumor-bearing mice resulted in greater CPT accumulation in the tumor.⁵⁹ The efficacy of IT-101 in different mouse tumor xenografts, including colon carcinoma, small-cell lung cancer, breast cancer, and pancreatic cancer, was subsequently studied.⁶⁰ Using a cycle of three weekly doses, IT-101 was found to display potent anticancer activity in all models, noticeably delaying tumor growth. In some models, complete tumor regression occurred. The results of these studies further indicate that the polymer–drug conjugate IT-101 may be valuable to the field of cancer therapeutics.

While functionalization of drugs with linear PEG is of continuing interest, inherent drawbacks should be noted. For example, the loading capacity of linear PEG is limited to the polymer chain-ends, giving a maximum of two biologically active agents per polymer. Drug release from these polymer conjugates relies on degradation of the linker in a continuous process, rather than a precisely triggered event. Simple PEGylated drugs also lack targeting functionality that would enable specific interactions with diseased tissue. Current research efforts striving to overcome these limitations have exploited advances in synthetic chemistry and bio-tailoring strategies. These efforts include covalent linkage of drugs to

branched polymer architectures, including PEG, dendrimers, hyperbranched polymers, graft copolymers, and functional micelles and capsules.

Cyclic polymers, produced by joining the chain-ends of a linear structure, offer another interesting possibility useful for drug delivery.⁶¹ The synthesis of cyclic polymers, once an academic curiosity, has been enabled recently by new catalysts that produce cyclic structures in high yield. Cyclic polymers behave differently from their linear analogs, due to the lack of end-groups that would alter their hydrodynamic properties in solution and reptation behavior.⁶¹ The application of cyclic polymers in therapeutics was reported recently by Fréchet, Szoka and coworkers, for the case of a cyclic random copolymer of α -chloro- ϵ -caprolactone and ϵ -caprolactone.⁶² The consequence of the difference in hydrodynamic volume of the linear vs. cyclic structures was seen by SEC-GPC, where the cyclic product elutes after the linear precursor (attributed to the cyclic molecule having a smaller hydrodynamic volume). The cyclic polyester backbone was modified to contain azides, allowing for further functionalization by click chemistry, including PEGylation. The pharmacokinetics of the PEG-grafted cyclic polyester were studied by radiolabeling with ¹²⁵I, and degradation occurred over the course of 10 days in PBS at physiological temperature. The cyclic polymers displayed longer half-lives than the linear analogs, which the authors attribute to the more facile reptation of the linear polymer relative to the cyclic structure.⁶²

Branched PEGylated polymer–drug conjugates

Branched macromolecules are well-suited for exploration in polymer therapeutics, as their multiple chain-ends (from three to hundreds, depending on the degree of branching and the number of monomer repeat units) can be functionalized with therapeutic agents, targeting ligands, and solubilizing groups such as PEG. Chain-end functionalization of linear PEG diols (i.e., HO-PEG-OH) with branched moieties increases the number of drugs that can be attached to each PEG chain. For example, carbodiimide coupling of aspartic acid dendrimers to one or both chain-ends of PEG diacid has been used to give four or eight carboxylic acid chain-ends per polymer.⁶³ These carboxylic acids were then used for covalent attachment of cytosine arabinoside (Ara-C) chemotherapy agents, for the treatment of leukemia and non-Hodgkin's lymphoma. A variety of molecules containing different spacer moieties were prepared, with each spacer attached through an amide bond in the N4 position of Ara-C. The PEG tetramer and octamer derivatives, containing four and eight Ara-C groups, respectively, were reported to inhibit tumor growth in an LX-1 solid lung tumor model with 66% and 78% tumor growth inhibition (TGI), improved over the free Ara-C (26% TGI), and the linear PEG version (50% TGI). The branched PEG-Ara-C conjugates exhibited a higher TGI against orthotopic pancreatic tumors, and the octamer derivatives were effective in a localized subcutaneous tumor model, which was unsuccessful using free Ara-C. The improved activity of the branched PEG-drug conjugates was attributed to multiple factors, including higher drug loading capacity, protection of Ara-C by the PEG chains from enzymatic

Fig. 4 (A) Camptothecin and derivatives (B) Camptosar[®] (irinotecan hydrochloride), (C) graft copolymers prepared by click cycloaddition of alkyne-substituted polyesters with PEG and camptothecin azides, (D) Prothecan[®], (E) multi-arm PEG star with four glycine-linked SN38 molecules, and (F) IT-101, a PEGylated linear cyclodextrin polymer bearing 2 glycine-linked CPT drugs per repeat unit.

degradation, and passive accumulation of the polymer-drug conjugate by the EPR effect. Taken together, this leads to a higher concentration of Ara-C near the tumor for longer periods, thus better inhibiting tumor growth.

Many types of branched PEG and PEG-like structures have been prepared for therapeutic purposes. One such example is a four-arm star PEG, capable of loading up to four irinotecan (7-ethyl-10-hydroxy-camptothecin; SN38) molecules at the chain ends.⁶⁴ This PEG-SN38 conjugate was found to be improved over the previous linear PEG-CPT conjugates due to the higher potency of SN38, as well as increased loading. PEG-SN38 (Fig. 4E) was prepared by functionalizing a 40 kDa four arm PEG-OH with carboxylic acid groups at each of the four chain ends. After protecting the phenol of SN38, which would interfere with the desired conjugation, SN38 was acylated selectively at the 20-OH position, yielding the desired PEG-SN38 conjugate. *In vitro* studies were done using colorectal (COLO 205, HT29), ovarian (OVCAR-3), and lung (A549) cancer cell lines. Additionally, *in vivo* efficacy studies were performed using MX-1 tumor xenograft models in mice, which showed the drug conjugates to have superior anticancer activity as compared to SN38 alone, attributed to

the combination of enhanced drug solubility, improved bio-distribution, and the EPR effect. The four arm star PEG conjugate containing glycine-linked SN38 was selected for more preclinical development, and has reportedly entered phase I clinical trials.⁶⁴

Another elegant example of a branched structure is the synthesis of a dendritic PEG conjugate,^{65,66} in which the stepwise dendrimer synthesis gives a truly monodisperse polymer, to which even narrow polydispersity PEG (prepared by anionic polymerization) cannot compare. More common examples of branched PEGylated molecules for therapeutics are graft or comb structures, in which the PEG chains are placed pendent to the polymer backbone. PEGylated aliphatic polyester graft copolymers provide one recent example, in which PEG, oligopeptides, and drugs can be attached as pendent groups to the polyester backbone. Such polymers augment the well-known PEG-poly(lactide) and PEG-poly(ϵ -caprolactone) diblock copolymers used for micellar encapsulation and delivery.^{67–75} The graft copolymer approach is appealing in principle for aliphatic polyesters, due to their known biocompatibility (*i.e.*, degradation to benign small molecules), but difficult in practice as polyester

backbone lability limits the range of chemistries feasible for the preparation of useful conjugates. Recent examples of aliphatic polyesters with pendent reactive unsaturated groups, prepared by ring-opening polymerization of functionalized lactones, appear promising for subsequent grafting chemistry that proceeds cleanly, revealing little-to-no polyester degradation.^{76–82} For example, pendent allyl groups serve as precursors to hydroxyl, halide, and silyl groups, and thiol-ene click chemistries, that alter the solubility, physical properties, and degradation rates of the polymers.^{76,83} Pendent cyclopentene groups can be converted to 1,2-diols to give polyesters with good shelf-stability, and that can be PEGylated, for example, by esterification of the hydroxyl groups with PEG-succinic acid ester derivatives.⁷⁷ Pendent alkynes on aliphatic polyesters provide a route to azide-alkyne click cycloaddition chemistry with azide-substituted molecules, a method that is proving efficient for the rapid preparation of narrow polydispersity PEGylated aliphatic graft polyesters.⁸¹ The opposite synthetic strategy (polyester-azide with PEG alkyne) is also feasible.⁸² Beyond simple PEGylation, click chemistry on aliphatic polyesters can be carried out for attachment of chemotherapeutic agents, an appealing approach given the biodegradable and biocompatible properties of aliphatic polyesters. For example, an azide-functionalized camptothecin was used in this fashion, to give a water soluble biodegradable polymer drug (Fig. 4C), in which acylation at the 20-OH position of camptothecin stabilizes the lactone form of the drug in aqueous solution, setting up hydrolysis *in vivo* during the course of circulation and accumulation in tumor tissue.⁷⁸

PEGylated polyolefins and methacrylates represent additional types of PEG-comb structures (Fig. 5A) for potential application to therapeutics. Examples of these are shown in Fig. 5. Prominent among such polymers is poly(PEG methacrylate) (poly(PEGMA)) (Fig. 5B), prepared by standard free radical polymerization, or controlled free radical techniques such as ATRP or RAFT.⁸⁴ These polymerization techniques are especially interesting for therapeutics, as the low PDI and end-group specificity (*e.g.*, aldehyde, NHS-ester, *etc.*) achievable results in polymers amenable to protein or drug conjugation,⁸⁵ as demonstrated for example with lysozyme.⁸⁶ Other methacrylate units can be integrated into these structures, including oligopeptide residues such as the RGD sequence that is used widely for cell recognition.^{87,88} For conjugation to proteins, and protein-based nanocage assemblies, it should be noted that “growth-from” methods are gaining popularity, by functionalization of the proteins with appropriate initiators, followed by polymerization radially outward from the protein.^{89,90} Mild, aqueous chemistries are most suitable for performing chemistry on proteins—controlled free radical conditions (ATRP and RAFT) seem well-suited in the examples demonstrated so far.

Less conventional PEGylated polyolefins with potential therapeutic applications prepared recently include PEGylated polynorbornene and polycyclooctene graft copolymer structures (Fig. 5C).^{91–94} The discovery of water and functional group tolerant ruthenium benzylidene catalysts for ring-opening metathesis polymerization (ROMP)^{95–100} is a key enabling factor for the preparation of such polymers. Several examples

of functional norbornene and cyclooctene macromonomers have been prepared and polymerized, to give structures with pendent PEG,⁹¹ sugars,⁹² and oligopeptides.^{93,94} The poly(cyclooctene)-*graft*-PEG amphiphiles strongly segregate to oil–water interfaces, giving oil-in-water capsules, which are easily filled with hydrophobic drugs, such as DOX (Fig. 5D) or camptothecin, during the course of interfacial assembly (*i.e.*, by simply shaking the drug/polymer solutions in water).¹⁰¹ Sizing of these interfacial assemblies by passage through track etch membranes is followed by cross-linking of the polymer shell either by cross-metathesis or UV irradiation to give robust capsules that can be tailored to increase their suitability for therapeutic applications in terms of drug leakage rates, and integration of targeting ligands at the capsule periphery.¹⁰¹

The branched macromolecular architecture is advantageous in polymer therapeutics not only for increasing drug loading, but also for integration of triggered drug release mechanisms and reporter molecules into the structures either by covalent attachment or physical sequestration. For example, star-like polymers have been used as implanted gels that release drugs over time by diffusion, and substantial efforts are underway towards applying PEGylated star and dendritic structures as intravenous drug delivery systems. PEG stars refer to the specific type of branched architecture in which linear PEG arms extend outward from a central core, affording the desired macromolecular structure. These polymeric nanostructures may have applications as drug delivery platforms, as demonstrated recently by Hawker and coworkers,¹⁰² who report the design and synthesis of PEGylated star copolymers as micellar-type structures, prepared using nitroxide-mediated radical polymerization (NMP) techniques. The hydrophobic core of these materials is a microgel composed of poly(*N,N*-dimethylacrylamide) (PDMA) that is crosslinked with divinyl benzene or ethylene glycol diacrylate. The inner shell is a copolymer of PDMA and poly(*N*-acryloylsuccinimide) (NAS). By incorporating the poly(NAS), the inner shell has been functionalized so that further modification (*i.e.* radio-labeling) in this region may be performed. The star copolymer is then PEGylated to provide water solubility and biocompatibility. Using controlled polymerization processes, these materials displayed the desired architecture, and monomer functional group tolerance. The functionality incorporated in the inner shell was exploited to conjugate DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), which enables ⁶⁴Cu labeling used in positron emission tomography (PET) imaging to study biodistribution. Additional examples of recent work on PEGylated star polymers include PEG stars with poly(L-lactide) or poly(L-lactide-*co*-glycolide) scaffolds as drug delivery platforms.^{103,104} These star molecules show desirable properties for delivery of physically sequestered drugs, including a moderately decreased initial degradation over two weeks (20% molecular weight loss for the 4-arm star, and 10% loss for the 8-arm star), followed by accelerated polymer degradation. In comparison, an ABA linear triblock copolymer analog gave 30% molecular weight loss over two weeks. Such an *in vitro* degradation profile is thought to result from a hydrophilic environment during release of a guest molecule followed by fast elimination of the remaining polymer.

Fig. 5 Examples of PEGylated comb and graft copolymer structures (A–C), and their use in interfacial assembly and encapsulation (D–E).

PEGylated dendrimer therapeutic platforms

Dendrimers possess important distinctions from linear polymers that make them especially interesting for polymer therapeutics.^{6,8,105} Similar to linear polymers, the chemical composition of a dendritic polymer is readily tunable towards achieving a desired biocompatibility, degradation rate, and pharmacokinetic profile. However, unlike linear polymers, the molecular weight and size of a dendrimer is well-defined; indeed many dendrimers have been prepared and purified as single “monodisperse” macromolecules. This exquisite level of structural control is a function of the stepwise dendrimer synthesis that differs from the statistically dictated step- and chain-growth polymerization methods used to prepare more conventional linear and branched polymer architectures. Taken together, the monodispersity, degree of branching, and size control of dendrimers provides access to precision polymer materials that are expected to limit or eliminate undesirable effects of size variation in polymer drug delivery systems. Moreover, dendrimers as nanomaterials with multiple chain-ends are also tunable in terms of functional group type and density. This multi-functional design provides an advantage over linear polymers for covalent attachment of drugs, imaging agents, targeting ligands, and other biologically relevant moieties.

Among the many types of dendrimers synthesized to date, poly(amidoamine) (PAMAM) versions are used extensively in therapeutic research, benefiting from the convenience of commercial availability of a range of dendrimer sizes (*i.e.*, generations) and chain-end functionality. PAMAM-based dendrimers have progressed considerably in drug and gene delivery, for example as anti-HIV (VivaGel[®]) and diagnostic (Stratus[®]) agents, and as the gene delivery reagent Superfect[®].¹⁰⁶ The core-shell architecture of PEGylated PAMAM dendrimers is useful for encapsulation of hydrophobic small molecule drugs, and the *in vivo* behavior of these systems as drug delivery agents has been evaluated. For example, amine-terminated PAMAM (G-4) dendrimers were

PEGylated using NHS activated carboxymethyl mPEG-5000, reacting with about 25% of the terminal amines.³⁶ Studies were performed on the PEGylated *vs.* non-PEGylated PAMAM delivery vehicles of the anticancer drug fluorouracil. The drugs were sequestered non-covalently into the dendritic core, and held there by hydrogen bonding. PEGylated PAMAM was seen to sequester an order of magnitude more fluorouracil than PAMAM itself, attributed in part to steric effects of the PEG corona that hinder drug release. The presence of the PEG coating led to a six-fold decrease in drug release rate and lower hemolytic toxicity, for a better overall performance relative to non-PEGylated PAMAM.

Fréchet and Szoka have demonstrated the benefits of combining state-of-the-art functional dendritic polymers with potent chemotherapeutic agents. For example, bow-tie dendrimers functionalized with both PEG and doxorubicin were prepared as drug delivery vehicles, to combine pH dependent drug release with passive tumor targeting.¹⁰⁷ This sophisticated synthetic strategy, combining different polymer architectures and compositions into one structure, also provides a pH-triggered delivery of the drug. The bow-tie polymer scaffold (Fig. 6A), consists of a PEG-terminated third-generation aliphatic polyester dendron (based on the 2,2-bis(hydroxymethyl)propionic acid repeat unit structure¹⁰⁷) attached covalently to a fourth-generation polyester dendron terminated with hydroxyl groups. Each component of this polymer therapeutic plays a role in the delivery design that seeks to minimize side-effects associated with DOX treatment. The hydroxyl chain-ends of the polyester dendrimer were used to conjugate DOX through a pH sensitive hydrazone linkage. The resulting polymer therapeutic, containing 8–10 weight percent DOX, will release the drug in the vicinity of tumor tissue due to the chemistry of the linkage. *In vitro* degradation studies showed that at pH 5, all of the hydrazone-linked DOX was released in 48 h, while at pH 7.4 less than 10% of the drug was released over the same time-frame. This dendritic polyester scaffold forms not only the delivery vehicle, but is also biodegradable, such that following delivery of the

therapeutic drug, the degraded dendrimer can clear from the bloodstream without accumulating in the liver or kidneys. The dendritic-linear hybrid structure allows the vehicle to benefit from the EPR effect and passively target solid tumors, while maintaining the characteristic high water solubility and low toxicity of PEG. Moreover, the dendrimer size was chosen to balance the polymer-to-drug ratio, such that the size of the PEG chains could tune serum elimination half-life, and the dendrimer could both carry and release enough drug to achieve a desirable therapeutic benefit. These dendritic-drug delivery systems are readily water soluble, carrying up to 6 mg mL⁻¹ of the hydrophobic DOX, a massive solubility increase over unmodified DOX (which has an aqueous solubility of <1 mg mL⁻¹). The concentration of DOX accumulated in the tumor 48 h after dosage was an order-of-magnitude higher for this dendritic-DOX conjugate relative to unmodified DOX. This enhanced accumulation is attributed to the much longer serum elimination half-life of dendritic DOX (16 h for the conjugate compared to 10 min for unmodified DOX), which allows the vehicle to take advantage of the EPR effect. When dosed once with 20 mg DOX per kg, all of the ten mice used in the study survived 60 days after tumor inoculation, displaying complete tumor regression. This result is remarkable considering that none of the mice treated with free DOX (at the maximum tolerated dose of 6 mg kg⁻¹) survived longer than 30 days. Additional studies by Fréchet and Szoka describe novel bow-tie dendrimers, (Fig. 6B), with symmetry about the core, generated by divergent growth of a bis-2,2-hydroxymethylpropionic acid (bisHMPA) from a pentaerythritol core.¹⁰⁸ The bisHMPA block was then functionalized using a trifunctional amino acid, by EDC coupling to the carboxylic acid. PEGylation of the terminal amines through carbamate linkages leaves the third functional group available for modification at the dendritic core. This final functional group has a range of possibilities, including radiolabeling, esterification, amidation, click chemistry, Pd coupling, or hydrazone formation at the core of the PEGylated dendrimer. To examine the capacity of the dendrimers for conjugation with a small molecule drug, DOX was conjugated again by a hydrazone linkage.¹⁰⁸ Biodegradability and biodistribution studies demonstrated increased half-lives relative to unmodified DOX. This drug delivery system was found to be comparable to DOXIL, the current PEGylated version of DOX, in terms of amount of drug delivered to the tumor. Additionally, less DOX was found to accumulate in healthy tissue as compared to DOXIL. Another advantage of the bow-tie system is that the material can be stored conveniently as a stable solid, and rehydrated easily when needed.¹⁰⁷

Another recent example of aliphatic polyester dendrimers for therapeutic applications was reported by Adronov and coworkers, detailing the synthesis of high-generation dendrimers from poly(2,2-bis(hydroxymethyl)propanoic acid) and a toluene-sulfonyl ethyl ester core.¹⁰⁹ This core was chosen due to its stability during the dendrimer growth process, as well as its facile deprotection under basic conditions. Deprotection followed by amidation with a bifunctional bis(pyridyl)amine ligand allowed for the chelation of ^{99m}Tc, a common radionuclide in diagnostics, beneficial for its long half-life (6 h), γ -energy of 140 keV, low dosing, and low cost.

It is also the preferred radionuclide in single photon emission computed tomography (SPECT) radio imaging, which allows for real-time, *in vivo* imaging of biodistribution.¹⁰⁹ This method allowed for the synthesis of well-defined, radiolabeled polyester dendrimers and sets the stage for further investigation of these macromolecules as drug delivery vehicles, eventually incorporating therapeutically relevant biomolecules.

Recently, an amino acid-based dendrimer was PEGylated and conjugated to camptothecin in an effort to provide a new drug delivery platform. Szoka and coworkers synthesized a second generation lysine dendron, which was subsequently functionalized with aspartic acid, providing two different functionalities at the periphery; an amine and a carboxylic acid.¹¹⁰ The amine was PEGylated, while the acid was coupled with a camptothecin derivative, yielding a 40 kDa PEGylated poly(L-lysine) dendrimer–camptothecin conjugate, typically loaded with 4–6 wt% camptothecin. The drug conjugate showed improved circulation time over the drug alone, as well as increased uptake in tumor tissue, and was shown to increase the survival rate of tumor-bearing mice.¹¹⁰ These exemplary studies, along with other ongoing work, indicate that polymer therapeutics is on target for making a major impact in medicine, not only for protein drugs but also for small molecule therapeutics.

A diverse array of dendritic polymers has been investigated in various capacities as polymer therapeutics.¹¹¹ For example, cascade-release dendrimer scaffolds prepared by de Groot and coworkers are designed for triggered fragmentation to release the conjugated drug.¹¹² The dendrimers degrade to their respective core units upon activation in the dendritic core, thereby initiating release of the terminal groups. The activation step can refer to any number of either chemical or biological processes. Release of the drug is dictated by the linker placed between the drug molecule and the cleavable carrier, termed the “specifier,” and electron delocalization from a masked amine to a leaving group triggers the release, as depicted in Fig. 7. The system is stable while the amine is protected. The study utilized paclitaxel as a model drug to demonstrate the cascade release properties of the delivery vehicle. Such cascade-release dendrimers may be useful for targeted drug delivery, and may be advantageous for their inherent high drug loading. They may also be useful for tumor-targeted delivery, by incorporating a specifier for tumor-related enzymes, as well as an anticancer agent at the dendritic termini.¹¹²

Other dendritic delivery systems include dendritic-linear hybrid copolymers that form core-shell globular structures in aqueous solution. Hybrid polymer vehicles reported by Simanek and coworkers¹¹³ are composed of a melamine-based dendritic core and a shell of PEG chains. These PEGylated dendritic-linear hybrids were seen to exhibit good biocompatibility, with no observed toxicity in mice at doses up to 2.5 g kg⁻¹ (intraperitoneal injection), or 1.2 g kg⁻¹ (intravenous injection).¹¹³ Another level of complexity introduced to dendritic architectures for polymer therapeutics is the placement of pH-sensitive linkers at the core-shell interface in dendritic-linear hybrid structures. Haag and coworkers reported the synthesis, uptake, and release profile of dendrimer-drug conjugates composed of a

Fig. 6 (A) Bow-tie polyester scaffold as a dendritic-DOX polymer therapeutic; (B) PEGylated dendrimer with core functionality for DOX loading.

Fig. 7 Cascade-release dendritic scaffold (first generation), shown with a nitro “masked” amine where reduction triggers release of paclitaxel.

poly(ethylene imine) (PEI) core and PEG corona.¹¹⁴ Covalent PEGylation in this system was accomplished through imine groups. Imine lability at pH < 6 is thus expected to give polymer fragmentation within the acidic regions associated with tumor vasculature and lysosomal compartments. To study the triggered release of a polar dye from these nanocarriers, congo red, was loaded into the core. This serves as an easily detectable polar model, and has also been proposed as a neuroprotective drug.¹¹⁵ The PEGylated dendrimers gave markedly enhanced drug loading, from 54 mmol per mole carrier for the PEI dendrimer, to 440 mmol per mole carrier

for the fully PEGylated PEI dendrimer. Shell cleavage and dye release was achieved upon lowering the pH to 5.

In addition to synthetic polymers, such as PEG, for enhancing drug delivery vehicles, dendrimers are also being explored as nanoprobe. Fine control over degree of branching and architectures with these materials, combined with the ability to tune fluorescence intensity by environmentally responsive linkers makes dendritic nanoprobe ideal for the biomedical field. Fréchet and coworkers recently reported a nanoprobe based on a dendritic scaffold, designed for imaging acidic tissue *in vivo*, as shown in Fig. 8.¹¹⁶

Fig. 8 A dendritic nanoprobes composed of a PEGylated polyester dendrimer from a pentaerythritol core labeled with Cypate (A), a near-infrared dye, conjugated through acid degradable hydrazone linkages formed from the reaction with hydrazine-modified Cypate (B).

The nanoprobes are pH sensitive, biodegradable, and PEGylated for biocompatibility. NIR dyes are covalently attached to the dendritic periphery by acid-degradable linkages, and the dyes aggregate, by π stacking, due to their close proximity. The stacking reduces the lifetime and intensity of the fluorescence signal. However, once the probe enters an acidic environment, the particle becomes activated causing the release of the NIR dyes. Upon release, the dye molecules are no longer stacked, allowing them to regain their original fluorescence lifetime and signal intensity. This change in the fluorescence from the dyes can therefore be detected as they change environments. The dendrimer was constructed from a pentaerythritol core with polyester branches. Each of the eight branches was conjugated to PEG chains for enhanced solubility and biocompatibility. The resulting particles were determined to be approximately 8–10 nm from dynamic light scattering measurements. Among the important attributes of these systems, the release of the NIR dyes can be monitored by change in fluorescence, giving insight to the kinetics of drug delivery systems.

Other examples of PEGylated dendritic drug delivery systems include those with poly L-lysine (PLL) cores, for example those reported by Porter and coworkers.¹¹⁷ PEGylation of the PLL dendrimers resulted in enhanced control over the pharmacokinetics and degradability of these materials, as well as the biodistribution, and rate of renal clearance. The circulation half life of the dendrimer could be manipulated based on the molecular weight of the peripheral PEG chains that are conjugated to the PLL core.

Polymer micelles as drug delivery systems

Polymer micelles can assemble spontaneously following placement of amphiphilic polymer structures into selective solvents. Hydrophobic–hydrophilic diblock copolymers in aqueous media are especially effective, producing core-shell structures in which the hydrophobic block collapses to generate the core, leaving the hydrophilic block as the surrounding corona. The core-shell morphology of polymer micelles makes them ideally-suited for drug delivery. The core can function as an encapsulating matrix, while the shell presents chemical functionality to control pharmacokinetic profiles and provide recognition or targeting. In some respects, polymer micelles

are similar to liposomal and dendritic structures in that (1) hydrophobic drugs can be sequestered into a hydrophobic region; (2) the structures are of appropriate size to exploit the EPR effect; and (3) the surface or corona can be functionalized with multiple targeting or imaging agents. However, polymer micelles also present unique opportunities as drug delivery platforms, as the core and shell-forming blocks are tunable, such that stimuli responsive linkages can be introduced, cross-linkable functionality can be embedded into the core or shell, the core can be excavated following assembly, and the size and shape of the micelle can be controlled.

Solution characteristics of polymer micelles, such as critical micelle concentration and aggregation number, are key determinants of their suitability for drug delivery. Early studies by Ringsdorf and coworkers examined antitumor cyclophosphamides covalently attached to poly(ethyleneimine)s (previously modified by Michael addition with acrylic acid to yield carboxyethyl groups), as well as cellular uptake of radiolabeled poly(ethylene oxide)-*block*-poly(lysine) micelles.^{118,119} Subsequent reports on applying synthetic polymer micelles to drug delivery platforms appeared rapidly, including studies geared towards understanding structure-property relationships and solution assembly, such that key structural features including aggregation number and micelle size could be optimized.^{10,120–123}

Block copolymer micelles with PEG coronas have emerged as systems with great potential in drug delivery, as they combine biocompatibility with the synthetic versatility of PEG. A variety of activated PEGs can be used exploited for these systems, such as block copolymer structures, providing control over the type and stability of covalent linkage formed. Relevant examples include poloxamers, also known by their trade name Pluronics[®], composed of triblock copolymers of poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide) (PEO-*b*-PPO-*b*-PEO) that form core-shell micelles in aqueous media, imparting the advantages of micellar platforms and also contributing unique *in vivo* properties.¹²⁴ Pluronics[®] nomenclature conveys its characteristic structure information. L, P, and F denote the room-temperature physical state as a liquid, paste, or flake, respectively. The first number (or two numbers if three digits are used) refers to the approximate molecular weight of the hydrophobic (PPO)

unit divided by 300, while the final digit multiplied by 10 is the weight percent PEO in the structure. For example, Pluronic[®] L61 is a liquid with a PPO molecular weight of 1,800 g mol⁻¹ and 10% PEO, while Pluronic[®] F127 is a solid flake with a PPO molecular weight of 3,600 g mol⁻¹ and 70% PEO incorporation. Interestingly, some Pluronics[®] (without encapsulated drug) have been found to inhibit P-glycoprotein expression in cancer cells, which when over-expressed leads to undesirable drug resistance.¹²⁵ In addition, some Pluronics[®] can traverse the small intestine and the blood-brain barrier. Studies on DOX-loaded SP1049C, a mixed micelle system of Pluronic[®] L61 and Pluronic[®] F127,¹²⁶ indicate activity against multiple DOX-sensitive cancer cell lines, with a 2-5 fold greater *in vitro* sensitivity of the cell lines (IC₅₀ values) relative to free DOX. Interestingly, these drug-loaded micelles also showed orders-of-magnitude higher activity compared to free DOX against cancer cell lines not normally sensitive to DOX. SP1049C is able to bind with DNA ten times more efficiently than unmodified DOX, due to a combination of factors including an increase in drug influx due to tissue accumulation by the EPR effect, inhibition of efflux, and changes in intracellular trafficking (*i.e.*, is able to enter the cell by endocytosis rather than relying solely on DOX diffusion). *In vivo* studies using small animal models with various tumor types showed that the micellar-based delivery system improved tumor inhibition over free DOX by 20–60%, and increased the life-span of tumor bearing mice by 35–170%. Thus, the ability of polymer micelles to sequester hydrophobic drugs within the core represents yet another pathway to aqueous drug solubility, and the size of the polymer micelle provides passive tumor localization by the EPR effect. While this system showed improved characteristics over free DOX, drug release relied on partitioning between the hydrophobic core and the external environment, which explained the similar amounts of DOX found in non-cancerous tissues for the Pluronics[®] delivery system *vs.* free DOX. This formulation entered phase II clinical trials in 2003.¹²⁷ SP1049C, an anticancer agent developed by Supratek Pharma, Inc., gained the FDA designation of orphan drug, a term reserved for products intended for diseases which affect less than 200 000 people in the United States, for the treatment of esophageal carcinoma, and has more recently gained the same FDA designation for the treatment of gastric cancer.¹²⁸

Another DOX-encapsulated polymer micelle delivery system, reported in 2001 by Kataoka and coworkers, used PEG-*b*-poly(aspartic acid) diblock copolymers, with DOX attached covalently to the aspartic acid block (Fig. 9).¹²⁹ When taken up in water, the diblock copolymer forms a micelle, in which the DOX-conjugated hydrophobic block facilitates additional physical sequestration of DOX into the hydrophobic core (the amide-linked drug is stable under physiological conditions). The delivery system functions by diffusion of the non-covalent, physically sequestered DOX out of the core, and in small animal studies, plasma and tissue analysis confirmed the expected prolonged release benefit of the micellar system. These DOX-loaded micelles proved very effective, resulting in cures against C-26 colon carcinomas (a model cancer cell line), while administration of the free DOX did not result in cures. Results from phase I clinical

trials were reported in 2004,¹³⁰ and phase II clinical trials were initiated and recently reported as ongoing.¹³⁰

Kataoka and coworkers added sophistication to this polymer-DOX delivery system by attaching the drug to the hydrophobic segment of the micelle through a hydrazine linkage,¹³¹ which, as the dendritic system, releases the drug by covalent bond cleavage at pH ~ 5. These polymer micelles were constructed from PEG-*block*-poly(β -benzyl-L-aspartate) (PEG-*b*-PBLA) chains functionalized with hydrazide moieties, following removal of the benzyl protecting groups from the aspartate repeat units. Conjugation of DOX with the hydrazine moieties gives PEG-*block*-poly(ASP)_{37-co}-poly(Asp-Hyd-DOX)₂₈. These polymer-drug micelles were characterized by dynamic light scattering to be ~65 nm average diameter, and displayed the desired pH dependant DOX release, with about 30% of the DOX released at pH 5. Incubation of these micelles with human small lung cancer SBC-3 cells revealed an effective inhibition of cell growth, approaching that found in experiments using unmodified DOX. Since DOX must enter the nucleus to inhibit cell growth, the experiments reveal the effectiveness of the pH-triggered release system.

Camptothecin may also benefit from delivery using PEGylated micelles. The uptake and release of camptothecin was studied *in vitro* with poly(ethylene glycol)-*b*-poly(benzyl L-aspartate) (PEG-*b*-PBLA) micelles, in which the hydrophobicity of the core was varied by controlling the percentage of benzyl ester protecting groups.¹³² Micelles richer in benzyl ester groups (~two-thirds benzylated) were seen to more effectively sequester the drug (~90%). The same micelle also showed the slowest camptothecin release (100 h were needed to release ~80% of the sequestered drug).

Micellization by spontaneous self-assembly of amphiphilic diblock copolymers can afford a variety of morphologies, including spherical micelles, vesicles, and worm-like micelles. Discher and coworkers report that the worm-like “filomicelles” can be obtained by simply decreasing the weight fraction of the PEO to under 50%, as seen in Fig. 10.¹³³ For example, filomicelles and spherical micelles were assembled from poly(ethylene oxide)-*block*-poly(ϵ -caprolactone) (PEO-*b*-PCL), and subsequently loaded with paclitaxel. Filomicelles were ultimately found to retain up to two times as much paclitaxel, and showed greater drug solubilization relative to the spherical micelles.¹³³

More recent findings by Discher support the benefits of such alternative micellar morphologies over more traditional spherical micelles in drug delivery.¹³⁴ They examined the biodistribution of the worm-like micelles *in vivo*, as well as compared them to traditional spherical micelles of the same diblock copolymer both in terms of maximum tolerated dose and therapeutic efficacy. To visualize the micelles *in vivo*, they were loaded with a near-infrared fluorophore. This study showed that the filomicelles are able to avoid rapid clearance, and circulate for at least 24 h post intravenous injection, and are expected to decrease drug accumulation in healthy tissue. As compared to spherical micelles having the same copolymer structure, filomicelles increase the maximum tolerated dose (MTD) and thus allow more paclitaxel to be administered at once. Paclitaxel-loaded filomicelles were also shown to slow tumor growth in mice up to 6 times more than the

Fig. 9 PEG-*block*-poly(aspartic acid) diblock copolymers for micelle formation and DOX sequestration in water.

Fig. 10 Formation of worm-like micelles from poly(ethylene oxide)-*b*-poly(ε-caprolactone) (weight fraction of PEO = 0.43), and visualization using fluorescence microscopy (with kind permission from Springer Science and Business Media: Pharmaceutical Research, Micelles of Different Morphologies - Advantages of Worm-like Filomicelles of PEO-PCL in Paclitaxel Delivery, 24, 2007, 2099-2109, S. Cai, K. Vijayan, D. Cheng, E. Lima, and D. Discher, Figure 1).

corresponding spherical micelles.¹³⁴ All of these results demonstrate the potential usefulness of filomicelles as novel effective PEGylated drug carriers.

Polymer micelles are also being considered as DNA carriers in polymer-DNA (“polyplex”) gene therapy, in attempts to provide potentially safer synthetic polymer alternatives to viral delivery methodology. Various types of synthetic polycations, prepared from either conventional or amino acid monomers, may be suitable for gene therapy; those most likely to advance will be prepared by facile syntheses, exhibit the ability to protect DNA from degradation, and have sufficient synthetic tunability to contain targeting features. Polycations studied for gene therapy include synthetic polymers such as polyethyleneimine, various poly(amino esters), and polylysine, and natural polycations such as chitosan and dextran.¹³⁵ One particular gene vector explored by Kataoka and coworkers involves the formation of micelles by electrostatic interactions between DNA and so-called “catiomers,” or cationic polymers.¹³⁶ Interestingly, adapting PEGylation concepts to gene therapy vectors can improve water solubility and reduce cytotoxicity, but also tends towards decreased transfection efficiency, referred to as the “PEG-dilemma.” To circumvent this, a “smart” PEGylated micelle was designed to respond to differing intracellular environments, by linking the PEG block to the polycation by a disulfide moiety. In this delivery system, PEG can escape the micelle upon disulfide cleavage, which can occur during any of a number of steps along the endocytic pathway (the cytoplasm is an effective reducing environment due to the presence of glutathione and a variety of redox enzymes). There are a number of possibilities for when the disulfide cleavage will occur, though enhanced transfection efficiency is expected regardless of the cleavage mechanism. This environmental responsiveness may prove to be important in novel effective gene therapy agents.¹³⁶ New innovations that utilize controlled architectures, click chemistry, and cyclodextrin core structures,

such as those reported by Reineke and coworkers, represent promising novel approaches to synthetic transfection reagents;^{137–140} PEGylation can help optimize biocompatibility while ideally maintaining effective transfection efficiency.

Although considerable success has been demonstrated with micellar drug delivery systems relative to conventional administration of small molecule drugs, the functional nature of polymer micelles opens additional opportunities for the preparation of highly sophisticated delivery systems by employing chemical, physical, or ionic cross-linking in the micellar core or corona. So long as these cross-linking processes do not alter the chemical structure of the drugs, they can in principle be very effective at tailoring drug delivery by imparting stability to the micelle, and fine-tuning uptake and release of guest molecules. Examples of micelles with cross-links in the corona are referred to as shell cross-linked micelles (SCMs), or shell cross-linked nanoparticles (termed SCKs, after the initially-coined term “shell cross-linked knedels”). Wooley and co-workers described the preparation and characterization of SCKs in 1996,¹⁴¹ and the concept has since been extended considerably to provide a suite of tailored nanoparticles for drug delivery,¹⁴² by the addition of biologically active moieties,^{143–147} imaging agents,^{148,149} stimuli-sensitive and degradable linkages,^{11,13,150,151} core excavation,¹⁶ and PEGylation.¹⁵² One recent detailed study compared PEGylated and non-PEGylated SCK’s with different hydrophobic cores, one consisting of the malleable poly(methyl acrylate), and the other composed of hard polystyrene. This SCK platform, shown in Fig. 11, was constructed from poly(acrylic acid)-*block*-poly(methylacrylate) (PAA-*b*-PMA) and poly(acrylic acid)-*block*-poly(styrene) (PAA-*b*-PS), with the hydrophilic PAA forming the corona in aqueous solution.¹⁵² Both larger and smaller diameter nanoparticles were assembled from each type of diblock copolymer by adjusting the PAA-to-PS block lengths, to give four SCKs: 24 nm particles from PAA₇₀-*b*-PMA₇₀, 37 nm particles from PAA₅₆-*b*-PMA₁₈₆, 18 nm

Fig. 11 SCKs prepared with poly(methyl acrylate) or poly(styrene) cores (highlighted in red), labeled with a covalently bound TETA group, and complexed with ^{64}Cu (highlighted in blue).

particles from $\text{PAA}_{56}\text{-}b\text{-PS}_{135}$, and 37 nm particles from $\text{PAA}_{101}\text{-}b\text{-PS}_{63}$. Cross-linking of the PAA shell, using diamines and carbodiimide coupling, consumed about half of the acrylic acid groups by amidation. Subsequently, about 5% of the acrylic acids were PEGylated by amidation with PEG-5000 amine. The biodistribution of these various SCKs was monitored *in vivo* by labeling the shell with a covalently attached TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid) group complexed with ^{64}Cu , allowing positron emission tomography (PET) tracking of nanoparticle uptake in biological tissue.

Interestingly, these studies revealed that both size and flexibility of the SCKs influences pharmacokinetic profiles, and that surface PEGylation was of lesser importance. The smaller nanoparticles with the styrenic core showed greater blood retention times (1.05% initial dose/g tissue at 24 h after injection) and lesser accumulation in the liver (4.20% initial dose/g tissue at 10 min after injection), attributed to the 18 nm hydrodynamic diameter and the retention of nanoparticle shape *in vivo*. For comparison, the smaller nanoparticles with the flexible acrylate cores showed lesser blood retention times (0.82% initial dose/g tissue at 24 h after injection) and greater liver accumulation (9.08% initial dose/g tissue at 10 min after injection). This study provides a good basis for the development of SCKs as drug delivery vehicles, and recent SCK efforts include those prepared from amphiphilic diblock copolymers, such as poly(methylacrylate)-*block*-poly(*N*-(acryloyloxy)succinimide-*co*-(*N*-acryloylmorpholine)) (PMA-*b*-P(NAS-*co*-NAM) diblock copolymers.¹⁵³ The NAS and NAM portion provides the copolymer with hydrophilicity, eliminates the need for protecting groups, and reduces surface charge density on the nanoparticle. SCKs were prepared from this polymer through crosslinking between primary diamines and *N*-hydroxysuccinimide (NHS) functional groups in the shell region of the particle.

Alternatively, micellar cross-linking can be performed in the core (to give core-crosslinked micelles, or CCMs). Early studies on such micelles in the 1970s^{154–158} have since been extended to many types of nanoparticles for drug delivery,

utilizing PEG as the basis of a hydrophilic shell.^{159,160} Di- and triblock copolymers of poly(ϵ -caprolactone) (PCL) and PEG assemble to give micelles in water, and can be cross-linked in the core by radical polymerization of the olefins that had been integrated into the hydrophobic core as chain-ends or interblock connectors.¹⁶¹ The cancer drug paclitaxel is of interest for micellar delivery, as it suffers from poor water solubility and deleterious side effects. Loading paclitaxel into PEG-block-PCL nanoparticles, followed by cross-linking by radical polymerization of double bonds throughout the PCL blocks, results in drug delivery systems with an average diameter under 200 nm.¹⁶¹ In the case of nanoparticles prepared from polymers with longer hydrophobic PCL chains, much higher drug loading capacities were achieved (4.3% paclitaxel for (PCL18K-*b*-mPEG5K)₂ compared to 0.2% paclitaxel for PCL1.2 K-*b*-mPEG2K). However, in order to tune the CCM for use as a drug delivery vehicle, both the size (less than 100 nm for effective penetration of endothelial cells) and paclitaxel loading capacity were considered. 100 nm nanoparticles built from mPEG5k-*b*-(PCL3.8k)₂-*b*-mPEG-5k triblock copolymers, loaded with 2.7% fluorescently labeled paclitaxel, were incubated with 3T3 mouse fibroblasts for 4 h, and fluorescent paclitaxel was observed by confocal microscopy to have crossed the cell membrane and entered the cytoplasm.¹⁶¹ This core cross-linked micellar system combines the advantages of a PEG-covered micelle, with the shape-stability offered by cross-linking, to give a novel and effective delivery vehicle.

Kabanov and coworkers have demonstrated the use of “block ionomers,” or block copolymers containing both ionic and nonionic blocks, and their use in solution-assembling core-cross linked micelles.¹⁶² Specifically, the core was composed of the polyanion polymethacrylate, with a surrounding shell of PEG chains. Crosslinking in the core was achieved through the use of 1,2-ethylene diamine, and the potential for drug encapsulation and subsequent release from the ionic core of the micelle was demonstrated with the anticancer drug cisplatin. This work represents another facet of PEGylated polymer micelles as efficient drug delivery vehicles, as in addition to controlling the size and crosslinking of the polymer

nanoparticles to control drug delivery vehicle properties, thermal responsiveness can be adjusted.¹⁶³

Summarizing remarks

Synthetic polymers provide vehicles for optimization of drug delivery of many varieties. The polymers provide tools to alter drug solubility and biodistribution, reduce side effects, and increase the efficacy of the treatment. While the chemical composition is important for biocompatibility and in some cases degradation, the polymer architecture is now known to be of considerable importance as well. Objectives that center on directing drug delivery vehicles to the disease are becoming more realistic through the use of polymers, and the application of novel synthetic polymers to medicine will continue to benefit from new synthetic methodology, and more efficient conjugation, encapsulation, and release chemistries. As the synthetic polymer materials and medical communities become increasingly connected, better exploitation of synthetic advances can be applied to the as yet unmet needs of patients.

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