Nanopolymeric Therapeutics

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Abstract

Polymers have been extensively utilized in the design of nanometer-sized delivery vehicles of chemotherapeutics for clinical cancer therapy. Polymeric nanoparticulate delivery vehicles, with chemotherapeutics being either conjugated or encapsulated, have been developed into a variety of different architectures, including polymer-drug conjugates with linear or branched polymers, micelles, and polymersomes. This review describes the progress that has been made in the field of polymeric nanomedicine that brings the science closer to clinical realization of nanopolymeric therapeutics for its application in cancer treatment.

Polymeric Nanomedicines in Cancer Therapy

Nanomedicines, a class of nanometer-sized therapeutic or diagnostic modalities (1 to sub-100 nm), have attracted much attention for their potential in clinical cancer treatment.[1] Although the term nanomedicine has appeared only in the last few years,[2–7] the practice of applying nanotechnology to cancer treatment dates back to the 1970s.[4] Polymeric nanomedicine, an emerging subfield of nanomedicine that involves the use of polymeric nanostructures that contain therapeutic and imaging modalities for the treatment and diagnosis of cancer, respectively, is anticipated to provide unprecedented precision and efficacy in cancer therapy[8] and eventually will alter the landscape of oncology.[9–11] Through numerous efforts, a handful of polymeric nanoparticulate modalities have been developed and evaluated in various preclinical[7,12–24] or clinical studies,[25–31] some of which have been approved for clinical cancer treatment.[1]

Development of Polymeric Nanomedicines Through a Conjugation and an Encapsulation Approach

Drug molecules can be either released through the cleavage of covalent linkages that connect drug molecules to polymers (the conjugation approach) or through the diffusion of drug molecules from polymer matrices (the encapsulation approach). Ringsdorf first introduced the covalent conjugation approach in 1975.[32] In his postulated model of a polymer-drug conjugate, multiple drug molecules are bound to polymer side chains through covalent but cleavable bonds. The cleavage of the polymer-drug linkers results in the release of attached drug molecules. This concept, once conceived and reported, received immediate attention. Numerous polymer-drug conjugates have been developed and evaluated preclinically and clinically.[33–36] A few systems have been approved for clinical cancer treatment.[37] The concept of the physical encapsulation approach controlling drug release from a polymer matrix originated from the seminal work by Folkman and Long in 1964.[38] They reported that hydrophobic small molecules could diffuse through the wall of silicone tubing at a controlled rate. Later, this concept was incorporated into the design of a polymer-based controlled-release system[39] to the development of Gliadel,[40] an FDA-approved, implantable wafer that can slowly release 1,3-bis(2-chloroethyl)-1-nitrosourea for treating malignant glioma. The physical encapsulation approach has been applied to the development of a variety of nanometer-sized delivery vehicles, many of which are based on the aggregation of hydrophobic polymers or the self-assembly of hydrophobic polymer segments of amphiphilic block-copolymers (copolymers containing both hydrophobic and hydrophilic polymer segments) to form ordered nanostructures with the drug encapsulated (e.g., polymeric micelles,[41,42] polymersomes[43–45]).

Hydrophobicity and hydrophilicity play important roles in drug delivery. By definition, hydrophobicity is the property of being water repellent; hydrophilicity is the tendency of a molecule to be solvated by water. Hydrophobic molecules/polymer segments prefer to stay with molecules/polymer segments with like hydrophobicity or hydrophilicity. These unique properties are crucial in drug delivery. For example, when an amphiphilic diblock copolymer is placed in water, the hydrophobic segments will phase separate from water and agglomerate to form an aggregated solid core, while the hydrophilic segments remain in the water phase and form a shell covering the hydrophobic core. This process is called micellation. During micellation, hydrophobic drug molecules can be encapsulated in the hydrophobic micelle cores; this process is called drug encapsulation.

The majority of the nanotechnology platforms for chemotherapy have involved repackaging traditional anticancer agents into various forms of nanometer-sized delivery vehicles previously mentioned. The development of the first-generation anticancer therapeutic nanomedicine has been focused on the formulation of delivery vehicles using well-developed biomaterials and formulation methodologies (conjugation, micellation, or encapsulation) and on targeting and treating of primary tumors based mainly on the enhanced permeation and retention (EPR) effect, which refers to the accumulation of nanoparticles in a tumor facilitated by the highly permeable nature of tumor vasculatures and poor lymphatic drainage of the interstitial fluid surrounding the tumor.[46] Some of these efforts will be highlighted first. We also will review papers published in the last three to five years on the development of promising new delivery technologies that are related to the design of next-generation nanomedicine for cancer therapy, including new types of delivery vehicles (e.g., polymersomes,[43,47] worm-like micelles,[19] nanoconjugates[48]) and new chemistries and fabrication technologies[6] that allow unprecedented, precisely controlled nanomedicine formulation to make it possible to evaluate nanomedicine with the

variation of one parameter (e.g., size, surface property, shape) at a time to provide insight into the fundamental understanding of the interplay of these parameters *in vitro* and *in vivo*.

Polymer Drug Conjugates

Conjugation of hydrophobic small molecule drugs to hydrophilic polymers has been actively pursued for improved pharmacological and pharmacokinetic properties of the therapeutic molecules. In general, therapeutic agents conjugated to polymers through cleavable bonds have increased aqueous solubility, reduced toxicity, and prolonged the plasma circulation half-life compared to free drugs from minutes/hours to tens of hours or even days. Polymer drug conjugation also may change the internalization pathway of small molecules by bypassing multidrug resistance.[49] Polymers that are particularly important and have track records of preclinical success for small molecule conjugation include a poly(ethylene glycol) (PEG),[50] N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer,[51–53] poly(glutamate),[54,55] dextran,[56–58] and a cyclodextrin-based polymer.[59–62] Conjugates of various anticancer drugs with these polymers are currently in clinical trials.[35] Other polymers that have been successfully developed include polymannopyranose[63] and albumin.[64]

PEG has been used for the conjugation and delivery of therapeutic drugs paclitaxel,[65] doxorubicin (DOX),[20] and camptothecin.[66] Conjugation of PEG to these hydrophobic drugs dramatically increased their solubility and improved their retention in circulation.[20] Enhanced antitumor efficacy in various preclinical studies[67,68] and clinical studies[69] was observed. Protracted antitumor activities observed with the use of PEG were attributed to the prolonged circulation and improved tumor accumulation due to the EPR effect.[46] One drawback of linear PEG is that it only has two terminal hydroxyl groups for the conjugation of drugs, which limits its drug-carrying capacity.[70] One strategy to increase drug loading is to use multiarm or branched PEG that has more functional groups for drug conjugation.[70] Synthesis of PEG with various functional groups is well-established. PEG, in general, can be produced in large quantities with extremely low polydispersity (distribution of the polymer molecular mass). A handful of PEG-protein conjugates have been approved for clinical use.

HPMA copolymers are another class of polymeric materials that have been extensively investigated as a drug carrier in the form of polymer-drug conjugates for drug delivery applications. They are water-soluble, biocompatible, and nondegradable, which resembles PEG to some extent.[33,71] HPMA drug conjugates evaluated clinically include HPMA-paclitaxel,[72] HPMA-camptothecin,[73] HPMA-doxorubicin,[26] and HPMA-platinate.[74] To ensure complete clearance of HPMA, most HPMA copolymers tested *in vivo* had molecular weights 30 kDa or lower. This molecular weight reflected a balance between using as high as possible a molecular weight while ensuring that it can be cleared by the body (less than 50 kDa).[26] Compared to PEG, HPMA has a larger number of pendent hydroxyl groups that allow the conjugation of many hydrophobic small molecules on each HPMA polymer chain. The drug loading capacity of HPMA is thus much higher than that of linear PEG.

Poly(glutamate)s represent a class of anionic polymers for small molecule drug delivery. They have a large number of pendant carboxylate groups, which make poly(glutamate)s very water soluble and have high loading capacities. As much as 30 wt% of paclitaxel[16,75] or camptothecin[54] can be conjugated to poly(glutamate) carboxylate side chains, which is much higher than the drug loading typically observed in PEG or other polymer conjugates (typically around 2–10%). Paclitaxel and camptothecin conjugated to poly(glutamate) showed enhanced preclinical antitumor efficacy in several preclinical tumor models presumably due to the EPR-mediated passive tumor targeting.[55,76] Positive responses in patients who are resistant to paclitaxel, an anticancer agent, were observed in several clinical studies. Taxane-resistant patients (patients who do not respond to the use of taxane) were observed with the use of poly(glutamate)-paclitaxel.[77] Syntheses of poly(glutamate)s with controlled molecular weights and narrow polydispersities have traditionally been very difficult. A few methods were recently established to allow the preparation of poly(glutamate) with any molecular weight and polydispersities as low as 1.02 through the controlled ring-opening polymerization of amino acid *N*-carboxyanhydrides.[78–82]

Linear cyclodextrin (CD) polymers are a relatively new class of hydrophilic biomaterials developed for drug delivery applications.[11,14] This class of polymers has excellent safety profiles,[83] high water solubility,[60] and the unique capability of forming an inclusion complex with hydrophobic molecules with compatible sizes. When used for the delivery of camptothecin (CPT), excellent *in vivo* results were obtained.[59,61,62]

Besides the polymers already mentioned, a handful of other hydrophilic polymers have been developed or utilized as vehicles for the conjugation and delivery of hydrophobic therapeutic agents. These polymers can be generally categorized as polysaccharides,[63,84] polypeptides,[85–88] or PEG-containing synthetic polymers,[89–91] some of which are in clinical trials.[92] The basic design principles are quite similar to those systems previously discussed.

Polymeric Micelles

Amphiphilic block copolymers can self-assemble at concentrations above the critical micelle concentration to form core-shell micellar nanostructures, namely polymeric micelles (Figure 1).[93] Such self-assembled structures have condensed, compact inner cores that serve as the nanocontainers for the encapsulation and as the devices for the controlled release of hydrophobic therapeutics. Hydrophilic shells of the self-assembled micelles are used for the reduction of micelle/tissue interaction, the minimization of nonspecific micelle uptake by the reticuloendothelial system, and the conjugation of targeting moieties.[93] Polymeric micelles (20–100 nm) are usually big enough to resist rapid renal clearance but are small enough to pass through the wall of the vessels of tumors. Numerous types of amphiphilic copolymers have been employed to form polymeric micelles for drug delivery applications.[13,41,94–96] PEG has been predominantly used as the shell-forming material because of its excellent physicochemical properties, such as flexible backbone, no charge, low toxicity, low tissue binding, remarkable hydrophilicity, and ease of introducing terminal functional groups for micelle surface modification. The core-forming hydrophobic segment, on the other hand, can be many different types of hydrophobic polymers, such as polyesters, polypeptides, and hydrocarbon-based polymers.[13,41,94–96]

Biodegradable polyesters, such as polycaprolactone (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(lactide-co-glycolide) (PLGA), have been extensively used as core-forming backbones for the preparation of drug-loaded polymeric micelles. Hydrophobic therapeutic agents typically are incorporated into these micelles through physical interaction. Because it is hydrophilic and cannot be entrapped in hydrophobic polymer matrices, DOX has been conjugated through covalent bonding to the terminal carboxylate group of PLGA prior to micellation.[97,98]

Kataoka[99] initially developed PEG-polypeptide micelles for drug delivery. The

polypeptides' backbones are biodegradable and can be readily modified for drug incorporation and manipulation of micelle stability for drug release in a controlled or even stimulus-responsive manner. In one system, DOX-loaded polymeric micelles with diameters in the range of 15–60 nm were prepared by physical encapsulation of DOX with DOX-conjugated PEG-*b*-poly(aspartic acid) (NK911).[93] The DOX molecules attached to the poly(aspartic acid) backbone were not subject to release. Instead, they enhanced the drug encapsulation and micelle stability through the π–π interaction with the physically encapsulated DOX. This strategy has been utilized to develop NK 105, a paclitaxel-loaded PEG-*b*-polypeptide micelle.[100] Both NK 911 and NK 105 are in phase II clinical trials in Japan.

The metal-polymer complexation also has been used as the driving force for the self-assembly of polymeric micelles. In the systems reported by Nishiyama et al.[101] and Cabral et al.,[102] platinum drug-loaded polymeric micelles were formed through the coordination of the platinum agents and the carboxylates of PEG-*b*-poly(glutamic acid) (Figure 2).[101,102] These formulations allow for the release of incorporated drugs in chloride ion-containing media due to the reversibility of the coordination bond. The micelles showed prolonged circulation and capabilities for their accumulation in the tumor. Cabral et al. recently demonstrated excellent anticancer efficacy against metastatic cervical cancer with the use of (1,2-diaminocyclohexane)-platinum(II) (DACHPt)-loaded micelles (Figures 3a–d).[103] The treatment group (DACHPt-loaded micelle) effectively inhibits tumor growth for an extended period of time, outperforming the clinically used DACHPt derivative, oxaliplatin, and control groups (Figure 3a).

Preparation of smart, stimuli-responsive polymeric micelles has been attempted by using environmentally sensitive core-forming polymers or linkers that connect the drug and the polymer. The mildly acidic pH in a solid tumor (pH = 6.8–7)[104] and in endosomes (a membrane-bound compartment inside cells, roughly 300–400 nm and pH 5–6)[105] have been utilized as triggers for the disruption of the micelle and the release of encapsulated cargos.[106] DOX-loaded polymeric micelles with DOX covalently conjugated to PEG-*b*-P(Asp) with an acid-labile hydrazone bond (Figure 4a) showed negligible DOX release at a physiological pH (pH of 7.4) (Figure 5a) but displayed accelerated drug release rates with a decrease of pH.[107] Thus, these micelles actively released doxorubicin in endosomes

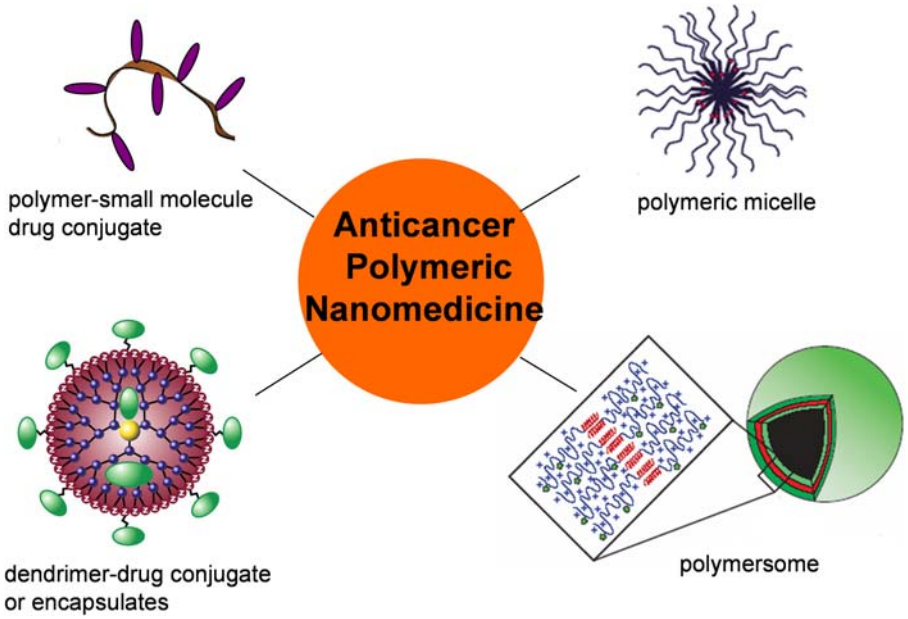

Figure 1. Illustration of various anticancer polymeric nanomedicines that have been developed and are used in cancer drug delivery. Polymer-small molecule drug conjugates are usually hydrophilic (water-soluble) polymers with covalently bound, releasable hydrophobic drug molecules. Polymeric micelles are core-shell micellar nanostructures with a hydrophobic core that can be used for the encapsulation of hydrophobic drug molecules and for the controlled release of hydrophobic therapeutics, and a hydrophilic shell can be used for micelle surface modification (e.g., incorporation of targeting ligands). Polymersomes are a class of hollow spherical nanostructures that enclose a solution and can be used to deliver hydrophilic therapeutics such as DNA and proteins. Dendrimer drug conjugate or encapsulates are a class of drug delivery systems with drugs conjugated to the periphery or encapsulated inside of monodisperse macromolecules with highly branched, symmetric, three-dimensional architectures.

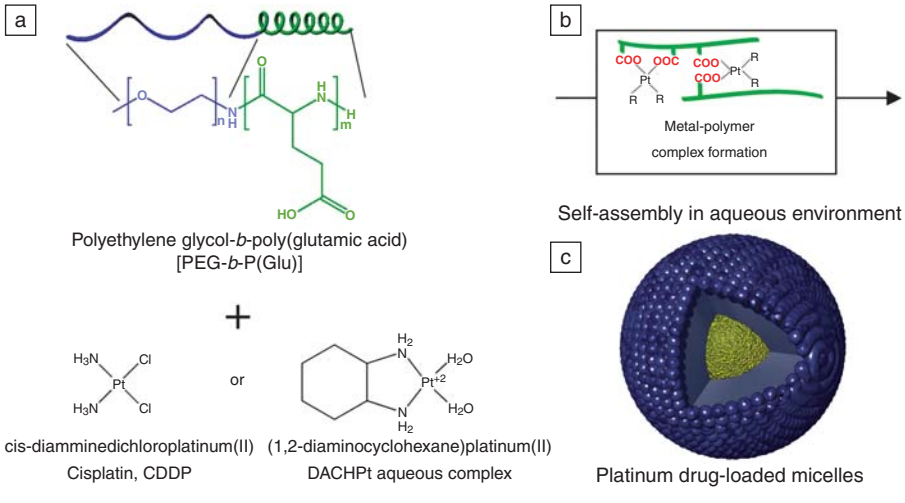

Figure 2. (a) Schematic diagram of proposed self-assembly of platinum drug-loaded polymeric micelles.[101,102] (b) The self-assembly is mediated by the coordination of the platinum (II) and the carboxylate groups (COO) of the poly(glutamic acid) segments. (c) Narrowly distributed polymeric micelles with dense drug-loaded cores are formed.

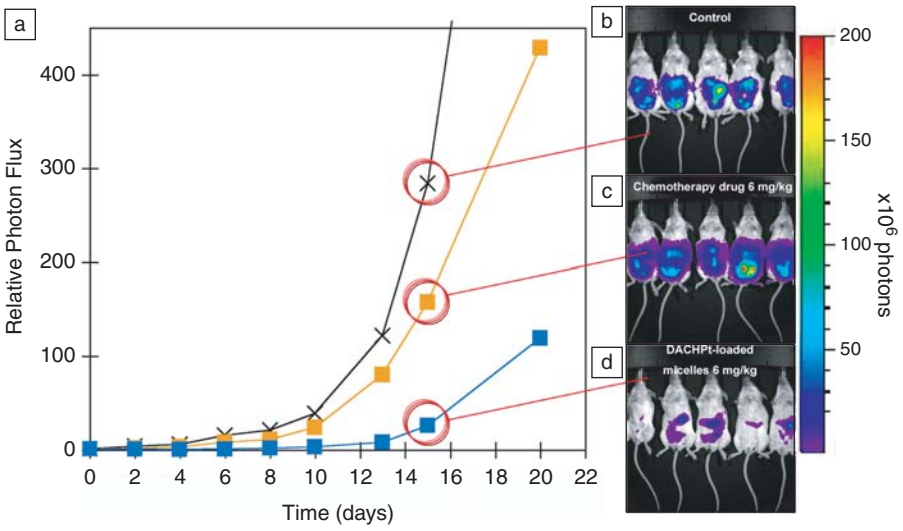

Figure 3. Platinum drug-loaded polymeric micelles.[103] (a) Antitumor activity measured as the relative photon flux, which is the ratio between the photon flux (photons/second) and the initial photon flux, from bioluminescent intraperitoneal (within the abdominal cavity) metastasis and the *in vivo* bioluminescent images corresponding to day 10. (b) Control (crosses), (c) the clinically used DACHPt derivative, oxaliplatin, 6 mg/kg (orange squares), (d) (1,2-diaminocycloheance) platinum (II) (DACHPt)-loaded micelle (blue squares).

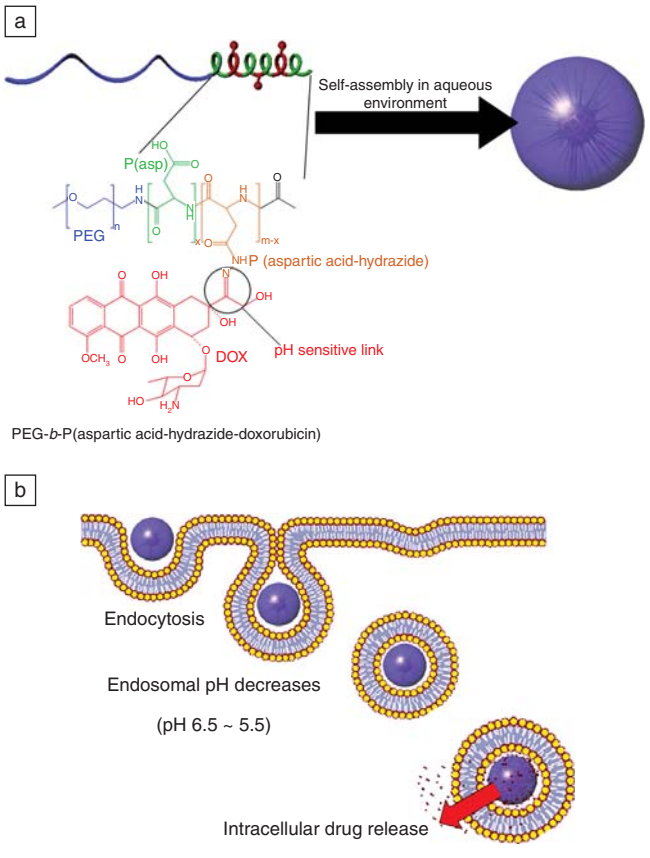

Figure 4. pH-sensitive doxorubicin-loaded polymeric micelles. (a) Molecular structure of PEG-poly(aspartic acid) conjugating with doxorubicin (DOX) by an acid-labile hydrazone bond. (b) Schematic diagram of selective drug release at endosomal pH.

(Figure 4b and 5b) and were very efficacious against solid tumors *in vivo* (Figure 5c and 5d), with negligible systemic toxicity due, in part, to the minimal drug leakage during circulation.[108]

The incorporation of molecules that target specific cellular signals on the outer surface of polymeric micelles is essential for designing carrier systems with specific cellular recognition.[109–111] Thus, the self-assembly of end-functionalized block copolymers will serve as a versatile platform for the construction of polymeric micelles with pilot molecules on their exterior. Moreover, specific drug delivery to the target tissue and specific activation of the delivered drug in the targeted cell may enhance the efficacy and minimize the side effects during drug targeting.

New Polymeric Nanostructures Developed for Drug Delivery Applications

Worm-Like Micelles and Polymersomes

Viruses have evolved to exploit morphology and/or morphological transitions in delivering their cargo efficiently into a target. Some viruses are filamentous rather than spherical in shape, and many, if not most, viruses seem to exploit controlled disassembly of the viral capsid for efficient release of the viral genome into the cell. Block copolymer amphiphiles are well-known to self-assemble in aqueous solutions to form super molecular weight aggregates of varying morphology, including filaments and spheres. The principles that govern assembly—as well as the disassembly elaborated on later in text—can be captured in a packing parameter (p) that is determined by the hydrated area of the hydrophobic chain relative to the molecular volume.[112] This parameter provides an initial prediction for whether the aggregate morphology of an amphiphile in water is spherical ($p < 1/3$), cylindrical ($1/3 < p < 1/2$), or a vesicle bilayer ($1/2 < p < 1$). Using a fully synthetic, strongly segregating diblock copolymer,[113] block copolymer assembly in water has been more thoroughly elucidated in terms of the hydrophilic volume fraction (f), which is inversely related to p. Experimentally, the morphology of the resultant aggregates in aqueous solution can be tuned for many amphiphiles to form spherical micelles ($f > 50\%$), worm-like micelles ($40\% < f < 50\%$),[114] and unilamellar polymer vesicles ($25\% < f < 40\%$) referred to as polymersomes (Figure 6a).[43]

Polymersomes and worm-like micelles composed of high molecular weight PEG-based diblock copolymers are robust structures capable of circulating *in vivo* for tens

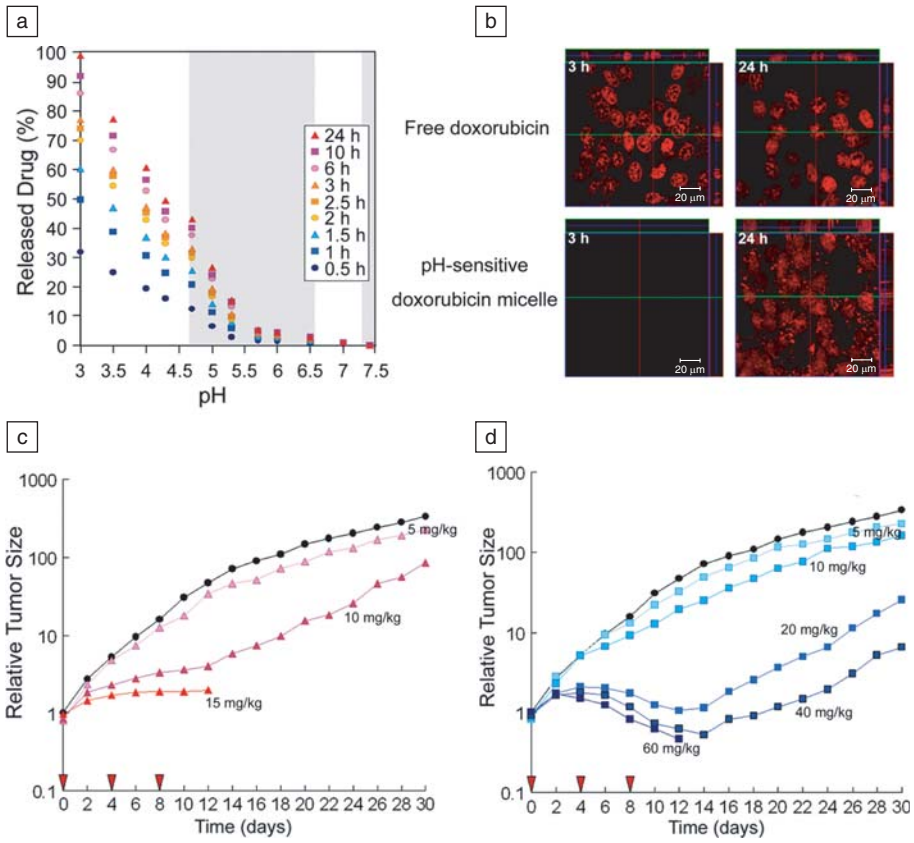

Figure 5. (a) Drug release profiles of pH-sensitive doxorubicin-loaded polymeric micelles at different pH values from 0.5 h to 24 h after release. Drug release amount increased with decreasing pH.[107] (b) Confocal microscopy of SBC-3 cancer cells incubated with free doxorubicin and pH-sensitive, doxorubicin-loaded polymeric micelles after 3 h and 24 h. Free doxorubicin rapidly penetrated the cancer cells by diffusion, while pH-sensitive, doxorubicin-loaded polymeric micelles were internalized by endocytosis and released the drug inside the cells. (c) Antitumor activity of free doxorubicin. The maximum tolerated dose was 15 mg/kg. (d) Antitumor activity of pH-sensitive doxorubicin-loaded polymeric micelles. The maximum tolerated dose was 60 mg/kg. Control (black circles); red arrows indicate intravenous injections.[107,108]

of hours or days due, in part, to the stealthy qualities imparted by a dense outer PEG brush.[19,115] Because of the intrinsic stability of these polymeric assemblies, release mechanisms have been engineered into their structures to allow for the delivery of encapsulated therapeutics. Some work has focused on shifting the polarity of the hydrophobic block to induce release,[116–119] while another common mechanism for release is hydrolytic degradation of hydrophobic polyester blocks such as PLA or PCL. Release takes advantage of the molecular shape-dictated morphology of block copolymer aggregates by increasing f (decreasing p) through chain-end hydrolysis.[19] For example, as PEG-polyester copolymer molecules that initially form bilayer vesicles degrade, f increases and induces a shift in the morphology to worm-like micelles and eventually spherical micelles (Figure 6b).[116,118] These shape transitions destabilize polymersomes or worm-like micelles, which allow for the release of therapeutics and also provides a mechanism for intracellular delivery by compromising the lipid membranes within the cell uptake pathway.[117] Polyester-based degradable polymersomes and worm-like micelles have, thus far, been formed from PEG-PLA, PEG-PCL, and PEG-PMCL.[46] By blending degradable diblocks with an inert diblock, the release rates of encapsulated molecules can be systematically varied.[120] More recent work has described both polymersomes and worm-like micelles made purely from poly(ethylene oxide) (PEO)-PCL,[116,121] although it seems that the crystallinity of pure PCL blocks at temperatures below 50°C adds significant complexity to the phase behavior and properties.

The stability and long-circulating properties of 100% PEGylated degradable polymersomes and worm-like micelles make these assemblies ideal for use as drug delivery vehicles that are capable of accumulating in solid tumor sites through the EPR effect.[46] The ability of polymersomes and worm-like micelles to encapsulate small molecules into either the aqueous lumen of the vesicle or the hydrophobic core of the vesicle membrane or micelle backbone has been thoroughly studied. Recent work has applied these carriers as anticancer treatments by encapsulating the soluble drug DOX into the polymersome lumen[122] with the hydrophobic drug paclitaxel in the core of the vesicle membrane[117,118] and paclitaxel in the worm-like micelles (Figure 7a).[123] By loading both DOX and paclitaxel simultaneously into degradable polymersomes, an effective, dual drug anticancer therapeutic device has been developed.[117,118] *In vitro* studies of these anticancer polymersomes demonstrated the delivery of both DOX and paclitaxel to their intracellular targets (Figure 7b). In combining passive tumor site accumulation (Figure 8a), endolytic escape, and the capacity to increase tolerable doses of two anticancer drugs in the same carrier, these polymersomes are able to effectively shrink tumors *in vivo* (Figure 8b).[117,118] Similarly, the ability of degradable worm-like micelles—or "filomicelles" in analogy to filoviruses—to deliver integrated paclitaxel to tumor cells was first demonstrated *in vitro*,[19,123] with subsequent tumor shrinkage *in vivo* proving dependent on micelle length. Surprisingly, long filomicelles appear superior to micron-length filomicelles as well as the free drug (Figure 8c).[19]

To better understand how the various polymer-based carriers deliver anticancer therapeutics to a tumor, methods for tracking their biodistribution *in vivo* are being developed. Hydrophobic near-infrared fluorophores (NIRFs) can be integrated into thick polymersome membranes to permit whole-body fluorescence imaging in mice,[121,122] while more recent studies[124] with amphiphilic NIRFs in filomicelles demonstrated persistent circulation *in vivo* and partial permeation into the tumor connective tissue. Such demonstrations are important because most nanoparticles are cleared by the liver and spleen within minutes, frustrating delivery to desired target sites such as tumors.

Polypeptides have diverse conformations (coils, α-helices, and β-sheets) and also have been utilized as building blocks of polymersomes.[47,125,126] In addition to the control on the relative length of hydrophilic and hydrophobic segments that are critical to the formation of vesicles, the conformations of polypeptides were found to play an important role in controlling the

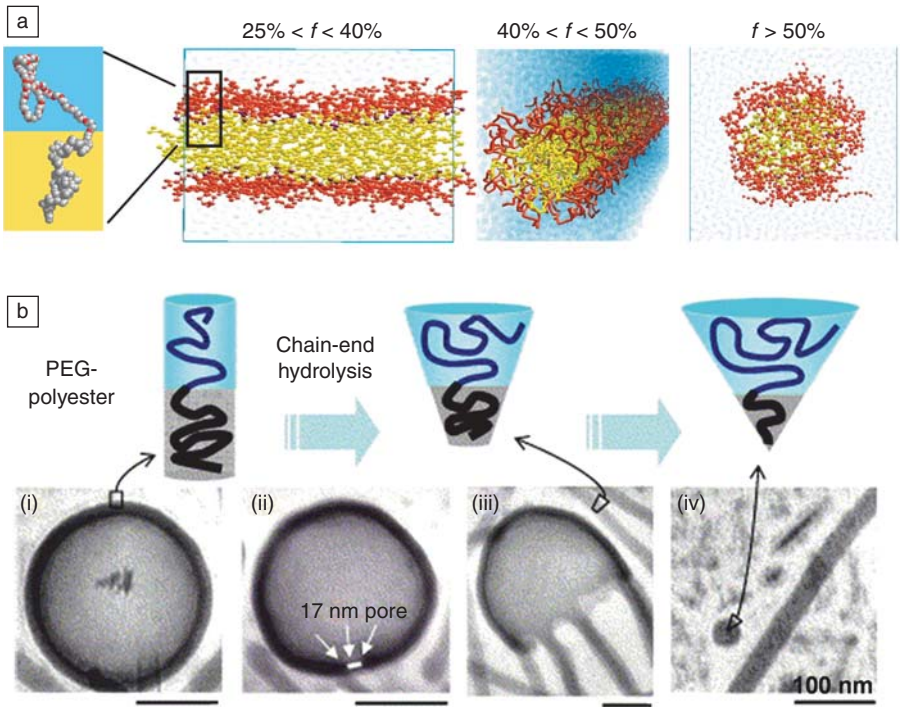

Figure 6. Self-assembly and degradation of block copolymer assemblies. (a) Amphiphilic block copolymers self-assemble to form micellar assemblies of varying morphology. Experimental results and coarse-grain simulation demonstrate the dependence of assembly morphology on the hydrophilic volume fraction (f) of the diblock copolymer. (b) Assemblies composed of PEG-polyester diblock copolymers undergo a morphological phase transition from polymersomes (i, ii) to "squids" (iii) to worm-like micelles and spherical micelles (iv) as the hydrophobic block is systematically shortened by chain-end hydrolytic degradation.[117] As polymersomes and worm-like micelles undergo degradative transitions, encapsulated therapeutics are released. All scale bars = 100 nm.

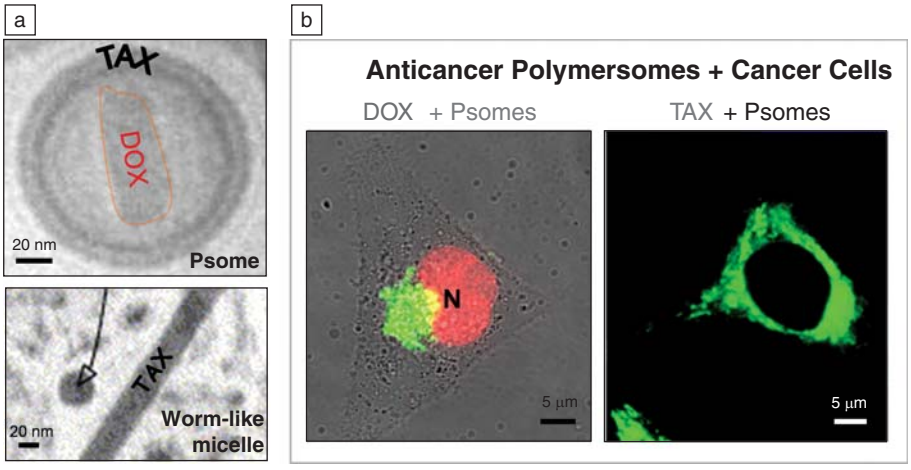

Figure 7. Anti-cancer polymersomes (psomes) and worm-like micelles. (a) Polymersomes encapsulating doxorubicin (DOX) in the lumen and paclitaxel (TAX) in the core of the vesicle membrane. Worm-like filomicelles carrying paclitaxel (filomicelles) can be imaged by cryo-transmission electron microscopy. Polymersome-encapsulated DOX forms a visible insoluble crystal. (b) The ability of degradable polymersomes to escape the endolysosomal pathway and deliver encapsulated therapeutics to the intracellular target is demonstrated *in vitro* for both DOX (red, intercalates into DNA within the nucleus) and TAX (green, binds to microtubules).

formation of polymersomes. Conventional uncharged amphiphilic block copolymer polymersomes require high hydrophobic contents (approximately 30–60 mol%) to form stable vesicles.[127] However, the block copolypeptides deviate from this trend and can form polymersomes with 10–40 mol% hydrophobic domains,[125] presumably because of the strong intermolecular interactions of rigid helical polypeptide[128] as compared to polybutadiene (PBD)-PEG or PLA-PEG vesicles with more flexible polymer segments. Copolypeptides can adopt rod-like conformations in both hydrophobic and hydrophilic domains due to the propensity for forming the α-helix structure.[129] These rod-like conformations provide a flat interface on hydrophobic association in aqueous solution, thus driving the self-assembly into polymersome structures.

Dendrimer and Dendritic Polymer Nanocarriers

Dendrimers are a class of monodisperse macromolecules with highly branched, symmetric, three-dimensional architectures (Figure 1)[130,131] and contain layered structures that extend outward from a multifunctional core on which dendritic subunits are attached.[132] The sizes of dendrimers range from 1–15 nm. Syntheses of multigeneration dendrimers involve alternative repetition of a generation growth and an activation step, which resembles solid-phase peptide synthesis. Depending on the direction to which dendrimers grow, the synthetic strategies can be classified as divergent[131] or convergent.[133] Dendrimers generally are utilized as soluble polymer drug carriers. Drug molecules can either be conjugated on the surface or encapsulated inside of dendrimers. The periphery of a dendrimer usually contains multiple functional groups amenable for the conjugation of drug molecules or targeting ligands. Surface conjugation is straightforward and easy to control; the majority of dendrimer-based drug delivery systems was developed using this covalent conjugation approach, a few of which have been evaluated *in vivo* (e.g., polyamidoamine dendrimers,[46,134,135] asymmetric dendrimer with bow tie–shaped architecture[17]) and demonstrated excellent antitumor efficacy.

Compared to micelles, dendrimer-drug conjugates have a more stable architecture due to their unimolecular structures and thus are easier to handle (formulation and sterilization). Compared to linear polymer-drug conjugates, dendrimer-drug conjugates with similar molecular weights, and surface properties tend to circulate for longer periods of time and have reduced

tendency of renal clearance.[136] Syntheses of monodisperse, high molecular dendrimers, however, can be challenging; conjugation of a large number of insoluble drugs to the surface of dendrimers may result in significantly increased peripheral hydrophobicity, which may subsequently lead to dendrimer aggregation and increased polydispersity. Although surface hydrophobicity-induced dendrimer aggregation may be reduced by encapsulating drug molecules inside of dendrimers, and there have been some efforts in developing dendritic nanocarriers that are capable of encapsulating drugs,[137] this approach is still in an early stage of development with insufficient results to give a full assessment. In conclusion, dendrimers are excellent carriers and hold great promise for the development of next-generation drug delivery vehicles.

New Chemistries and Fabrication Techniques

Polymeric micelles and nanoparticles, prepared by micellation or nanoprecipitation, may lead to the formation of nanostructures with poorly controlled physicochemical properties, such as low drug loading, uncontrolled drug release kinetics, heterogeneous particle sizes, and broad particle size distributions.[138] To address these challenges, Tong and Cheng recently developed a drug incorporation strategy by using Zn-paclitaxel complex as the catalyst to mediate the controlled polymerization of lactide (LA), thus allowing quantitative incorporation of paclitaxel to PLA (Figure 9).[48] When bulky chelating ligands are used, the Zn-catalyst can only interact with the least sterically hindered 2'-hydroxyl group of paclitaxel and regulate the initiation and polymerization at this hydroxyl position, which resulted in paclitaxel-PLA conjugates with precisely controlled composition and molecular weights and low polydispersities (as low as 1.02). At a low monomer/initiator (LA/paclitaxel) ratio, the nanoparticle derived from the paclitaxel-PLA conjugates had extremely high loadings (close to 40 wt%) and displayed controlled-release kinetics with negligible "burst" drug release. Recently, this technique has been extended to the formulation of PLA conjugates of drugs with more complex structure, such as DOX. DOX can be incorporated to the terminus of PLA with its 14-hydroxyl group in DOX-initiated LA polymerization with no need of protecting the intrinsic 3'-amine group.[139]

Bottom-up formulation strategy usually gives rise to nanostructures with relatively broad particle size distributions and an almost exclusively spherical shape. Euliss et al. developed a top-down nanofabrica-

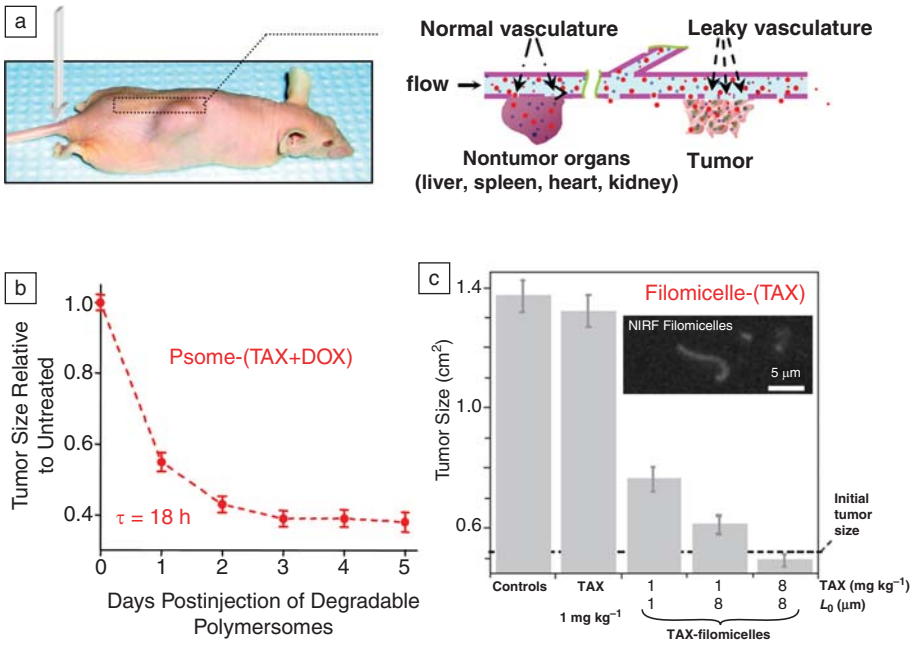

Figure 8. Tumor shrinkage *in vivo*. (a) Anticancer polymersomes and filomicelles are injected into tumor-bearing mice, and the long-circulating properties of both leads to their passive accumulation in the tumor through the leaky tumor vasculature. (b) Anticancer polymersomes loaded with both DOX and TAX effectively shrink solid tumors.[119] A fit to the rate of tumor shrinkage yielded a time constant (τ) of 18 h. (c) The efficacy of TAX-loaded filomicelles to shrink solid tumors is shown to increase with increasing worm-like micelle contour length L_0.[19] Inset: fluorescent image of near-infrared fluorophore (NIRF)-labeled filomicelles.

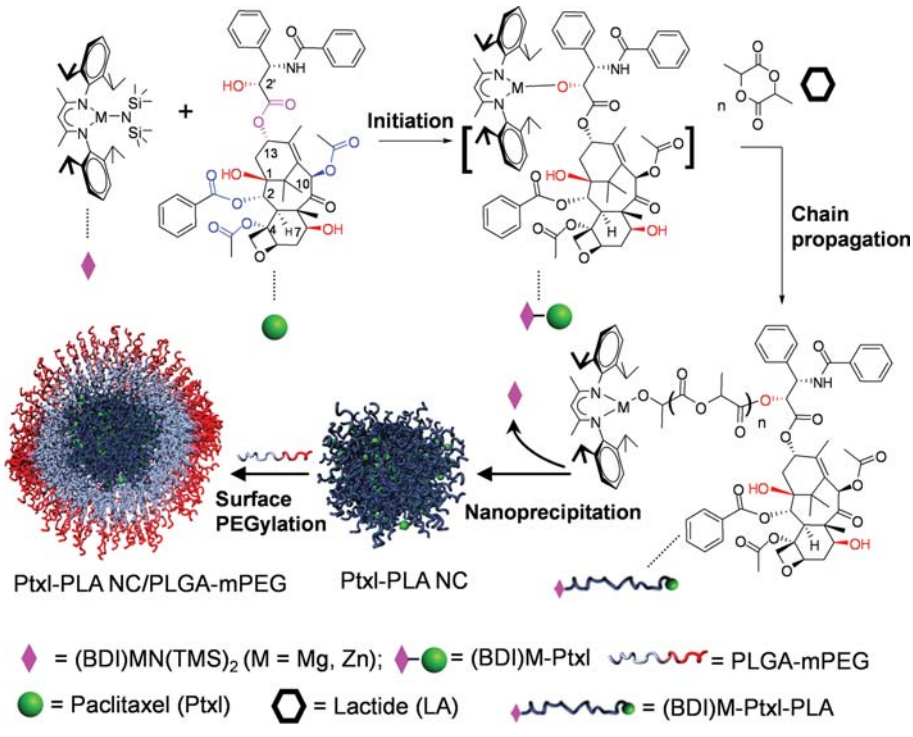

Figure 9. Preparation of poly(ethylene glycol) (PEG)-coated poly(lactic acid) (PLA)-paclitaxel nanoconjugates by paclitaxel-initiated site-specific lactide polymerization in the presence of (BDI)ZnN(TMS)$_2$ (chain propagation), nanoprecipitation, and noncovalent surface modification with poly(lactide-co-glycolide) (PLGA)-mPEG.

tion technique called particle replication in nonwetting templates (PRINT) that addressed these limitations and allowed large-scale formulation of polymeric nanoparticles with precisely controlled sizes and shapes (e.g., cylinder, cube, disc) using a soft lithographic molding technology.[6] They used photocurable perfluoropolyether molds to emboss liquid precursor compounds using highly fluorinated surfaces that are nonwetting to organic materials, which enables the fabrication of isolated objects with superior shape and composition control.

Advanced Targeting Techniques

Tumor targeting has been extensively evaluated using traditional targeting ligands, such as small molecules, peptides, and proteins. Aptamers or single-stranded DNA or RNA that can fold into unique conformations capable of binding to specific targets with high affinity and specificity recently emerged as a new class of targeting ligands that showed some unique abilities unattainable from antibodies or small molecules.[140] Farokhzad et al. demonstrated for the first time that intratumorally administered polymeric nanoparticles with surface-coated aptamers specific for prostate-specific membrane antigen (PSMA) could successfully recognize and target PSMA-positive LNCaP cells (human prostate cancer cells) and eradicate the tumor more effectively than nanoparticles without aptamers.[141–143] When injected systemically, these nanoparticle-aptamer conjugates could target a subcutaneously implanted LNCaP tumor, and the *in vivo* targeting efficiency correlated well with the surface density of the aptamer ligands. An aptamer-based targeting strategy has attracted much interest in the past three to four years; the clinical benefit of using aptamers over antibodies and targeting ligands to create cancer-targeting modalities is yet to be demonstrated.

Future Perspectives

In parallel to the development of lipid-based drug delivery, the advancement of modern polymer chemistry has made it possible for the preparation of a large variety of synthetic polymeric materials with structures tailored to accommodate the specific needs for systemic drug delivery for cancer treatment. As the field of cancer nanotechnology further matures with an increasing number of nanotechnologies moving closer to clinical applications, there is room for continued efforts in developing the polymeric nanometer-sized carrier for the prevention of disease progression and dissemination. To achieve personalized nanomedicine, there are still many obstacles to overcome. Formulations of nanocarriers with precisely controlled parameters (drug loading, size, release kinetics) in large quantity are still challenging. Techniques that can be broadly utilized for the incorporation of therapeutics to a variety of polymers with all translational issues fully addressed are significantly lacking. Much information has been accumulated for the correlation of various physiochemical properties of nanocarriers (e.g., size, surface functional groups, shape) with the systemic biodistribution, and long circulating nanocarriers can be prepared in some specific systems. However, long-circulating nanocarriers may not exhibit maximized anticancer effects if these long-circulating nanocarriers cannot homogeneously distribute in solid-tumor tissues and get internalized into the target cancer cells. In fact, drug delivery nanocarriers that can successfully penetrate the extracellular matrix of tumor tissues are rare. Cancer targeting by incorporating homing ligands to the surface of nanocarriers has been attempted for many years. However, the formulation of nanocarriers containing protein-based targeting ligands (e.g., antibodies) is extremely difficult to control and may only be made in small scales. Incorporation of antibodies to nanocarriers also may result in a substantially increased immune response and rapid accumulation of nanocarriers in organs such as the liver or spleen with a large number of macrophage cells. Solid formulation of polymeric nanoparticles often resulted in aggregation during postformulation processing (e.g., lyophilization), which substantially reduced clinical applicability. Although these challenges are difficult to address, synergistic integration of the efforts of chemists, materials scientists, chemical and biomedical engineers, biologists, and physicians may facilitate the development of anticancer polymeric nanomedicine at an unprecedented pace and eventually may make it possible for chemotherapy to be achieved in a time-, tissue-, and patient-specific manner with the use of polymeric nanomedicine.

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