Polymer Nanostructures Synthesized by Controlled Living Polymerization for Tumor-Targeted Drug Delivery

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Abstract

The development of drug delivery systems based on well-defined polymer nanostructures could lead to significant improvements in the treatment of cancer. The design of these therapeutic nanosystems must account for numerous systemic and circulation obstacles as well as the specific pathophysiology of the tumor. Nanoparticle size and surface charge must also be carefully selected in order to maintain long circulation times, allow tumor penetration, and avoid clearance by the reticuloendothelial system (RES). Targeting ligands such as vitamins, peptides, and antibodies can improve the accumulation of nanoparticle-based therapies in tumor tissue but must be optimized to allow for intratumoral penetration. In this review, we will highlight factors influencing the design of nanoparticle therapies as well as the development of modern controlled “living” polymerization techniques (e.g. ATRP, RAFT, ROMP) that are leading to the creation of sophisticated new polymer architectures with discrete spatially-defined functional modules. These innovative materials (e.g. star polymers, polymer brushes, macrocyclic polymers, and hyperbranched polymers) combine many of the desirable properties of traditional nanoparticle therapies while substantially reducing or eliminating the need for complex formulations.

1. Scope

In recent years, polymeric nanoparticles have joined established liposome technology (e.g. Doxil) as clinically approved anticancer drug delivery vehicles. The first approved formulation, Genexol-PM, a polyethylene glycol-*b*-poly(*D,L*-lactide) (PEG-PDLLA) micelle encapsulating paclitaxel, is available in Korea and is undergoing Phase II clinical trials in the US.[1] Several PEG-polypeptide micelle formulations are also in mid- to late-stage clinical trials for delivery of cisplatin, paclitaxel, and doxorubicin,[1] and a targeted micelle formulation for docetaxel delivery (BIND-014) is currently in Phase II trials. These aforementioned micelles are self-assembled structures that mitigate the toxicity profiles and improve solubility of highly hydrophobic drugs. However, ongoing challenges with such systems include the need for a formulation step during

manufacturing and incomplete control of drug release profiles. For example, studies with fluorescent dye-loaded PEG-PDLLA micelles showed that the micelles were destabilized and their cargo rapidly released as early as 15 minutes post-injection due to interactions with α - and β -globulins in the blood.[2] Other polymeric formulations, including linear polymer-drug conjugates such as CRLX101 (a camptothecin-cyclodextrin polymer conjugate), are progressing in clinical trials.[3-5]

Advances in controlled polymerization techniques have led to the development of new polymers with well-defined and controllable nanoscale size, morphology, and composition. These materials, called “polymer nanostructures” in this review, are well suited for drug delivery applications. Polymers with advanced architectures synthesized by controlled polymerization can approach the size of micellar systems without the need for self-assembly. Drugs and targeting ligands can be readily conjugated to the polymers via both reversible and stable bonds, respectively. Here, we review design guidelines for nano-sized, tumor drug delivery systems that have been defined by experimental reports from the literature. We then discuss controlled polymerization techniques and summarize examples of polymer nanostructures synthesized using these methods, emphasizing synthesis methods to generate materials meeting the aforementioned design guidelines. Finally, we highlight potential advantages of polymeric nanostructures as tumor drug delivery vehicles and present our thoughts on the future of this area.

2. Designing polymer carriers with desired pharmacokinetics and biodistribution for anticancer drug delivery.

The “enhanced permeability and retention” (EPR) effect, first reported by Maeda and colleagues in 1986, has been widely exploited for drug delivery to solid tumors.[6] The EPR effect results from the unique pathophysiology of the tumor, particularly the tumor vasculature, and comprises several factors (Figure 1).[7] First, tumor cells induce angiogenesis as a means of supplying oxygen and other nutrients to the growing tumor, leading to hypervascularization within tumors. However, this neovasculature is often abnormal in morphology or leaky, with large fenestrations in the endothelium that allow for increased permeation of macromolecules. In addition, solid tumors have impaired lymphatic drainage, leading to prolonged retention of macromolecules in the tumor tissue or tumor interstitium.[8]

Figure 1. Design parameters for drug delivery to solid tumors. Drug carriers in circulation (1) are passively targeted to the tumor site by the “enhanced permeability and retention” effect, which encompasses extravasation of the carrier into the tissue via leaky tumor vasculature (2) and prolonged residence due to defective lymphatic clearance. Diffusional barriers often prevent vehicles from penetrating into the tumor tissue (3). Carrier functionalization with “active” targeting ligands can facilitate uptake of drug carriers by cancer cells (4). Finally, release of drug cargo can occur intracellularly or in the extracellular space in response to stimuli such as pH or protease activity (5).

To use the EPR effect for preferential tumor delivery, the concentration of drug in plasma must remain high for >6 h.[7,9] This can be achieved with anticancer drugs or drug carriers with sizes above the renal clearance threshold (>40 kDa, or diameter >5 nm[10]). Another factor in prolonged drug circulation is avoidance of the reticuloendothelial system (RES, also known as the mononuclear phagocyte system, or MPS) in the liver and spleen. Larger particle sizes as well as greater surface charges (either positive or negative) are subject to increased surface opsonization, complement activation, and ultimately scavenging by Kupffer and liver endothelial cells, along with other phagocytic cells of the RES. In addition, cell membranes and blood vessel lumens are highly anionic; cationic drug formulations that interact strongly with these surfaces are therefore expected to have poor stability and short plasma half-lives.[9] A common strategy to reduce opsonization and increase circulation half-life is modification with poly(ethylene glycol) (PEG).[10-12] PEGs with molecular weights of 2,000 to 10,000 Da and at a high grafting density (such that the PEG chains adopt a “brush” conformation rather than a low density “mushroom” conformation) are typically used to prevent protein adsorption and prolong circulation.[13]

The first step in cancer drug delivery is extravasation of the drug or drug carrier out of the vasculature and into the tumor. At the lower bound, drugs should be >2-6 nm in diameter to avoid extravasation into normal tissue[14]. As mentioned previously, the leaky vasculature of the tumor can allow for the extravasation of large macromolecules (diameters up to 400 nm[15] or even >1 μm [16] in some models), although it should also be noted that the extent of this effect has been found to be highly heterogeneous both within a single tumor as well as between different tumor models and patients.[17-

19] In addition, this upper size limit must be balanced against the requirements for effective drug penetration into the tumor tissue.

After initial accumulation due to the leaky vasculature, large macromolecules can have prolonged residence time in tumor tissues. This results from the defective lymphatic drainage system within tumors and the difference in clearance rate between solid tumors and normal tissues.[8] Gradual accumulation of drug at the tumor results in a form of passive localized drug delivery. However, a key limitation in nanoparticle-based drug delivery to solid tumors is poor penetration into the tumor due to limited diffusion past cell-cell junctions and the extracellular matrix. This is an important consideration because many small molecule anticancer drugs exhibit limited tissue penetration, and the resulting drug concentration gradient likely plays a role in drug resistance and tumor recurrence.[20-22] Tumor penetration has been shown to be highly dependent on vehicle size. Vehicles smaller than ~50 nm in diameter are generally most effective for tumor penetration, while larger vehicles may be restricted to the perivascular space.[23-25] Vehicle charge is an additional consideration; neutral or slightly negatively charged particles may have improved penetration as compared to their slightly positive counterparts[26], due to the negative charge of the extracellular matrix.

While the EPR effect forms the basis of "passive targeting" to tumors, "active targeting" using various targeting moieties has also been widely investigated. Targeting ligands can include small molecules, peptides, proteins, or aptamers that bind specifically to receptors expressed (or overexpressed) on the cells or tissues of interest. Ligands that have been commonly used for anticancer targeting include the small molecule folate[27], the protein transferrin[28], and antibodies to tumor markers such as HER2[19].

Selection of an appropriate targeting ligand requires the consideration of several parameters:

- a. Binding affinity. The binding affinity between targeting ligand and receptor should be high enough to permit ligand recognition at therapeutically-relevant doses and in the possible presence of natural ligands, while being reversible enough so that tumor penetration can occur.[29] Studies with single-chain variable fragments (scFvs) of antibodies found that tumor retention required a minimum binding affinity (K_d) of $\sim 10^{-8}$ M but plateaued at affinities $>10^{-9}$ M, and that scFv with $\sim 10^{-11}$ M affinity was only able to penetrate 2-3 cell diameters into a solid tumor.[30,31] One strategy to enhance targeting by low affinity ligands is by using multivalent display of targeting ligands to increase avidity.
- b. Size. The size of the targeting ligand impacts the final size of the targeted construct as well as the ligand density that can be achieved. For example, full antibodies are ~10 nm, relatively large in the context of the requirements for tumor penetration (sub-50 nm). Antibody derivatives such as scFvs have reduced size while retaining antibody specificity. Small molecules and peptide ligands have minimal effect on overall vehicle size and can therefore be conjugated at greater densities.

- c. Ease of synthesis, modification, and conjugation. Small molecules can be chemically synthesized with varying functional groups and at relatively low cost. At the other extreme, proteins and antibodies require recombinant expression, which adds to cost, and have a limited range of site-specific conjugation chemistries and reaction conditions. Peptides and aptamers typically offer a compromise between small molecules and proteins in terms of manufacture cost, storage stability, and binding affinity.
- d. Receptor-mediated endocytosis. Targeting ligands can often mediate internalization of drug carriers into the target cells via receptor-mediated endocytosis. Effective internalization is dependent on nanoparticle size, shape, as well as the target receptor.

3. Considerations in integrating drugs with polymeric carriers

a. Drug loading

The drug loading capacity and efficiency of potential carriers are important parameters in determining clinical relevance. Low drug loading necessarily increases the amount of material required to achieve a therapeutic dose of drug. This problem is further exacerbated by the fact that delivery formulations are often less effective than free drug, due to incomplete drug release or inactivation of the drug. Methods for drug loading in polymer carriers vary based on the structure of the drug carrier and the physicochemical properties of the drug, such as size and solubility. The three most common methods rely on hydrophobic interactions, ionic attraction, and covalent conjugation.

Micelles, formed from assemblies of amphiphilic block copolymers or other polymers with hydrophobic domains, can be particularly useful as carriers of poorly water-soluble drugs. Hydrophobic drugs can be physically entrapped within the micelle core; drug loading capacity and efficiency depends on the miscibility of polymer and drug, as well as the length of the hydrophobic block.[32,33]

Polymer-drug conjugates based on hydrophilic polymers are another class of drug carriers. Drugs can be conjugated to the polymer termini (although loading is limited with this approach) or to pendant groups along the polymer backbone. Drug content is dependent on the molecular weight of the polymer itself and the number of functional groups incorporated for drug attachment. A high density of hydrophobic drugs can lead to polymer aggregation into micelles or other supramolecular assemblies[34]; this should be taken into account when designing polymer nanostructures, e.g. by shielding hydrophobic drugs within a polymeric brush.

b. Drug release

Ideally, a drug delivery vehicle should protect its payload during circulation and allow for drug release only upon reaching the target site. For delivery formulations such as polymeric micelles in which drug is physically but not covalently encapsulated, drug release can occur due either to premature vehicle disassembly or via passive diffusion, leading to drug leakage in circulation. The structural stability of micelles has been improved by introducing covalent crosslinks in the hydrophilic shell, hydrophobic core,

or core-shell interface.[35] Stimuli-responsiveness must then be introduced to allow for particle destabilization in response to pH, temperature, or reducing conditions. For example, polymeric micelles have been reported that destabilize under acidic pH (e.g. poly(*L*-histidine)-containing polymers) or increased temperature (e.g. poly(*N*-isopropylacrylamide) (pNIPAAm)-containing polymers).[36,37] In addition, micelles that are based on disulfide-containing polymers or that contain reducible crosslinks can exploit the highly reducing environment of the cytosol for triggered intracellular degradation.[38,39]

Covalent drug attachment can reduce drug leakage during circulation. However, drugs often have limited potency while attached to their polymeric carriers, so release of the active drug at the target site is necessary. Various conjugation chemistries have been employed for triggered drug release in tumor tissue or intracellularly. The pH-sensitive hydrazone linkage is often used because it hydrolyzes under acidic conditions.[40,41] This allows for intracellular drug release primarily in the late endosomes and lysosomes (pH ~5.5). Some extracellular release may also occur as the tumor microenvironment is known to be mildly acidic (pH ~6.8)[42], although hydrolysis of the hydrazone bond is slower at this pH. Enzyme-cleavable linkages are also attractive because *in vivo* degradation occurs in very specific environments. Efficient intracellular drug release has been demonstrated using peptide-based linkers that are substrates for the lysosomal protease cathepsin B; these linkers are stable in circulation but are rapidly cleaved following endocytosis.[43-45] As another example, matrix metalloproteinases (MMPs) are often overexpressed in tumors due to their role in extracellular remodeling and tumor progression[46]; consequently, MMP-sensitive peptide linkers have also been used for triggered drug release within the tumor microenvironment.[47]

4. Controlled Polymerization

Natural polymers such as albumin, chitosan, and heparin have been used in FDA-approved drug delivery formulations. Despite this precedence, the heterogeneity, cost, and difficulty of working with biopolymers has generated interest in developing synthetic polymers with enhanced drug delivery potential.[48] Historically, the preparation of monodisperse polymers with controlled, spatially-defined functional groups for drug delivery applications has been quite challenging. Conventional addition and chain growth polymerization techniques typically yield polymers with broad molecular weight distributions ($M_w/M_n \sim 2$)[49] and cannot be used to prepare advanced polymer architectures such as block copolymers and polymeric stars. In order to prepare these controlled architectures, it has been necessary to employ living ionic polymerization which requires stringent reaction conditions and is only accessible with a small number of relatively nonfunctional monomers. Common functional groups such as amines for complexing nucleic acids or hydroxyl groups for enhancing serum stability are not directly accessible by these techniques without laborious protection-deprotection steps.[50]

With the advent of controlled “living” polymerization methods such as atom transfer radical polymerization (ATRP), reversible addition-fragmentation chain-transfer (RAFT)

polymerization, and ring opening metathesis polymerization (ROMP), the preparation of synthetic polymers for drug delivery applications has been greatly simplified.[51,52] These methods are applicable to a wide range of functional monomers and can be conducted in most solvents, including water, using commercially available reagents. This unprecedented synthetic latitude is for the first time allowing for the preparation of water-soluble or amphiphilic architectures with precise dimensions and appropriate functionality for the attachment and targeted delivery of diagnostic and therapeutic agents. Pseudo-living techniques maintain most of the advantages of traditional living polymerizations while significantly extending the scope of functional architectures that can be prepared under relatively simple synthetic conditions. These methods can be roughly divided into controlled radical polymerization (e.g. ATRP and RAFT) and metathesis polymerization (e.g. ROMP and REMP) (Figure 2).

Figure 2. Schematic representation of controlled polymerization methods that have been widely employed to prepare sophisticated polymer architectures for drug delivery.

Controlled radical polymerization (CRP) methodologies are rapidly moving to the forefront in construction of drug and gene delivery vehicles. ATRP, first reported by Matyjaszewski and coworkers in 1995, is an extremely versatile technique for preparing sophisticated polymer nanostructures with dense multivalent polymer structures.[53] In ATRP, the active-dormant equilibrium is established between non-propagating alkyl halide initiators (R-X) and radicals that are produced by the homolytic cleavage of the R-X bond. This cleavage is accomplished with the use of a redox-active transition metal complex, which is raised to a higher oxidation state with the transfer of a coordinated (pseudo) halide ligand. The transition metal catalyst is generally copper but other metals have also been reported.[54] The resultant radicals ($P_n\cdot$) can then react with monomer (M) to produce polymeric radicals ($P_n\cdot$) before undergoing deactivation with the higher

oxidation state metal halide complex regenerating the original catalyst and reversibly terminating the growing polymer chain. Because ATRP is a radical process, the activation-deactivation equilibrium must be shifted to yield predominately dormant chains in order to minimize radical termination events. This equilibrium is determined by a number of factors including the solvent polarity, ligand composition, temperature, solvent polarity, alkyl halide, and catalyst. Especially careful control is required when conducting ATRP in polar solvents such as water where excessive radical formation can lead to a loss of “livingness”.

Another CRP technique that has been widely employed to prepare sophisticated polymer nanostructures for drug delivery applications is RAFT. This technique was first reported by Commonwealth Scientific and Industrial Research Organization (CSIRO) in 1998.[55] Since that time RAFT has become one of the premier methods to prepare sophisticated polymers for biotechnology applications because of the elegant simplicity of this technique, broad chemical compatibility, and ease of use. RAFT employs a thiocarbonyl thio compound as a degenerate chain transfer agent (CTA), which is most commonly a dithioester or trithiocarbonate.[55] By simple manipulation of the initial monomer, CTA, and radical initiator stoichiometry, it is possible to prepare near monodisperse materials over a range of predefined molecular weights. These agents contain the general structure shown in Figure 2, where R is a radical initiating group and Z stabilizes the thiocarbonyl towards radical addition. Through a series of chain transfer steps, polymerizations proceed in a controlled process with most polymer chains containing R and Z groups at their α and ω chain termini, respectively. Following polymerization of a given monomer or monomers, the resultant macroCTA can be isolated for use in subsequent block (co)polymerization steps. Because this methodology does not require the use of any metal catalysts, it is particularly well suited for use in biotechnology applications. RAFT polymerizations are also well-controlled in water, assuming appropriate pH conditions are maintained, making them ideal for many bioconjugation applications.[56,57]

Similar to ATRP and RAFT, ring-opening metathesis polymerization (ROMP) is a chain growth polymerization process that can be used to yield well-controlled polymers with narrow molecular weight distributions.[58] In ROMP, cyclic olefins are converted to polymers via a metal-mediated double bond exchange process. Unlike radical initiated polymerizations, where the thermodynamic driving force for propagation is the conversion of double to single bonds, the driving force in ROMP is the relief of ring strain and associated steric considerations. The mechanism of ROMP is based on the formation of a transition metal alkylidene complex that undergoes [2+2] cycloaddition with cyclic olefins to yield a four membered metallocyclobutane intermediate. The resultant intermediate then undergoes a cycloreversion reaction to yield a new alkylidene of larger molecular weight because of the previous monomer addition. This alkylidene can then undergo subsequent propagation reactions through an analogous series of addition and cycloreversion reactions. Cyclic olefin monomers commonly employed in ROMP usually possess considerable ring strain and include cyclobutenes, cyclopentenenes, cis-cyclooctenes, and norbornenes. A variety of ROMP catalysts have been developed using range of metals but the most commonly employed catalysts are the ruthenium-based 1st and 2nd generation Grubbs catalysts.[59] These catalysts are

highly active and relatively tolerant of protic impurities. A variation of ROMP is ring expansion metathesis polymerization (REMP), which can be employed to prepare macrocyclic polymers. In REMP, the catalyst contains a short tether that forms a cyclic structure with the alkylidene. Ring opening reactions with the cyclic catalyst leads to the formation of larger macrocyclic structures.

5. Polymer architectures for drug delivery

Controlled polymerization can be used to synthesize sophisticated new polymeric materials that retain the desirable properties of traditional nanoparticle therapies (e.g improving drug solubility and increasing therapeutic indices) while substantially reducing or eliminating the need for complex formulations. This section summarizes the impact of controlled polymerization techniques on the synthesis of advanced polymeric architectures: star polymers, polymer brushes, macrocyclic polymers, and hyperbranched polymers (Figure 3). The synthetic advantages and disadvantages of these sophisticated polymer architectures are outlined in Table 1.

Figure 3. Schematic representation of advanced macromolecular architectures that are being investigated for drug delivery.

Table 1. Summary of the currently-known advantages and disadvantages of sophisticated macromolecular architectures

Macromolecular architecture	Advantages	Disadvantages
Star polymer	<ul style="list-style-type: none"> – Can be prepared in a single step with good control over segment length and number. 	<ul style="list-style-type: none"> – Multifunctional initiators/CTAs must be synthesized. – Limited number of arms possible before star-star coupling becomes significant. – Molecular weight is limited by the small number of polymeric arms.
Polymeric brush	<ul style="list-style-type: none"> – High number of arms possible. – Discrete macromolecular segments can be precisely positioned. – High molecular weights possible. 	<ul style="list-style-type: none"> – Brush-brush coupling can be significant. – Multistep synthesis is required.
Macrocyclic polymer	<ul style="list-style-type: none"> – Unique physiochemical properties may provide enhanced biological activity. – Smaller hydrodynamic size can enhance tumor penetration. 	<ul style="list-style-type: none"> – Synthesis via ring closing methods generally requires dilute conditions/slow addition during ring closing. – Ring expansion methods are limited in their ability to control the final molecular weight and dispersity.
Hyperbranched polymer	<ul style="list-style-type: none"> – Synthesis straightforward from readily available precursors. – Can incorporate a large number of hydrolytically degradable linkages at branch points to facilitate <i>in vivo</i> clearance. 	<ul style="list-style-type: none"> – Materials usually have large molar mass distributions. – The final degree of branching is dependent on monomer conversion.
Linear polymer	<ul style="list-style-type: none"> – Readily synthesized from diverse commercially available monomers. – Excellent molecular weight control under facile synthetic conditions. 	<ul style="list-style-type: none"> – Condensation polymers have limited molecular weights and high molar mass dispersities. – Polymers prepared from vinyl polymerizations cannot be degraded <i>in vivo</i>, limiting their molecular weight.

a. Star polymers

Star polymers have been investigated for biomedical and pharmaceutical applications because of their well-defined globular structures, low solution viscosities, and core-shell architectures.[60] Star polymers consist of at least three linear macromolecular segments radiating from a single central core. Based on this definition, star polymers can further be divided into homo-arm stars, where the radiating arms have identical chemical composition and similar molecular weights, and mikto-arm stars, where the arms are composed of two or more arm species with different chemical compositions and/or molecular weights.[61] Early examples of star polymers for drug delivery were largely based on polyethylene glycol and poly(L-lactide).[62,63] Typically these polymers were made using anionic ring opening polymerization.[64]

The controlled synthesis of star polymers was greatly simplified by the introduction of controlled radical polymerization techniques. Star polymers are generally prepared using one of three synthetic strategies: the core-first approach, the coupling-onto approach, and the arm-first approach.[65] In the core-first approach, a multifunctional ATRP initiator or chain transfer agent is attached to a central scaffold from which polymerization of the arms proceed. Liu and coworkers employed this approach to synthesize 40-arm star polymers for cancer targeted drug delivery and MR imaging applications.[66] In these studies, the authors employed hydroxyl-functionalized fourth-generation hyperbranched polyester to grow polycaprolactone (PCL) arms to which ATRP initiators were grafted. Following copolymerization of PEGMA and azidopropyl methacrylate from the polyfunctional initiator, azide-alkyne click chemistry was employed to graft both gadolinium-based contrast agents and folate targeting ligands.

Synthesis of star polymers by RAFT via the core-first approach can be accomplished by covalently conjugating multiple CTAs to a central core via either the R or Z group. The R group strategy is advantageous in that the growing polymer chains propagate outwards away from the central core, reducing steric hindrance and increasing accessibility of the thiocarbonyl thio groups to growing polymeric radicals. However, the R group approach can show increased levels of radical-radical coupling because of the close proximity of growing polymeric radicals. This coupling is minimized in the Z group approach, where growing polymer chains propagate in solution away from the central scaffold. Thang and coworkers have employed the R group first method to prepare a series of 4-arm star polymers from a biodegradable core.[67] Vana and Boschmann used the Z group approach to prepare poly(vinyl acetate) (PVA) and poly(vinyl propionate) (PVP) star polymers with low protein adsorption, excellent biocompatibility, and high water solubility.[68]

In contrast to the core-first approach, star polymers can also be synthesized using the arm-first crosslinking method. In this approach, linear copolymers with “living” chain ends are linked together by conducting a subsequent copolymerization in the presence of divinyl monomers to form a crosslinked core.[69,70] Davis et al. have employed this approach to prepare theranostic star polymers containing conjugated doxorubicin (Dox) as well as gadolinium-based MRI contrast agents.[70] In these studies, the resultant nanoparticles were shown to have excellent serum stability, IC₅₀ values similar to the free drug, and highly effective MRI contrast capability.

b. Polymer brushes.

Cylindrical polymer brushes generally consist of a linear macromolecular scaffold from which polymeric segments radiate. Because of the large amount of steric repulsion between the polymeric arms, the scaffold adopts an elongated anisotropic conformation.[71] This unique conformation, which has a large directional persistence length similar to DNA, may provide substantially enhanced *in vivo* circulation times and cellular uptake.[72] Brush copolymers are prepared using some of the same general approaches as star polymers (i.e. core-first, arm-first, and coupling-onto); however, the large number of arm segments can lead to high levels of brush-brush coupling when using radical-based polymerization techniques. For this reason, particularly low

instantaneous radical concentrations in combination with limiting the overall monomer conversion are commonly employed to mitigate termination. Brush copolymers prepared from fast propagating monomers generally show lower dispersity than slower propagating monomers.

ATRP is well-suited for preparing dense polymer brushes via the core-first approach because macromolecular scaffolds can be easily functionalized using commercially available α -haloesters or benzyl halides, and because the instantaneous radical concentration can be carefully controlled to minimize brush-brush coupling.[73] For example, Caruso et al. employed a poly(hydroxyethyl methacrylate) (pHEMA) scaffold as the starting point for the synthesis of a library of cylindrical polymer brushes (CPBs) for *in vivo* evaluation.[74] The resultant hydroxyl-functionalized scaffolds were then modified with ATRP initiators from which copolymerizations of PEGMA and glycidyl methacrylate were conducted. While the syntheses of brush copolymers via CRP have predominately employed ATRP, examples using RAFT have been recently reported.[75] In this example, the macromolecular scaffold was prepared by RAFT copolymerization of HEMA and polyethylene glycol monomethyl ether methacrylate macromonomer (Figure 4). Polyfunctional graft chain transfer agents (gCTAs) were subsequently synthesized from these copolymers and used to generate brush copolymers with potent endosomal-release activity due to their ability to spontaneously assemble/disassemble in response to pH.

Figure 4. (a) Schematic representation of polymeric brushes synthesized by RAFT and (b) cryoTEM image supporting the pH-induced assembly of these materials into polymersomes. These materials are composed of a methacrylate scaffold from which both hydrophilic poly(ethylene glycol) and endosomolytic poly(DEAEMA_{co}BMA) (EB) segments radiate. At physiological pH conditions, the hydrophobic (EB) segments induce self-assembly of these materials into polymersomes capable of encapsulating hydrophilic drugs. Subsequent internalization of the polymersomes into acidic intracellular compartments induces disassembly and disruption of the endosomal membrane. Reproduced from Ref. [75] with permission from Polymer Chemistry and The Royal Society of Chemistry.

ROMP has been widely employed to prepare brush copolymers because of its ability to polymerize large macromonomers with controllable molecular weights and low molar mass dispersities.[76,77] Examples of brushes prepared by ROMP for drug delivery have been reported by Johnson and coworkers.[76,77] In these studies, the authors polymerized a norbornene-terminated macromonomer followed by *in situ* cross-linking with a bis-norbornene derivative. Dox was incorporated into the hydrolytically degradable nanoparticles using a Dox-based branched macromonomer that has been shown to undergo enzymatic hydrolysis followed by rapid 1,6-elimination.[76,77]

The grafting onto approach has also been employed to prepare brush copolymers for drug delivery applications.[78] In one example, RAFT was employed to synthesize poly(propylacrylic acid) (PPAA), a pH-responsive polymer with potent membrane destabilizing activity, with a telechelic alkyne functionality. The linear PPAA chains were then grafted onto poly(azidopropyl methacrylate) (PAPMA) scaffolds using copper catalyzed azide-alkyne click chemistry, yielding a brush polymer with ~10 times the molecular weight of each linear chain. *In vivo* studies with the PPAA brushes showed significantly enhanced accumulation in tumor tissue relative to linear PPAA and PEG controls (% injected dose/g = 0.79 ± 0.12 for brush PPAA vs. 0.28 ± 0.8 for linear PPAA).

c. Macrocyclic polymers

The development of drug delivery systems based on macrocyclic polymers has recently become an area of active research because of their unique macromolecular topologies which include a lack of chain ends, smaller hydrodynamic diameters, and lower radii of gyration compared to their linear analogs. Macrocyclic polymers have been shown to have increased circulation time *in vivo* compared to their linear analogs.[79] Here, enhancements in both the elimination half-life ($t_{1/2,\beta} = 20.9 \text{ h} \pm 4.4 \text{ h}$ vs. $14.7 \text{ h} \pm 1.5 \text{ h}$) and AUC (587.1 ± 13.6 vs. 218.6 ± 1.0) were observed for the cyclic polymer relative to the linear control at comparable molecular weights. The increased circulation time also leads to high tumor accumulation of cyclic polymer compared with linear polymers.[80] The synthesis of macrocyclic polymers can be achieved by the ring closure (RC) or ring expansion (RE) methods.

In the RC method, linear polymers containing telechelic functional groups undergo intramolecular coupling to form the macrocyclic polymers.[81] A major advantage of this strategy is that the backbone with telechelic functional groups can be prepared from a wide panel of commercially available functional monomers via CRP using, for example, a functional chain transfer agent (CTA) or ATRP initiator. Following polymerization, the chain end is then modified to introduce the appropriate functional group at the ω chain terminus for end-to-end cyclization. Despite these advantages, the RC method is limited by the need for dilute cyclization conditions and/or the need for preparative size exclusion chromatography to purify the final cyclic polymers.

Ring-expansion metathesis polymerization (REMP), which was first reported by Grubbs and coworkers in 2002, provides an attractive alternative to ring closing methods for producing cyclic polymers.[82] In this technique, initiation occurs via insertion of cyclic

olefin monomers into a Ru-alkyl-indene bond, expanding the cyclic catalyst ring. A significant advantage of REMP relative to ring closing techniques is that it can be used to prepare large quantities of pure cyclic polymer with high molecular weights even at high polymer concentrations. Additionally, REMP is amenable to a wide range of commercially available cyclic olefins with a range of useful functionalities. This technique can show some broadening of the molar mass dispersity as a result of competing intramolecular chain transfer reactions.[83]

d. Hyperbranched polymers

Dendrimers and dendrimer-like polymers have attracted considerable interest as potential platforms for drug delivery. These materials have a high degree of branching and can be designed to incorporate a large number of functional groups at spatially-defined positions within the globular structure. Dendritic materials can also be designed to be hydrolytically or enzymatically degradable by incorporating degradable linkages at branch junctions. Traditionally, dendrimers have been prepared via a stepwise synthesis from a central core to yield materials with a highly regular branching pattern, low molar mass dispersities, and controllable functional group density.[84]. The high cost and limited selection of commercially available dendrimers has led to the development of hyperbranched polymers with many of the same properties as dendrimers but with simpler preparation methods.[85]

Hyperbranched polymers can conveniently be prepared via the use of initiator-monomers (inimers). Polymers synthesized via this method have larger molar mass dispersities; however, their constituent segments are still somewhat controlled.[86] Examples of inimers include hydroxyl-substituted lactones, acrylic-substituted ATRP initiators, and acrylic-substituted RAFT agents. The latter two inimers are conveniently prepared by the esterification of the acid halide and carboxylic acid groups present on many ATRP initiators and RAFT CTAs, respectively. These agents can then be incorporated into CRPs where they serve as branch points along the growing polymer chains. The average degree of branching/crosslinking can then be controlled by simple manipulation of the inimer to monomer ratio and overall monomer conversion. For example, inimers have been employed to prepare architecturally distinct antigen carriers with pH-responsive endosomal-releasing segments.[87] In these studies, dendritically branched copolymers were synthesized using a methacrylate-functionalized RAFT CTA. Antigen delivery with the hyperbranched and cross-linked polymer architecture enhanced *in vitro* MHC-I antigen presentation relative to free antigen, whereas the linear construct did not have a discernible effect.

6. Potential advantages of advanced polymer architectures

a. Formulation-free polymeric nanoparticles

The clinical translation of nanoparticle-based therapies is challenging because of the three-dimensional structure and multicomponent nature of these systems.[88] The *in vivo* properties of nanoparticles depend significantly on a number of critical physiochemical properties including size, size distribution, surface charge, and particle morphology. The identification of analytical tests to fully characterize these systems with respect to both their physiochemical and biological properties is a significant challenge

from both a technical and regulatory perspective. Additionally, the pharmaceutical industry currently lacks a robust set of *in vivo* assays to accurately predict the circulation and therapeutic properties of these complex systems, necessitating the use of extensive preclinical animal studies. These problems are compounded for systems that are designed to be prepared at the "bedside," where the reproducibility of the self-assembly process by individual clinicians is questionable.[88]

In order to overcome the complex formulation and reversible nature of self-assembled polymeric drug carriers, a number of innovative approaches have recently been employed to prepare polymers for tumor delivery with nanoscale dimensions similar to micelles but with precisely defined architectures. For example, Gong and coworkers employed ATRP to prepare targeted, theranostic polymer brushes containing a HEMA backbone with PLGA arms prepared by a combination of ATRP (backbone) and ring opening polymerization (arms).[89] A monoclonal antibody against CD105 (TRC105) was conjugated to the resultant PLGA arms. The resultant brushes showed significantly higher cellular uptake in CD105-positive (human umbilical vein endothelial cells) relative to CD105-negative control cells (MCF-7 human breast cancer). Furthermore, positron emission tomography (PET) studies using 4T1 murine breast tumor-bearing mice showed significantly higher uptake of the antibody-targeted ^{64}Cu -labeled polymer brushes relative to the untargeted controls.

Although high molecular weight polymers typically show enhanced *in vivo* circulation times and are relatively simple to synthesize, these materials can present clearance problems. In order to circumvent this limitation, we recently reported the synthesis of degradable 2nd generation brushed-brushes with narrow dispersities and controllable macromolecular architectures (Figure 5).[90] Here, hydroxyl-functionalized scaffolds were prepared by RAFT copolymerization of *N,N*-dimethylacrylamide (DMA) and hydroxyethylacrylamide (HEAm), followed by grafting of functional CTAs to scaffolds using carbodiimide chemistry to yield a series of graft chain transfer agents (gCTAs) with different numbers of linked CTAs. The gCTAs were then employed to prepare 1st generation brushes with poly(DMA-*co*-HEAm) arms. A second round of CTA grafting reactions and RAFT polymerization yielded 2nd generation gCTAs, which were then used to prepare the final 2nd generation brushed-brushes. The resultant materials have molecular weights in excess of 1 million g/mol with low molar mass dispersities.

Figure 5. (a) Schematic representation of 2nd generation brushed-brushes prepared via RAFT polymerization. (b) GPC chromatograms supporting the successful synthesis of 2nd generation brushed-brushes. The macroCTA shows weak absorbance at 310 nm because of the single trithiocarbonate functionality at the chain end. Grafting of additional RAFT CTAs to the poly(DMA_{co}HEAm) scaffold significantly enhances the UV absorbance at 310 nm. Following copolymerization of DMA and HEAm a significant shift in elution time is observed supporting the formation of polymeric brushes. The resultant polymer brush was then subjected to a second round of CTA grafting followed by copolymerization of DMA and HEAm to yield well-defined 2nd generation brushed-brushes. Reproduced from Ref. [90] with permission from Polymer Chemistry and The Royal Society of Chemistry.

While the 2nd generation brushed-brushes had hydrodynamic diameters similar to micelles (30-35 nm), these materials were molecularly soluble and thus not susceptible to premature disassembly in circulation. Additionally, the CTA groups, from which the polymeric arms were grown, were tethered with ester linkages, providing a potential route by which the high molecular weight single polymer nanoparticles could be degraded *in vivo* to polymers with molecular weights around 10 kDa. Carbazate and biotin groups for conjugation of Dox and streptavidin-antibody moieties, respectively, were introduced directly through copolymerization of DMA with pre-functionalized methacrylate monomers. This approach provided a modular route for preparing polymeric drug conjugates with a range of antibodies specifically tailored to the target tissue. Enhanced release of the Dox from hydrazone linkages under acidic conditions was confirmed via drug release studies, and chemotherapeutic activity was established in SKOV3 ovarian cancer cells.[90]

b. Polymers with finely controlled nanoscale dimensions

As discussed in section 2, systemically administered drug vehicles for solid tumor delivery require properties that facilitate long circulation, tumor extravasation, tumor penetration, and controlled drug release. Advanced polymer architectures synthesized by controlled polymerization allow for high control over particle size (by degree of polymerization), particle charge (by selection of monomers), and triggers for drug release (by linker chemistry).

For example, macrocyclic “sunflower” polymers incorporating folate ligands for tumor targeting and linkers for drug conjugation have been developed using controlled radical polymerization.[91] These materials consist of a macrocyclic “core” from which polymeric “petals” radiate. This architecture combines the structural complexity of dendrimers with the ease of synthesis and scalability of conventional polymers. The size of the resulting sunflower polymers depends on the degree of polymerization of the polymer “petals.” Interestingly, receptor-mediated delivery to cultured cells was demonstrated using sunflowers with folate conjugated to petal termini but not with analogous folate-conjugated comb polymers.[91]

Macrocyclic polymers with precisely defined nanoscale dimensions with potential drug delivery applications have also been prepared via REMP.[92] In these studies, the authors first prepared high molecular weight macrocyclic polymers with pendant hydroxyl groups via polymerization of exo-norbornene-based functional monomers. The resultant hydroxyl-functional scaffolds were then used as initiators for the triazabicyclodecene-catalyzed ring-opening polymerization of cyclic ester monomers. Three biodegradable cyclic ester monomers were selected to demonstrate this method including δ -valerolactone (VL), L-lactide (LLA), and ϵ -caprolactone (CL).

7. Looking ahead

Recent developments in polymer synthesis as well as various highly efficient “click” reactions have enabled the development of increasingly sophisticated polymeric architectures including macrocyclic polymers, dendronized polymers, polymeric brushes, block copolymers, star polymers, and polymeric neo-glycans. [49,93-96] These systems can now be easily synthesized with defined chemical compositions, low molar mass dispersities, engineered three-dimensional architectures, and discrete spatially defined functional segments. Additionally, the high valency of these systems allows for the creation of drug conjugates with high drug carrying capacities (relative to encapsulating systems) without the need for complex formulations. Reversible addition-fragmentation chain transfer (RAFT)[97] polymerization and atom transfer radical polymerization (ATRP)[52] in particular have allowed previously unattainable polymeric architectures to be prepared under facile synthetic conditions using a diverse panel of commercially available monomers. This unprecedented synthetic latitude is for the first time allowing for the preparation of water-soluble or amphiphilic architectures with precise dimensions and appropriate functionality for attachment and targeted delivery of diagnostic and therapeutic agents.[51] Application of these materials for drug delivery is in its nascent stage, but we anticipate rapid growth in translation of these polymeric nanostructures for tumor targeted drug delivery and imaging.

Another growth area is in precision polymeric materials with sequence control.[98,99] While the development of powerful controlled polymerization techniques such as ATRP, RAFT, and ROMP have greatly simplified the process of creating sophisticated polymer architectures for drug delivery applications, they are not currently able to create materials with precisely defined sequences. In contrast, the macromolecules of biology (e.g. DNA, RNA, and proteins) are composed of a large number of chemically distinct

monomers that are precisely placed in a predefined sequence throughout the polymer backbone. These organizations allow for complex structural and functional interactions between biomacromolecules. The most common method for preparing sequence-defined polymers involves the stepwise addition of single monomer units to a solid support. While these approaches can be used to efficiently synthesize polymers with defined sequences, they require a large number of synthetic steps with expensive reagents as well as extensive purification. Recently, significant progress has been made in the application of RAFT to prepare sequence-defined multiblock copolymers that could one day be extended to give full monomeric control of the polymer sequence.[99]

Current synthetic methods are also limited in their ability to prepare polymers with degradable backbones. This shortcoming limits the molecular weight of polymers that can be employed in drug delivery application. The development of sequence-defined polymers could be combined with materials prepared by CRP to overcome this limitation by engineering biologically degradable linkages into synthetic polymer backbones. Thus, we expect that in the near future, sequence and degradation control will also be added to the current suite of controllable polymer nanostructure properties. This will then enable more precise drug delivery and tuning of drug pharmacokinetics using polymeric delivery systems.

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Polymeric
Star

Polymeric
Brush

Macrocyclic
Polymer

Macrocyclic
Brush

Hyperbranched
Polymer