Current Opinion in Solid State and Materials Science 16 (2012) 323–332

Contents lists available at SciVerse ScienceDirect

Current Opinion in Solid State and Materials Science

journal homepage: www.elsevier.com/locate/cossms

Polymeric nanomedicines based on poly(lactide) and poly(lactide-co-glycolide)

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ARTICLE INFO

Article history:
Available online 31 January 2013

Keywords:
Cancer
PLA
PLGA
Drug delivery
Nanomedicine
Chemotherapeutics
Polymer–drug conjugates
Nanoconjugates

ABSTRACT

Small molecule chemotherapeutics often have undesired physiochemical and pharmacological properties, such as low solubility, severe side effect and narrow therapeutic index. To address these challenges, polymeric nanomedicine drug delivery technology has been routinely employed, in particular with the use of biodegradable and biocompatible polyesters, such as poly(lactide) (PLA) and poly(lactide-co-glycolide) (PLGA). Here we review the development and use of PLA and PLGA for the delivery of chemotherapeutic agents in the forms of polymer–drug conjugates and nanoconjugates.

1. Introduction

Small molecule chemotherapeutics often have undesired physiochemical and pharmacological properties, such as low solubility, severe side effects, and narrow therapeutic index [1]. These drawbacks limit their applications in clinical cancer treatments. Two strategies have emerged in the past 2–3 decades to address these intrinsic drawbacks [2]. One approach is to design and develop new derivatives of chemotherapeutics with improved physiochemical and pharmacological properties so that these modified chemotherapeutics can be better used to modulate the molecular processes and pathways associated with tumor progression [3]. The other widely used approach is to use drug delivery technology to prepare nanomedicines to improve the efficacy and reduce the side effects of the existing chemotherapeutic agents [4–6].

1.1. Nanoparticles for drug delivery

The first-generation anticancer nanomedicines focused on preparation of delivery vehicles with well-developed biomaterials and methodologies, and on targeting and treating primary tumors based on the Enhanced Permeation and Retention effect (EPR effect) [7]. The EPR effect refers to the accumulation of nanoparticles (NPs) in tumor tissue facilitated by the highly permeable nature of tumor vasculature and poor lymphatic drainage of the surrounding interstitial fluid (Fig. 1). NPs based on passive targeting mechanisms have been evaluated in the clinic since the mid-1980s, with the first sets receiving FDA approval in the mid-1990s [8]. In the development of second generation of nanomedicine, a greater emphasis is placed on novel strategies, such as bypassing biological barriers at the systemic, tissue, and cellular levels, and targeting metastatic lesions (active targeting) [6]. Recent development of new chemistry (e.g., click chemistry [9–11]) and fabrication technologies [12–15] is expected to allow for unprecedented, precise control of nanomedicine formulation, thus making it possible to evaluate delivery vehicles with the variation of one parameter (e.g. size, surface property, and shape) at a time [16].

1.2. Physiochemical properties of PLA and PLGA

Since the 1960s, efforts have been focused on developing different polymers for drug delivery to improve therapeutic efficacy. The main criteria in selecting polymer materials for drug delivery are bioavailability, biocompatibility, straightforward production, sustained release and degradation rate [17]. Lipid and polymer-based systems are among the most extensively explored in nanomedicine, accounting for more than half of the treatments approved for clinical use.

Polyesters, alone or in combination with other polymers, have been widely adapted for the formulation of NPs. Poly(lactide) (PLA) and poly(lactic-co-glycolic acids) (PLGA) are among the most well-known choices due to their high biocompatibility and biodegradability. These aliphatic polyesters have been used for surgical

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http://dx.doi.org/10.1016/j.cossms.2013.01.001

Nomenclature

PLA	poly(lactic acid) or poly(lactide)
PLGA	poly(lactic-co-glycolic acid)
PEG	poly(ethylene glycol)
NP	nanoparticle
NMR	nuclear magnetic resonance
MS	mass spectrometry
IR	infrared
HPLC	high-performance liquid chromotography
NC	nanoconjugates
PCL	poly(ε-caprolactone)
ROP	ring-opening polymerization
MW	molecular weight
MWD	molecular weight distribution
T_g	glass transition temperature
T_m	melting temperature
Cpt	camptothecin
Doxo	doxorubicin
Ptxl	paclitaxel
Dtxl	docetaxel
Apt	aptamer
NPP	nanoprecipitation

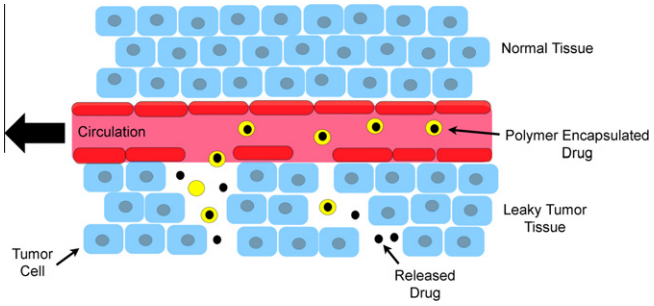

Fig. 1. Schematic illustration of 'Enhanced Permeability and Retention' (EPR) effect.

procedures since being patented as a resorbable suture material in 1967 [18]. Before discussing different PLA and PLGA systems for drug delivery, it is necessary to examine the structure of PLA and PLGA (Fig. 2). Lactic acid contains an asymmetric α-carbon which is typically described as the *D* or *L* form in classical stereochemical terms (sometimes as the *R* and *S* form, respectively). The development of single-site organometallic catalysts has made it possible to synthesize stereoselective PLA [19–23]. The physicochemical properties of optically active PDLA and PLLA are nearly the same, whereas racemic PLA has very different characteristics (e.g. T_g and T_m) [24]. For instance, racemic PLA is completely amorphous with T_g of 57 °C, while PLLA is highly crystalline with a T_m of 170 °C. Such stereo-regular concerns affect the mechanical, thermal, and biological properties of PLA [25]. PLLA is considered more biocompatible since the naturally occurring lactic acid is in the L form. From a physical level of understanding, PLA homopolymers degrade more slowly than PGA homopolymers on the basis of crystallinity as well as steric inhibition by the PLA methyl group to hydrolytic attack. PLA, PGA, and PLGA degradation does not conform to a simple model [26]. Vert and coworkers have demonstrated that hydrolytic degradation is size dependent for PLA systems, with PLA-derived microparticles degrading faster than the corresponding NPs [27–29].

1.3. Development of PLA and PLGA as biomedical materials

Drug delivery research using PLGA and PLA polymers throughout the 1970s and 1980s was largely confined to contraceptive steroids and small peptides such as luteinizing hormone releasing hormone (LHRH) analogs. In 1989, the FDA approved the first PLGA system for drug delivery, a PLGA matrix capable of slowly releasing LHRH analogs (molecular weight (MW) ~ 1200 Da). In the ensuing years, the device became the most widely used system for treating advanced prostate cancer, endometriosis, and precocious puberty. Meanwhile, research shifted to the delivery of emerging large molecular weight drugs (e.g. recombinant proteins) that required sustained dosage in unaltered form. PLGA was first investigated by the Langer group for the delivery of high molecular weight proteins in 1990 [30,31]. Their PLGA microspheres had diameters in the range of 55–95 μm, with protein encapsulation efficiencies greater than 90%, and were able to achieve slow release profiles over 100 days *in vitro*. In 1994, the same group demonstrated the simple formulation of a PLA–PEG copolymer NP system capable of small molecule drug delivery with controlled release and long circulation profiles [32]. The inclusion of PEG in the copolymer reduced the effects of non-specific protein adsorption and colloidal aggregation, further facilitating extended circulation [33].

The initial successes of the Langer group led to the rapid application of PLA, PLGA and various other copolymers in the formulation of microparticle and NP systems for a wide range of biomedical therapeutics delivery. As the potential active agents grew to include small molecules [34], proteins [35] and genes [36,37], the delivery routes also expanded from injection [32] to pulmonary [38], oral [39], and recently targeted delivery strategies

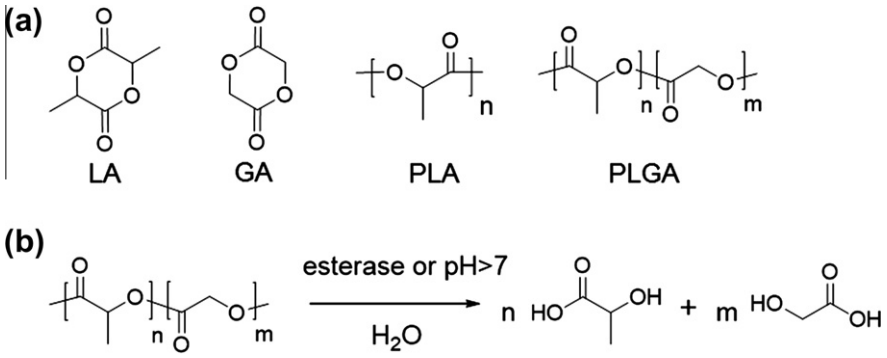

Fig. 2. (a) Schematic illustration of LA, GA, PLA and PLGA and (b) scheme of PLGA hydrolysis.

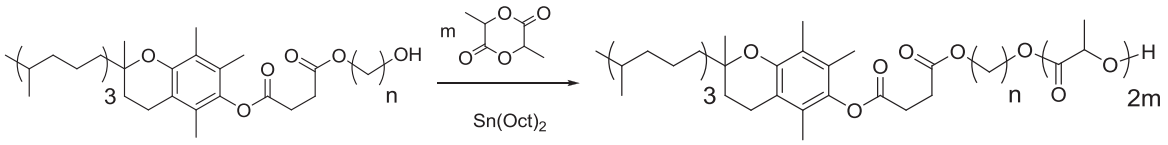

Fig. 3. Scheme of the synthesis of TGPS–PLA.

[40]. One recent application of PLA and PLGA is in the emerging area of stem cell and siRNA delivery. In 2002, human embryonic stem cells were shown to be successfully cultured and differentiated on supportive 3D PLGA scaffolds [41,42]. In 2009, Saltzman and coworkers used PLGA NPs as a siRNA delivery vehicle for *in vivo* gene silencing therapy [43]. They showed that a single dose of siRNA-loaded NPs to a female mouse reproductive tract was sufficient to yield effective and sustained gene silencing. Another intriguing work published in 2009 by the Cheng group showed that PLA–mPEG micelles can effectively repair traumatically injured spinal cord through intraveneous injection [44].

Progress in nanotechnology and microfabrication has produced many advanced nanodevices based on PLGA and PLA [45,46]. For example, the morphology of nanocarriers has moved beyond simple spherical shapes. In 2005, DeSimone and coworkers [47–49] prepared polymeric NPs (including a PLA copolymer) with various shapes (e.g. cylinder and cube) using their PRINT (Particle Replication In Nonwetting Templates) technique, a top-down technique to synthesize NPs with well-defined size and shape; they also demonstrated *in vitro* that shape greatly impacts the cellular uptake of NPs. Mitragotri and coworkers engineered an intriguing type of PLGA NPs that mimic the natural shape and size of red blood cells [50], allowing these particles to flow through capillaries smaller than their own diameters. Increased understanding of amphiphilic polymer self-assembly has yielded new delivery vehicles such as polymersomes [51,52] (polymer based liposome) and filomicelles [53]. The flexible, cylinder-shaped filomicelles (20–60 nm in cross-sectional diameter and a few micrometers in length) have circulation times up to one week after intravenous injection in rodents, roughly 10-times longer than their spherical counterparts. In addition to shape control, substantial attention has recently been focused on designing smart biomaterials using the simple structure of PLA and PLGA. These include a thermosensitive hydrogel composed of PLA and PLGA [54,55] and PLA/polyurethane [56] possessing shape memory properties [57–59].

In this paper we review the development of PLGA and PLA NPs. We present strategies for the preparation of PLA (PLGA)/drug encapsulates (Section 2) and conjugates for nanomedicine (Section 3). The newly developed chemistry to regioselectively conjugate drugs onto PLA or PLGA polymers is highlighted in Section 4. Finally, we briefly discuss the development of PLA (PLGA) NPs for cancer targeting (Section 5).

2. Development of PLA/PLGA drug encapsulates

The encapsulation and controlled release of a drug in a polymeric matrix allows the drug level to be maintained within a desired range, increase its therapeutic activity, decrease its side effects, and reduce the number of administrations necessary [60]. A wide variety of biologically active agents, from low-molecular-weight steroids to high-molecular-weight polypeptides, have been formulated via encapsulation. The emerging strategy of using nanometer sized amphiphilic PLA–PEG (or PLGA–PEG) polymeric micelles is believed to provide a controlled and targeted way to deliver encapsulated anticancer drugs, alter pathways of drug biodistribution, and increase the amount of the agent delivered to tumor cells [61]. Conventional PLGA–PEG or PLA–PEG NPs can be prepared by three methods: (1) emulsification and solvent evaporation methods; (2) solvent displacement methods (nanoprecipitation); and (3) salting out. All have been comprehensively reviewed elsewhere [62–65]. One exciting progress for the clinical transportation of PLA/PLGA micelles is the Genexol micelle system, which uses methoxy-PEG-*b*-PLA to encapsulate paclitaxel (Ptxl). The Genexol system is the first micelle system to enter clinical trials [61,66,67] and is currently under Phase II clinical evaluation [68–70].

The cellular uptake mechanism of hydrophobic molecules loaded in PEG-polyester micelles has been studied by the Cheng group using Forster resonance energy transfer (FRET) technology [71]. Their results show that during micelle–cell membrane interactions, hydrophobic molecules are transferred to the plasma membrane for endocytosis or passively diffuse through the cell membrane. Even high-density PEGylation of the micelle was also shown to bridge the transfer of hydrophobic molecules from the micelle core to the lipid membrane. Their observation explains the reported fast disassociation process of Ptxl from micelles to the blood: Ptxl might transfer from the micelle core to the blood during *in vivo* circulation [72].

In light of this, an alternative drug loading strategy (e.g. conjugation) that avoids rapid drug release during *in vivo* circulation would be desirable for clinical use, as shown in the next section.

3. PLA–drug conjugates for nanomedicine

3.1. Conventional conjugation for nanomedicine

Conjugation of therapeutic drugs to hydrophilic polymers has been actively pursued to improve the pharmacological and pharmacokinetic properties of therapeutic molecules. The hydrophobicity of PLA/PLGA as well as the lack of functional groups on the polymer chains has limited the development of conjugates. In 2002 the Stupp group described a synthetic method [73,74] to functionalize PLA chain ends by coupling the secondary alcohol terminus of PLA with carboxylic acids using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling reagents. In 2005 Sasisekharan and coworkers reported a co-delivery strategy to sequentially release two drugs for anticancer therapy [75]. Their carrier, named nanocell, consisted of a nanoscale PEGylated–phospholipid block-copolymer envelope coating PLGA NPs. The amine group on doxorubicin (Doxo), an important chemotherapeutic agent, was conjugated to PLGA through the terminal hydroxyl of PLGA. NPs were first formed from PLGA–doxorubicin conjugates using an emulsion–solvent evaporation technique and then added to a resuspension buffer containing lipids and the angiogenesis agent combretastatin. (Fig. 4). The nanocell formulation enables a temporal release of two drugs: the outer lipid layer rapidly released combretastatin within 12 h, causing vascular shutdown. The inner NPs released Doxo for tumor regression over 15 days at a noticeably slower rate. This strategy was shown to inhibit tumor growth and increase survival in animal models of melanoma and Lewis lung carcinoma (LLC) with an improved therapeutic index compared to groups treated with either single agent.

In another example, Benny and coworkers developed a promising oral formulation of mPEG–PLA–TNP-470 conjugates (Lodamin)

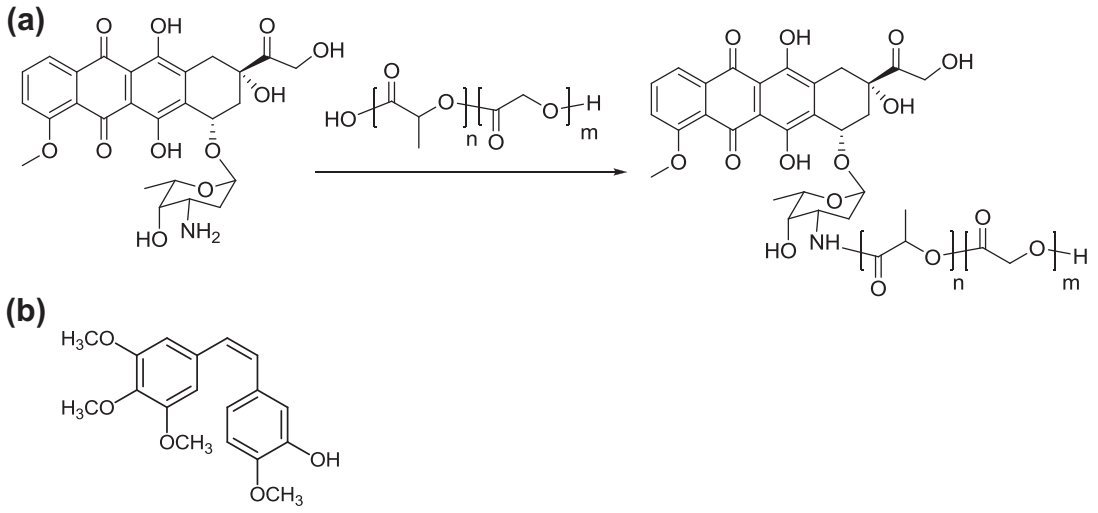

Fig. 4. (a) Scheme of the synthesis of Doxo–PLGA and (b) the chemical structure of combrestatin A4.

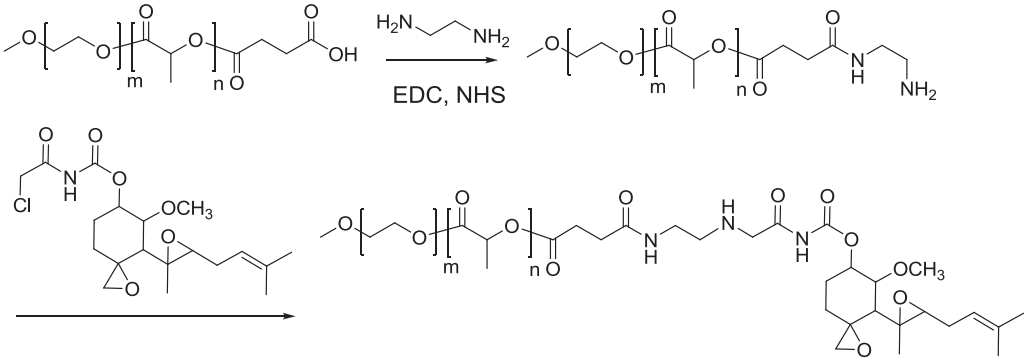

Fig. 5. Synthesis of mPEG–PLA–TNP-470 (Lodamin).

as a long-term anti-angiogenesis agent for tumor therapy [39]. Inhibition of angiogenesis is becoming one of the most promising modalities to suppress tumor growth and metathesis. Although it is one of the most potent angiogenesis inhibitors in many animal models, the clinical use of TNP-470 is limited because of poor oral availability. In the synthesis of Lodamin, ethylenediamine was first conjugated to succinated mPEG–PLA (Fig. 5). Next, the terminal chlorine of TNP-470 was reacted with the amine containing mPEG–PLA, thereby providing mPEG–PLA–TNP-470 (Lodamin) conjugates (MW = 3687 Da). The conjugates were dialyzed against water to form polymeric micelles, with a majority portion in the 7–8 nm size range and a small population of large particles (200–400 nm). *In vitro* release study of Lodamin showed the continuous release of TNP-470 over one month. Furthmore, Lodamin (60 nM TNP-470) could be taken up by the endothelial cell line HUVEC within 2 h, thereby completely inhibiting their growth. In subsequent biodistribution studies (oral administration), Lodamin had a prolonged blood circulation time of at least 72 h compared to 2 h for free TNP-470. Furthermore, no tissue abnormalities were detected (including liver, intestine, lung, and kidney) after administration of Lodamin over 20 days. The *in vivo* efficacy study in murine models bearing LLC tumors showed that mice orally treated with Lodamin (15 mg/kg TNP-470 equivalents per day) formed very small and undeveloped vessels while untreated mice possessed both large and small vessels. Preferential accumulation in tumor tissue via the EPR effect and an enhanced level of apoptosis in the Lodamin-treated tumors was also found. One of the most encouraging progresses of oral administration of Lodamin is the prevention of liver metastasis in mice. Liver metastases are very common in many tumor types and is often associated with a poor prognosis and survival rate. In an *in vivo* model using B16/F10 cells—cells which cause liver metastasis—the group without Lodamin treatment had low survival rates. However, all oral Lodamin-treated mice survived up to study completion and had a normal liver and spleen morphology with no apparent side effects. These results suggest that Lodamin can prevent the development of metastasis in the liver and point to the promising therapeutic technologies being developed in the treatment of solid tumor progression and metathesis using NP technology [76].

3.2. PLA-small molecule conjugates by ring opening polymerization

For lactones and lactides (e.g., LA), ring opening polymerization (ROP) can control a polyester's MW with narrow MWD through the coordination–insertion mechanism. Several excellent reviews have already covered the selection and range of initiators and catalysts tested [22]. ROP of PLA proceeds via the coordination of LA to a Lewis acidic metal alkoxide complex, allowing the activation and attack of lactone at the carbonyl carbon. Acyl bond cleavage results in a ring opening event and the generation of a novel metal alkoxide species. By judicious choice of initiating alkoxide complex, it is possible to instill functionality to the chain ends with an ester bond linkage. Notably, the majority of ROP studies focus on the use of simple alcohols such as ethanol or benzyl alcohol (Fig. 6) except a few examples we will discuss below.

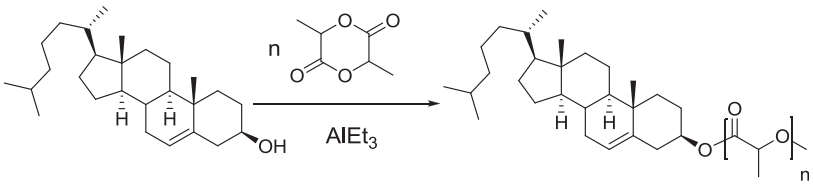

Fig. 6. PLA polymerization initiated by cholesterol catalyzed by $AlEt_3$.

In 1994, Kricheldorf and Kreiser-Saunders reported an interesting synthesis strategy that small molecules and metal catalysts can be used as the co-initiators for polyester polymerization [77]. In the study, the reactive initiators were in situ prepared by the reaction of triethylaluminium ($AlEt_3$) with drugs containing hydroxyl groups (Fig. 6A). Polymerization of L-LA with these in situ prepared initiators yielded oligo- or poly(L-lactide) (P_LLA) with covalently bound drugs. The first batch of drugs used in their study included geraniol, stigmasterol, tocopherol, testosterone, pregnenolone, ergocalciferol, cortisone and quinine. It is noted that the ketone groups of steroids did not undergo red–ox reactions during the polymerization process according to ^{13}C NMR spectroscopy, which indicated the chemoselectivity of the Al catalysts towards hydroxyl groups in cholesterols.

Kricheldorf's strategy was later investigated by the Stupp group to initiate LA polymerization with cholesterol [78]. Their functionalized oligo(lactide)s (OLA) were synthesized by initiating ROP from cholesterol with either $AlEt_3$ or $Sn(Oct)_2$ (Fig. 6B). The MW could be controlled by the L-LA/cholesterol ratio with narrow MWD ($M_w/M_n \sim 1.1$) using $AlEt_3$ catalyst with a high degree of polymerization ($n \geqslant 20$). Lower MW OLA ($n < 20$) could be synthesized in bulk at 150 °C using $Sn(Oct)_2$ and cholesterol, providing oligomers with controlled MW and reasonable MWD ($M_w/M_n \sim 1.2$). The covalently bound cholesterol-OLA (n from 10 to 40) oligomers were characterized and found to possess liquid crystalline behavior. The self-organized layered liquid crystalline structures were found to promote improved fibroblast adhesion and spreading. The ability of self-assembling PLA to present ordered and periodic bulk structures to cells could be a useful strategy in tissue engineering.

Using this conjugation strategy Fraser and coworkers developed intriguing PLA–difluroboron dye conjugates with superior *in vivo* imaging properties to the parental materials [79–81]. Hydroxyl-functionalized difluoroboron dibenzoylmethane (BF_2dbmOH) was used as an initiator in the ring opening polymerization of LA to produce BF_2dbm end-functionalized PLA (BF_2dbm-PLA) mediated by $Sn(Oct)_2$ [81]. The solvent-free reaction was stopped at ~50% monomer conversion to avoid higher MWD due to transesterification and thermal depolymerization.

Boron difluoride compounds are light emitting materials with impressive optical properties with intense fluorescence used in molecular probes, lasers, and photosensitizers, however, their phosphorescence is typically observed only at low temperatures. The BF_2dbm–PLA conjugates resulted in a highly sensitive single-component oxygen sensor with enhanced fluorescence quantum yields and temperature-sensitive delayed fluorescence. Interestingly, oxygen sensitive room temperature phosphorescence was found for BF_2dbm–PLA but not for the free BF_2dbmOH, allowing BF_2dbm–PLA to serve as a powerful tool for quantitative oxygen detection through calibrated RTP spectroscopy. The BF_2dbm–PLA conjugates with sufficient fluorescence and phosphorescence intensities have been first used for *in vivo* ratiometric tumor hypoxia imaging, thus allowing the furthered understanding of the relationship among hypoxia, tumor progression, metastasis and treatment resistance [80].

A novel formulation strategy to encapsulate drug in PLGA/PLA NPs has been reported by the Feng group [82–85]. They synthesized a PLA–TPGS polymer by ring-opening polymerization (ROP) using a Sn(II) octanoate catalyst (Fig. 3). TGPS is a water soluble derivative of Vitamin E which inhibits P-glycoprotein mediated multi-drug resistance, thus enhancing the cytotoxicity of chemotherapy agents. PLA–TPGS acts as a stabilizer during the emulsion formulation of NPs, enhancing drug loading and improving emulsification efficiency compared to PVA [82]. PLA–TPGS NP loaded with Ptxl was able to increase the survival rate with reduced tumor growth rate in a HT-29 xenograft tumor model compared to the treatment of Taxol. A new formulation of PLA–TPGS with montmorillonite for docetaxel (Dtxl) oral delivery was also developed by the same group [85]. The use of PLA conjugates as the stabilizer without conventional surfactants might provide a new emulsion formulation strategy for PLA NPs.

4. Nanoconjugates

4.1. Rationales of nanoconjugates design

Aforementioned studies used hydroxyl-containing small molecule drugs to initiate LA polymerization can provide the desired PLA–drug conjugates [82,86]. However, this strategy has a few limitations when applied to the broad spectrum of complex drugs with hydroxyl functional groups. First, hydroxyl-initiated LA polymerization mediated by Sn(II) or Al(III) catalysts require high temperature (50–150 °C); drugs may degrade during the high-temperature reaction conditions. Furthermore, the obtained PLA conjugates usually have low drug incorporation efficiency and undesired side reaction (e.g. transesterification or depolymerization). The MWD is slightly broad for the resultant polymer–drug conjugates. Besides, for multi-hydroxyl drugs, this polymerization strategy lacks regioselectivity control with regard to the conjugation site on drugs. Such chemical heterogeneities, which may be the present bottleneck in the clinical translation of polymer-drug conjugates, also limit the scope of the conjugation strategy for the important therapeutics.

The clinical application of many hydrophobic drugs is often accompanied with severe, undesirable side-effects. Current nanoencapsulates usually have low drug loading, uncontrolled encapsulation efficiencies, and notable drug burst release when used *in vivo* [17,34,64,87]. These formulation challenges significantly limit their potential clinical applications.

Many of these issues have been addressed in our newly developed nanoconjugates (NCs) system. We are able to conduct efficient polymerization at room temperature within hours and give PLA–drug conjugates with controlled MWs and narrow MWD with no or negligible side reactions. A particularly promising group of catalysts that overcame bottlenecks described above was developed by the Coates group [20,21,88–92]. The catalyst is in the form of L_nM–OR, where L_n is a ligand called β-diiminate (BDI), and M is the active metal center; we use Mg or Zn for NCs synthesis since Mg and Zn are nutrient elements and trace amounts of Mg or Zn in the PLA formulations will not raise significant safety concern. The –OR group can be readily generated by reacting L_nM–$N(TMS)_2$ with a hydroxyl containing molecule (R-OH, TMS = trimethylsilyl). During the

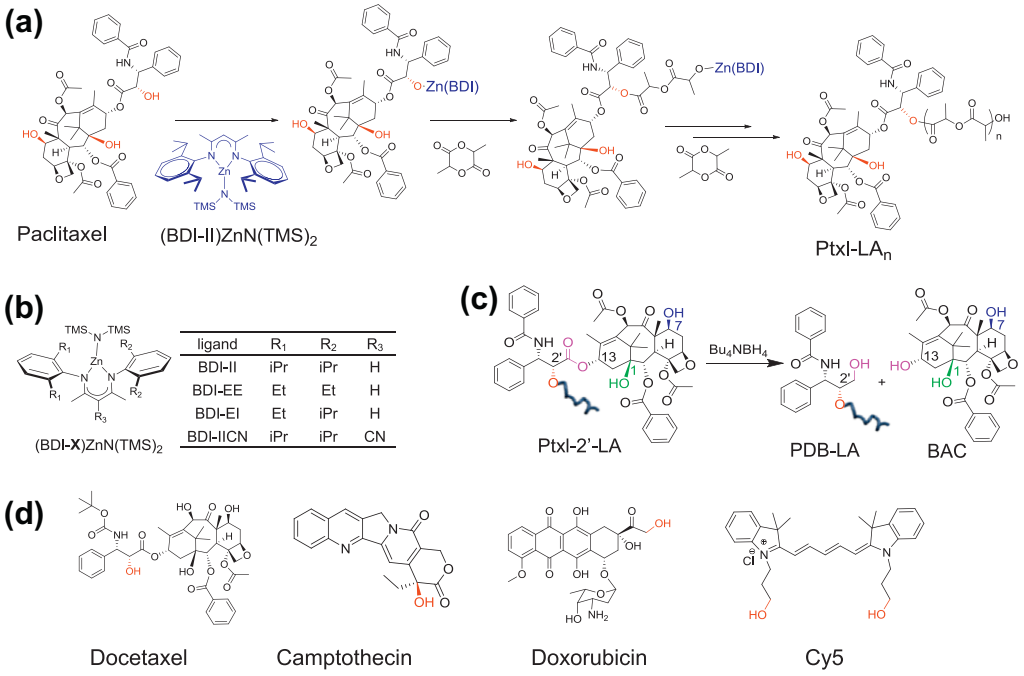

Fig. 7. (a) Synthesis of PLA–PtxI nanoconjugates with regioselectivity; (b) structures of (BDI-X)ZnN(TMS)$_2$ catalyst; (c) scheme of PtxI–LA$_n$ reduction to confirm the regioselective conjugation of PLA onto PtxI; (d) drugs and dyes can be incorporated to PLA to form nanoconjugates. The hydroxyl group highlighted by the red color in (d) indicated the PLA conjugation site of the molecule.

polymerization LA is ring-opened by this metal alkoxide to form a RO-terminated LA–metal alkoxide. After successive ring-opening polymerization steps, RO is linked to the PLA terminus through an ester bond (RO–(C(O)CH(CH$_3$)O)$_{2n}$–M(BDI)) [20–22].

Based on these well-accepted principles, our group postulated that hydroxyl-containing drug molecules could potentially be used to make the corresponding (BDI)M–drug alkoxides. These (BDI)M–drug alkoxides could in turn initiate LA polymerization to make PLA–drug conjugates [93,94] (Fig. 7).

4.2. PLA–camptothecin conjugates

We chose a mono-hydroxyl drug, 20(*S*)-Camptothecin (Cpt), to demonstrate the concept. Cpt is a topoisomerase I inhibitor exhibiting a broad range of anti-cancer activity in various animal models [95–97]. Polymer-Cpt conjugation through its C20-hydroxyl group is usually accomplished in a stepwise manner: Cpt is first converted to a Cpt-amino ester, followed by conjugation with a carboxylate-containing polymer [98,99].

We first tested the feasibility of forming Cpt-metal alkoxide complexes. Since studies have shown that subtle changes in the BDI ligand strongly affect the activity of the catalyst for polyester and polycarbonate polymerization [90,92,100], variable BDI-Zn catalysts were synthesized with modifications on the *N*-aryl ring and backbone of BDI in search of controlled polymerization activities with complete incorporation of Cpt (Fig. 7B). The Cpt–PLA synthesized by (BDI-IE)ZnN(TMS)$_2$ exhibited an M_n nearly identical to its theoretical value with a relative narrow MWD, outperforming both (BDI-II)ZnN(TMS)$_2$ and (BDI-EE)ZnN(TMS)$_2$. The polymerization of LA/Cpt mediated by (BDI-IE)ZnN(TMS)$_2$ showed linear correlation of MW versus [LA]/[Cpt] feeding ratio and gave Cpt–PLAs with monomodal GPC elution curves [101].

The lactone ring of Cpt should be preserved during the polymerization in order to maintain its antitumor efficacy. The initiation step was investigated in detail by using succinic anhydride (SA) to replace LA, as the resulting Cpt-SA adduct (a small molecule) has characterizable structure compared to a Cpt–PLA conjugate (a polymer). The reaction of Cpt with SA mediated by (BDI-IE)ZnN(TMS)$_2$ could rapidly produced Cpt-SA in a nearly quantitative manner. ^{1}H NMR analysis confirmed that the lactone ring remained intact in the Cpt-SA adduct; while the carboxylate form of Cpt-SA was not observed. This stands in sharp contrast to a control reaction mediated by (BDI-II)MgN(TMS)$_2$ [101]. These observations suggest that Cpt/(BDI-IE)ZnN(TMS)$_2$ mediates living polymerizations of LA, and Cpt structure remains intact in the obtained Cpt–PLA conjugates.

4.3. PLA–paclitaxel conjugates

Paclitaxel (PtxI) is a potent mitotic inhibitor that has severe, undesirable side effects. It has three hydroxy groups at its C2′, C1, and C7 positions, which cause difficulties in the synthesis of PtxI-polymer conjugates with precisely controlled structures. Among the three hydroxyl groups of PtxI, the C2′–OH is least sterically hindered. The C1–OH is surrounded by several bulky groups and is generally considered non-reactive [102,103]. The C7–OH can potentially compete with the C2′–OH group for coordination with metal catalysts to initiate LA polymerization, resulting in PtxI–PLA conjugates with one or two PLA chains being attached to each PtxI. We found out that PtxI–PLAs with expected MWs and narrow MWDs could be readily prepared by LA polymerization mediated by the (BDI-II)ZnN(TMS)$_2$/PtxI complex formed in situ (Fig. 7). In addition, we further determined that C2′–OH was the only initiation group of PtxI in this polymerization (Fig. 7A) using a selective and quantitative reduction reaction of PtxI. In such reaction, tetrabutylammonium borohydride (Bu_4NBH_4) quantitatively reduces the 13-ester bond of PtxI to give baccatin III (BAC, containing 1-OH and 7-OH) and (1*S*, 2*R*)-*N*-1-(1-phenyl-2,3-dihydroxypropyl)benzamide (PDB, containing 2′-OH, Fig. 7C) [104]. The HPLC analysis of PtxI–LA$_5$ prepared by (BDI-II)ZnN(TMS)$_2$ showed that initiation and polymerization occurred exclusively at the C2′–OH group of PtxI [105]. Recently, the regioslective activation of PtxI

and other multi-hydroxyl drugs was applied to synthesize various prodrugs, with site-specific modifications achieved through acylation of drugs with anhydrides and carboxylic acid [105]. Broad applications of such prodrug conjugates for medicinal chemistry and drug delivery are under investigation by our group.

4.4. PLA–doxorubicin conjugates

Doxorubicin (Doxo) is another well-known chemotherapeutic agent. It has more complex functionalities than Ptxl, containing three hydroxyl groups at its C4′, C9 and C14 sites, a primary amine group at its C3′ position and a ketone group at its C13 position. One commonly used conjugation strategy is through the reaction between the C13-ketone group of Doxo and hydrazine groups of polymeric carriers to form conjugates with acid-labile hydrazone linkers [106–114]. However, clinical studies of several Doxo–antibody immunoconjugates prepared using this conjugation method gave poor response *in vivo* [115]. An alternative strategy is to link Doxo to carboxylate-containing polymers through its hydroxyl group(s) [116]. Although direct *O*-acylation of Doxo was achieved in a serine endopeptidase-mediated reaction [117], there has been no report of efficient *O*-acylation of Doxo to prepare polymer–Doxo conjugates.

It is interesting to note that metal alkoxides (M-ORs) are more reactive than metal amides (M-NHRs) for the polymerization of LA or other cyclic esters [22,118]. Such reactivity difference was demonstrated by using pyrene-1-methylamine and pyrene-1-methanol as the corresponding model initiators in (BDI)ZnN(TMS)$_2$ mediated LA polymerization. We utilized the sharp difference in reactivity between M-ORs and M-NHRs to control chemo-selectivity in Doxo initiated LA polymerization. To evaluate the regio- and chemoselectivity, Doxo/(BDI-II)ZnN(TMS)$_2$ were mixed with SA to mimic the initiation step of the Doxo/(BDI-II)ZnN(TMS)$_2$-mediated LA polymerization. As expected, and confirmed by ^{1}H NMR analysis, Doxo-14-SE was the predominant product (Fig. 8). Polymerization of LA mediated by Doxo/(BDI-II)ZnN(TMS)$_2$ resulted in Doxo–PLA conjugates with narrow MWD (PDI < 1.2) and expected MWs. In this manner, Doxo–PLA conjugates can be achieved in a single step with highly controlled regio- and chemoselectivity without the need for C3′$-NH_2$ protection [94].

4.5. Particulate formulation of PLA–drug conjugates

Although the ideal physicochemical properties of NPs for *in vivo* applications have not been mapped out, a general consensus about several important parameters of NPs, such as particle size, drug loading, loading efficiency, and release kinetics, have been reached. The sizes of NPs should be typically less than 200 nm with narrow size distribution. During nanoprecipitation (NPP), both the solvent and the concentration of the polymer have dramatic effects on the size of the NPs. At a fixed concentration of Ptxl–PLA conjugates, the size of the NCs prepared by NPP from DMF to water is typically in a range of 60–150 nm with monomodal distribution. Sizes of PLA NCs can also be precisely tuned from 60 nm to 150 nm by changing polymer concentration. For Ptxl–LA$_{100}$, the size of NCs showed a linear correlation with concentration [93].

Drug loading is one of the most important aspects of NPs. It has been found that low drug loadings in NPs (typically in a range of 1–5% in most NPs) formulated by nanoencapsulation [62,86,119] requires large amounts of delivery vehicles to be administered [120,121]. For instance, the volume of a solution intravenously administered to mice with 20–30 g body weights should be controlled to below 100 μL [122]. Intravenous administration of NPs with 1 wt.% drug loading in a 100-μL solution at a dose of 50 mg/kg to a nude mouse with 20-g body weight requires the formulation of a concentrated, 1 g/mL NP solution. This is far too viscous to formulate and inject intravenously [119,123]. These formulation challenges prohibit the clinical translation of NPs prepared through the co-precipitation of polymers and drug [64]. In our NCs formulation, we could easily increase drug loadings to 20–30 wt.% and therefore we could prepare reasonable NPs solution for systemic administration [94].

The time scale and kinetics of drug release from polymeric nanocarriers are worth studying to further evaluate the potential efficacy of the nanocarriers. Typically 70–90% of encapsulated drugs are released from PLA/drug NPs during the first few to tens of hours [32,119]. The burst release of encapsulated drugs, also known as "dose dumping," causes severe systemic toxicities [124]. For Ptxl–PLA NCs, the Ptxl release kinetics of NCs is determined not only by diffusion, but also by the hydrolysis of the Ptxl–PLA ester linker. The release of Ptxl from NCs is more controllable over weeks, with significantly reduced burst release.

5. PLA/PLGA NPs for targeting cancer therapy

To achieve tumor targeting, nanocarriers must overcome systemic barriers to reach tumor sites, especially clearance via phagocytic uptake and hepatic filtration. They are then expected to extravasate the tumor vasculature and penetrate the tumor tissues so that even cancer cells situated distal to the tumor vessel can be exposed to the anticancer agent at effective concentrations [125].

The first examples of targeted NPs were reported in 1980. Despite nearly three decades of research, targeted NPs have made a limited impact on human health. This, in part, is because the optimal bio-physicochemical properties of NPs, including the choice of a suitable targeting ligand, have remained elusive. Aptamers (Apts) are single-stranded DNA or RNA oligomers that can fold into unique conformations capable of binding to specific targets with high affinity and specificity. Recently, they have emerged as a new class of targeting ligands that show some unique abilities unattainable from antibodies or small molecules. Apts are non-immunogenic and exhibit remarkable stability in a wide range of pH (4–9), temperature, and organic solvents without the loss of activity. The synthesis of Apts is an entirely chemical process, thus minimizing batch-to-batch variability [126,127]. These advantages of Apts are superior to immunogenic or labile antibodies, which have significant batch-to-batch variability due to their dependence on biological systems. Farokhzad et al. demonstrated for the first time

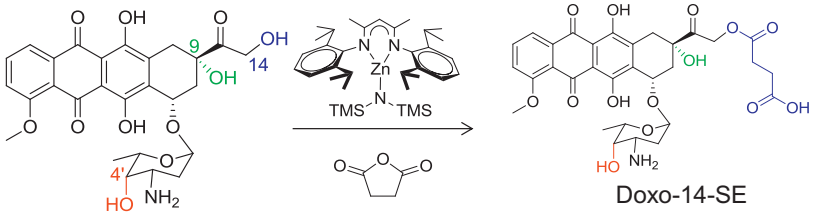

Fig. 8. Regioselective reaction of Doxo and succinic acid, mediated by (BDI-II)ZnN(TMS)$_2$, to yield Doxo-14-SE.

that intratumorally administered polymeric NPs with surface-coated Apts specific for prostate-specific membrane antigen (PSMA) could successfully recognize and target PSMA-positive lymph node carcinoma of prostate (LNCaP) cells and eradicate the tumor more effectively than NPs without Apts [128]. When injected systemically, the NP–Apt conjugates could target subcutaneously implanted LNCaP tumors [40].

One unique feature of Apts is that their activity can be strongly inhibited by their complementary DNAs (cDNAs). Complementary base pairing disrupts the Apts' target-binding conformation, rendering them ineffective. Therefore, the cDNA of the targeting Apt can serve as a "universal" antidote to reduce the efficacy of encapsulated or conjugated drugs towards target cells in targeted cancer therapy, since neutralized Apt-based delivery systems lose their disease-targeting capability (Fig. 9). We recently utilized these features and demonstrated that a nucleolin (NCL) Apt-mediated cancer-targeting strategy is highly specific for targeting the NCL overexpressing breast cancer cell line MCF-7. Moreover, the efficacy of cisplatin-loaded Apt-liposomes can be modulated for desired drug-delivery applications by using the cDNA antidote strategy mentioned above [129]. Our preliminary results showed that the cDNA-antidote caused reduction in drug-delivery efficiency and could be valuable for reversing adverse drug effects in targeted cancer therapy.

In another study we used Apts as targeting molecules for fluorescent NCs [130]. Cy5 (a fluorescent dye) was used to initiate PLA polymerization to prepare NCs (Cy5–NCs) which were subsequently analyzed *in vitro* using fluorescence microscopy. A10 Apts were conjugated to the PLA–PEG–COOH/Cy5–NCs surface through a carboxylic acid–amine coupling reaction. In a time course uptake study, the internalization of Cy5–NCs/Apts to LNCaP cells was significantly enhanced compared to control Cy5–NCs without Apt. In PSMA-negative PC3 cells, very low internalization efficiencies of NCs were observed for both Cy5–NCs/Apts and Cy5–NCs. This *in vitro* study confirmed that NCs could be conjugated with Apt for targeted cancer therapy.

In addition to Apts, the anti-[human epidermal growth factor receptor 2] (HER-2) affibody has been recently applied by Farokhzad and coworkers for drug delivery to HER-2-positive breast cancer cells [131]. The HER-2 affibody is a small protein with relatively low MW ($\sim$ 15 kDa) compared to anti-HER-2 monoclonal antibody ($\sim$ 15 kDa) but still shows high binding affinity ($K_D \sim 22$ pM) to HER-2 protein. PLGA–NP-affibody bioconjugates are also relatively easy to prepare compared to anti-HER-2 antibody. The HER-2-targeted affibody-NP delivery platform is being explored for its ability to encapsulate a variety of drug types with various affibody surface coverage for targeted cancer therapy.

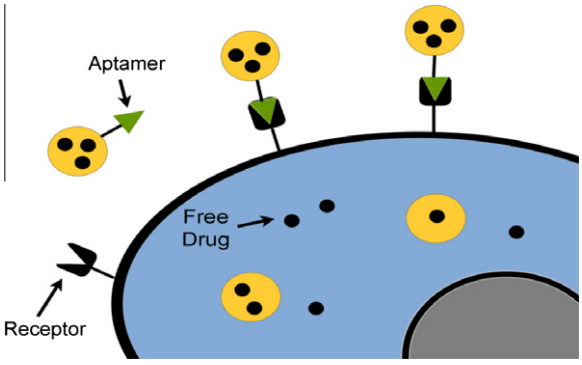

Fig. 9. Schematic illustration of aptamer-nanoparticles targeting strategy.

6. Summary

Nanotechnology is making a significant impact on cancer drug delivery. In conjunction with the development of lipid based drug delivery, the advancement of modern polyester chemistry make it possible for the preparation of a large variety of synthetic PLA/PLGA materials with structures tailored to accommodate the specific needs of systemic drug delivery. We reviewed the properties and current understanding of PLG/PLGA polymeric nanocarriers for cancer chemotherapy. It is anticipated that synergistic integration of the efforts of chemists, materials scientists, chemical and biomedical engineers and physicians will facilitate the design and development of polymeric nanomedicine at an unprecedented pace, and eventually allow for cancer therapy in a time-, tissue-, or even patient-specific manner.

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