Functional Polylactide-based Systems for Delivery of Cancer Therapeutics

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ABSTRACT

A series of studies are performed to develop functional polylactides (PLAs) and investigate their applications in anticancer therapeutic delivery. The synthetic approaches for a broad variety of functional PLAs are established. Several types of functional PLA-based drug delivery systems, including brush-like polymer-drug conjugates (PDCs), PDC-based nanoparticles and zwitterionic PDCs, have been prepared, and in vitro results suggest that they may potentially serve as potent anticancer nano-therapeutics. Novel cationic PLAs (CPLAs) have been synthesized, and their application as transfection agents in the delivery of anticancer small interfering RNA (siRNA) has been demonstrated. Unique CPLA-based nanocapsules enabling drug delivery, gene delivery, and drug-gene co-delivery have also been achieved. These nanocapsules can effectively evade drug resistance of cancer cells. Synergistic treatment effects are observed in the drug-gene co-delivery via the nanocapsules.

Keywords: polylactides, cancer, drug delivery, gene delivery, drug-gene co-delivery

1 FUNCTIONAL PLAS

Integration of degradability in biological environment with delivery scaffolds is critical for therapeutic delivery systems especially when polymeric scaffolds with hydrodynamic sizes larger than the threshold size for kidney clearance, because otherwise there can be remarkable side effects from these scaffolds.[1, 2] As an important class of biodegradable polymers, PLAs have been approved by FDA for clinical applications.[1] Conventional PLAs have only terminal functional groups available. Although some important studies on biomedical applications of PLAs have been performed by engineering through their terminal groups,[3] overall the lack of functionalities of conventional PLAs significantly limits their applicability in biomedical field.

Therefore, in recent years we have made significant efforts in developing functional PLAs via post-polymerization functionalization strategy (**Figure 1**).[4] Thiol-ene and alkyne-azide reactions were selected for functionalization because they are click reactions with broad applicability.[5] There are three key steps in the general routes for the synthesis of functional PLAs. At first, alkene and alkyne-functionalized lactides were prepared by multi-step organic synthesis. Then, ring-opening polymerization (ROP) of these monomers, with or without using the non-functionalized conventional lactide as the comonomer, was performed using 4-dimethylaminopyridine (DMAP) as the organocatalyst to yield well-defined PLAs with alkene or alkyne groups. Finally, click functionalization reactions of the alkene/alkyne-functionalized PLAs with thiol/azide-containing functionalization agents were performed to access PLAs carrying various biomedical related functionalities, including drug moieties, cationic groups, solubility-enhancing structures, and dyes. Among them, drug moieties are employed for drug delivery; cationic groups are utilized for gene adsorption; solubility-enhancing structures can improve water-solubility or water-dispersity of the PLA-based systems; dyes are used for bioimaging. In some cases, intermediate functional groups were introduced through click functionalization, and then converted to the final biomedical relevant groups.

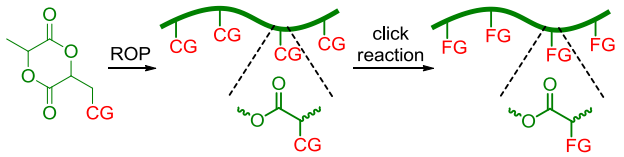

Figure 1. Scheme for the synthesis of functional PLAs

Because cancer is a leading cause of death of human beings, the functional PLA-based systems in our work are designed specifically for the delivery of cancer therapeutics. To capitalize the enhanced permeability and retention (EPR) effects for passive targeting, nanoscopic dimensions (10-100 nm) are targeted in the design of functional PLA-based systems.[6]

2 FUNCTIONAL PLA-BASED DRUG DELIVERY SYSTEMS

To construct drug delivery systems, drug can be incorporated with delivery scaffolds through either encapsulation or conjugation strategies. Because unfavorable burst release of drug is common for drug-encapsulated systems, we paid more attention to develop drug-conjugated systems. Several types of functional PLA-based drug-conjugated systems, including brush polymer-drug conjugates (BPDCs), polymer-drug conjugate (PDC) nanoparticles (NPs) and zwitterionic PDCs, have been synthesized, characterized and evaluated for drug delivery applications (**Figure 2**).

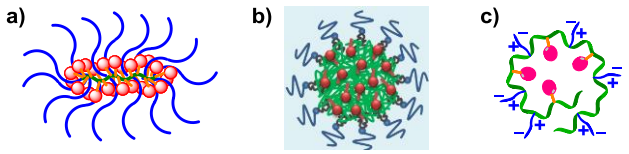

Figure 2. Schematic illustrations of functional PLA-based drug delivery systems: a) BPDC, b) PDC NP, and c) zwitterionic PDC

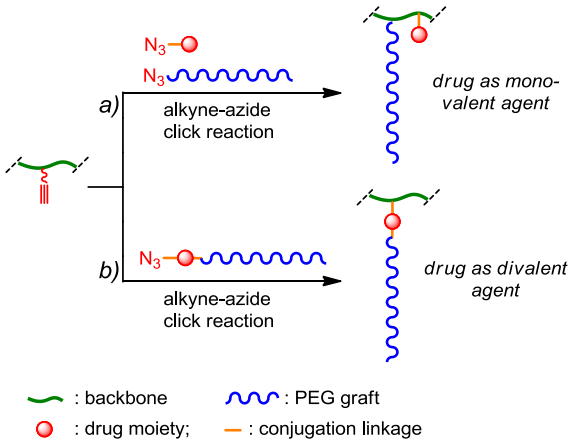

Figure 3. Scheme for the synthesis of functional PLA-based BPDCs with a) drug as mono-valent agent, and b) drug as divalent agent

Functional PLA-based BPDCs were designed to integrate nanoscopic degradable brush polymer templates with anticancer drug moieties.[7-9] Poly(ethylene glycol) (PEG)-based grafts, as solubility-enhancing structures, were used in these BPDCs. Two different types of grafting structures were prepared and studied (**Figure 3**). The first type of BPDCs possess both drug moieties and PEG chains as mono-valent agents (route a).[7, 8] The second type of BPDCs has drug moieties as divalent agents linking both backbones and PEG chains (route b).[9] Among them, the first type is the optimal structural design, because drug release from BPDCs requires the cleavage of only one conjugation bond. The BPDCs with the first type of grafting structures and carrying paclitaxel (PTX; 23 wt%)[7] or doxorubicin (Dox; 9 wt%; via alkyne-azide click reaction, followed by aldehyde-amine reaction)[8] showed sustained drug release behavior in PBS buffers and remarkable therapeutic effects in killing MCF7 breast cancer cells. The Dox-containing BPDCs with Schiff base conjugation linkages further demonstrated acid-triggered Dox release.[8] On the other hand, drug release from the BPDCs with drug moieties as divalent agents was sluggish and could not be readily controlled because the cleavage of two conjugation bonds with different sensitivity was involved.[9]

PLA-based PDC NPs were prepared from functional PLA with Dox side moieties (PLA-*g*-Dox), followed by nanoprecipitation with the presence of a PEG-based surfactant (**Figure 4**).[10] The NPs with 26 wt% of Dox showed well-controlled nanoscopic dimensions (~100 nm) and significant colloidal stability in aqueous solutions. With Schiff base acid-liable conjugation linkage, Dox release was promoted at acidic conditions (~70% release at pH 5.5 vs. ~20% release at pH 7.4 within 48 h). The NPs could readily internalize MCF7 cells. Relative to free Dox (as HCl salt), the NPs exhibited enhanced anticancer effects towards MCF-7 cells.

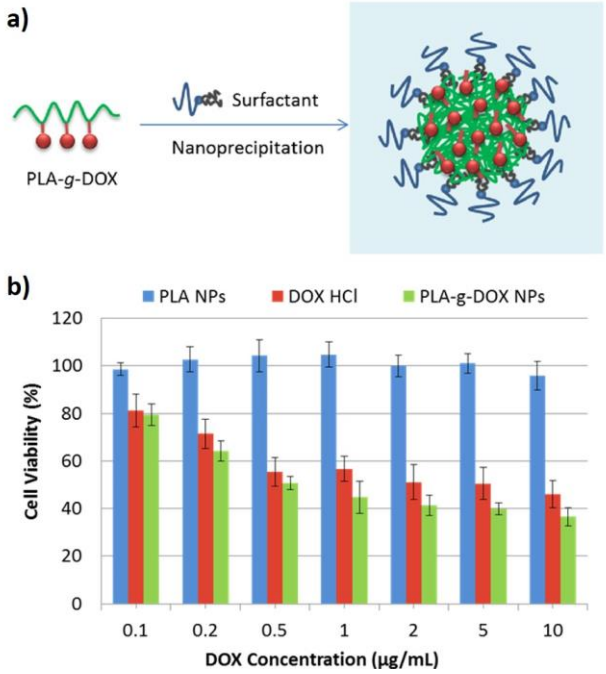

Figure 4. a) Scheme for the preparation of functional PLA-based PDC NPs; b) cytotoxicity of the PDC NPs towards MCF-7 cells (48 h; PLA NPs and Dox•HCl as controls). Adapted with permission.[10] Copyright 2014, American Chemical Society.

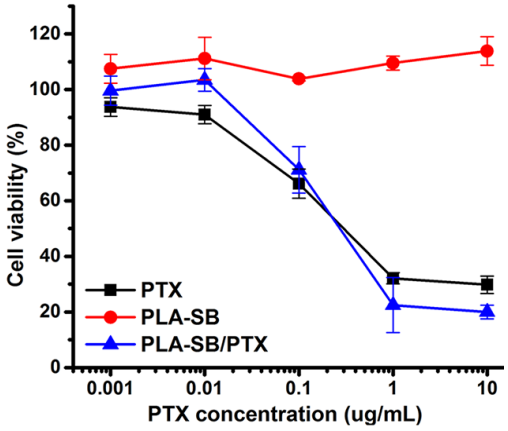

Figure 5. Cytotoxicity of zwitterionic PDC, PLA-SB/PTX towards A549 lung cancer cells (72 h; PTX and PLA-SB as controls). Adapted with permission.[11] Copyright 2017, Elsevier

Zwitterionic PDCs with PLA backbone and sulfobetaine (SB) and PTX side groups (i.e., PLA-SB/PTX) were prepared by thiol-ene functionalization of an alkene-functionalized PLA.[11] PLA-SB/PTX showed remarkable comprehensive biomedical relevant properties, including biodegradability, suppressed non-specific interaction with biomolecules, sustained PTX release, and ready cellular uptake. *In vitro* study exhibited significant anti-cancer efficacy of the zwitterionic PDC (**Figure 5**).

3 FUNCTIONAL PLA-BASED GENE DELIVERY SYSTEMS

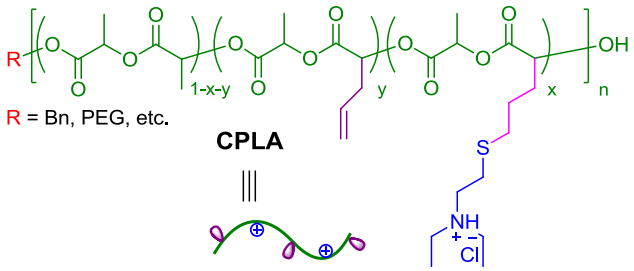

Figure 6. Chemical structure of cationic PLA

Cationic PLAs (CPLAs) with well-controlled mol% of positively-charged tertiary amine groups were synthesized by thiol-ene functionalization of alkene-functionalized PLAs which may carry either an alkyl group or a PEG block at α-terminals (**Figure 6**).[12, 13] Their hydrolytic degradation rate increases with amine mol%, and their cytotoxicity is insignificant. They can readily complex with negatively-charged genetic materials. The resulting nanoplexes can effectively protect genes against enzymatic degradation. The effectiveness of delivery of anticancer IL-8 siRNA was demonstrated (IL-8 is an a growth factor). The CPLA-IL-8 siRNA nanoplexes can be readily taken up by PC3 prostate cancer cells, leading to significant IL-8 gene silencing. The transfection efficiency of the nanoplexes positively correlates with the amine mol% of CPLAs, and can be comparable or even higher than that of the delivery system using Mirus TransIT, a commercial transfection agent. In vivo delivery of IL-8 siRNA by CPLAs can lead to significant inhibition of growth of aggressive prostate cancer (CaP).[14]

4 CPLA NANOCAPSULES FOR INDIVIDUAL AND CO-DELIVERY OF DRUG AND GENE

CPLA nanocapsules (NCs) were prepared by UV-induced thiol-ene crosslinking of CPLAs in transparent miniemulsions (**Figure 7**).[15] Sizes of CPLA NCs can be readily controlled. They possess remarkable hydrolytic degradability, without showing noticeable cytotoxicity towards MCF7 cells. From the perspective of structural design, they can serve as valid delivery scaffolds for individual and co-delivery of drug and gene for the following reasons: 1) their inner cavities can be used to encapsulate hydrophobic drugs; 2) their positively-charged CPLA shells can adsorb genetic materials for delivery; and 3) their hydrolytic degradability can minimize long-term side effects of the scaffolds. With the presence of Dox in the oil phase before miniemulsification, 12 wt% of Dox relative to scaffold was readily loaded into CPLA NCs with 74% loading efficiency. Aqueous solutions of CPLA NCs, with or without Dox loading, were obtained by removal of low boiling-point oil (i.e. $CHCl_3$). The loading of IL-8 siRNA was easily accomplished by mixing aqueous solutions of CPLA NCs and IL-8 siRNA.

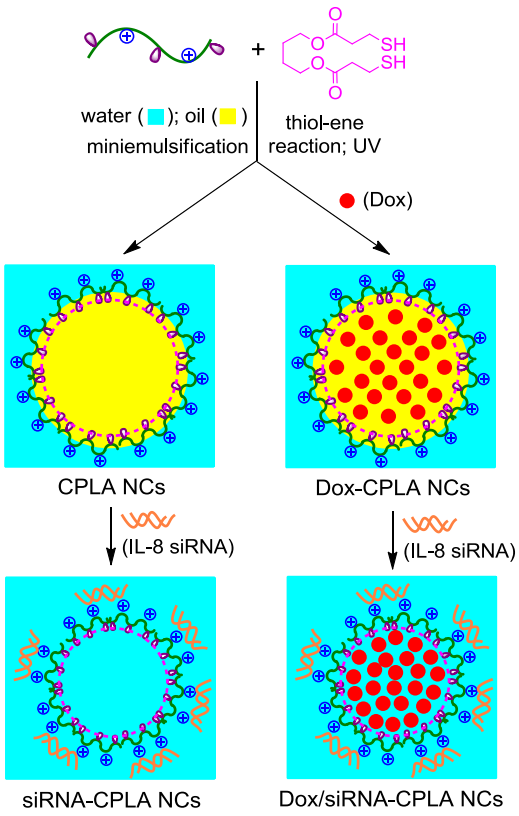

Figure 7. Scheme for preparation and therapeutic loading of CPLA NCs. Adapted with permission.[15] Copyright 2013, Royal Society of Chemistry

The effectiveness of individual delivery of drug and gene to cancer cells by CPLA NCs were demonstrated.[15] Relative to free Dox, the Dox-encapsulated NCs resulted in increased intracellular drug concentration and reduced cell viability of MCF7/ADR cancer cells, due to the encapsulated drug can bypass P-glycoprotein (Pgp)-mediated multidrug resistance. By using IL-8 siRNA as the genetic material for PC3 cells, CPLA NCs led to significant gene silencing efficiency similar to that via commercial transfection agents, i.e. lipofectamine and Mirus TransIT.

Co-delivery of Dox and IL-8 siRNA into PC3 cancer cells was successfully achieved by using CPLA NCs as the delivery scaffold

(**Figure 8**).[15] Both Dox and IL-8 siRNA were delivered into PC3 cells in the same timescale. CPLA NCs loaded with both agents exhibited noticeably higher cytotoxicity than CPLA NCs loaded with only Dox towards PC3 cells at low Dox concentrations (≤ 1 μM), presumably because the silencing of IL-8 gene made the cells more sensitive to Dox.

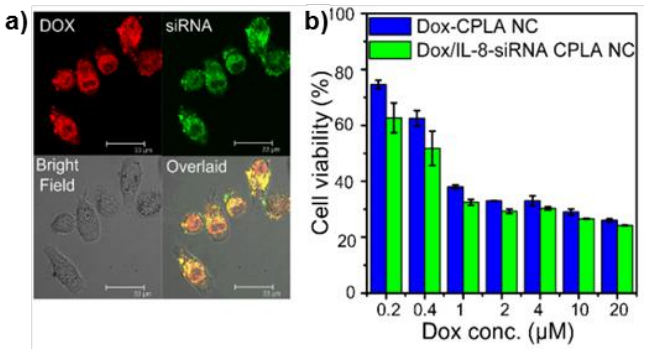

Figure 8. a) Confocal images of PC3 cells treated with CPLA NCs carrying both Dox (red) and dye-labelled IL-8 siRNA (green) for 4 h. (f) Cell viability data of PC3 cells treated with Dox-loaded CPLA NCs and Dox & IL-8-siRNA-loaded CPLA NCs for 72 h. Adapted with permission.[15] Copyright 2013, Royal Society of Chemistry.

5 SUMMARY

A variety of well-defined functional PLAs carrying biomedical relevant functional groups through side chains were prepared by ring-opening polymerization of alkene/alkyne-functionalized LAs, followed by post-polymerization modifications based on click chemistries. Drug-conjugated PLAs and CPLAs were studied for the delivery of anticancer drugs and IL-8 siRNA, respectively. CPLA NCs were investigated for individual and co-delivery of anticancer drugs and IL-8 siRNA. The preliminary biomedical results obtained from these systems encourage further biomedical studies to explore their significant potentials for cancer treatment.

6 ACKNOWLEDGEMENTS

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