Drug delivery

# Hierarchical Supermolecular Structures for Sustained Drug Release**

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In this study, we report the formation of a hierarchical supermolecular structure, a fiber-shaped block copolymer/paclitaxel complex, from the self-assembly of stereoregular amphiphilic block copolymer mixtures with paclitaxel, a well-known anticancer drug. The behavior of the unusual self-assembled structure in drug loading and in vitro release is explored for potential biomedical applications such as a localized and sustained drug delivery system. Paclitaxel, which is extracted from the bark of the Pacific Yew tree (*Taxus brevifolia*), is a natural taxane effective against various tumors like ovarian and breast cancer.[1] It prevents cancer cells from dividing by binding and stabilizing the microtubes and inhibiting the disassembly into tublins at the end of the mitosis process. However, it has limited solubility in water (0.3 mg $L^{-1}$ at 37 °C), making application of paclitaxel in cancer therapy difficult.[2] Currently, Taxol is the only formulation available for clinical use, where paclitaxel is dissolved in a 50:50 mixture of Cremophore EL (polyethoxylated castor oil) and dehydrated alcohol. Unfortunately, the organic medium Cremophore EL has been reported to cause a migration of suspected carcinogens such as di-(2-ethylhexyl)-phthalate (DEHP) from the infusion bags frequently used in hospitals,[3] and severe side effects such as hypersensitivity reactions, nephrotoxicity, neurotoxicity, and cardiotoxicity have been documented.[4] Thus, various polymer-based carriers like microspheres,[5–8] nanoparticles,[9–10] micelles,[11–13] and electrospun fibers[14–16] have been explored with a growing need to develop an alternative delivery system. Although the microparticles[5–8] and electrospun fibers[14–16] suggested therapeutic efficacy due to the sustained release and localized delivery of paclitaxel,[17] these systems composed of hydrophobic poly(lactic-*co*-glycolic acid) (PLGA) or poly(L-lactic acid) (PLLA) could not avoid plasma protein adsorption and fibrosis formation, common problems associated with implantation devices.

Efficient drug absorption and physical stability are two critical factors in determining the clinical efficacy of drug delivery systems. Amphiphilic block copolymer micelles have received a great deal of attention for application in the biomedical field because of their small size (<200 nm) and prolonged activity in systemic circulation without the accompanying adsorption of plasma protein on the hydrophilic surfaces of the block copolymers or the recognition by macrophages of the reticuloendothelial system (RES). However, the loading efficiency of paclitaxel into micelles is low (below 30%),[18] and the polymeric micelles are equilibrium systems that are susceptible to infinite dilution arising from their administration.[14,16] The chemical diversity and precisely tunable structure of block copolymers combined with new synthetic methodologies and efficient organic reactions can lead to the use of noncovalent interactions in polymeric assemblies to control polymer design for functional materials and complex architectures.[19–22] For example, Kang et al. showed that spherical nanometric micelles formed from stereocomplexation of stereostructure-controlled block copolymer mixtures, that is, poly(ethylene glycol)-*block*-poly(L-lactide) (PEG-*b*-PLLA) and poly(ethylene glycol)-*block*-poly(D-lactide) (PEG-*b*-PDLA), have enhanced stability with the potential for the delivery of drugs.[21] While the most common micellar structure consists of a spherical core surrounded with corona chains, a variety of supramolecular assemblies including elongated cylindrical micelles, vesicles, and helices can also be realized from specific amphiphilic block copolymer micelles in selective solvents.[22] Here, we, for the first time, report hierarchical supermolecular structures self-assembled from PEG-*b*-PLLA or PEG-*b*-PDLA or a mixture of PEG-*b*-PLLA and PEG-*b*-PDLA with paclitaxel.

Figure 1 illustrates the general procedure to produce supramolecular structures from the self-assembly of block copolymers and paclitaxel through a membrane dialysis method using dimethylformamide (DMF) and water as a

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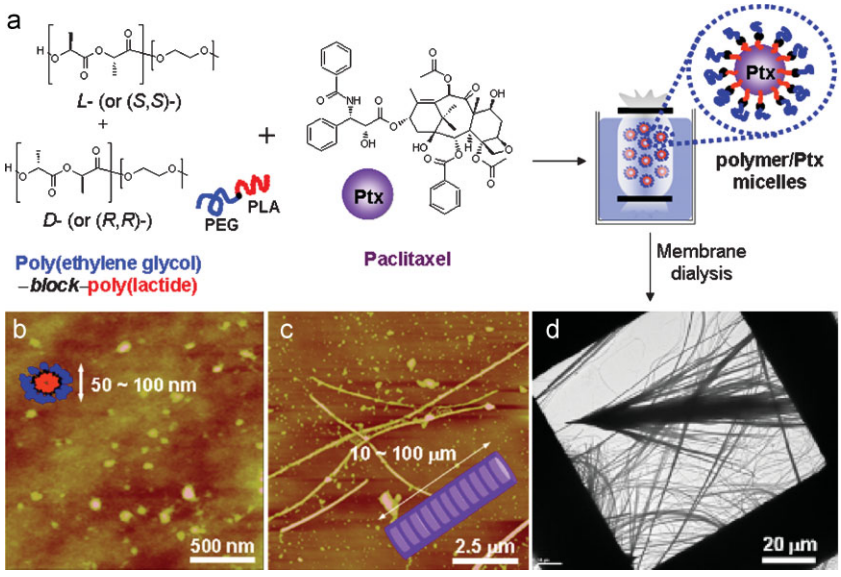


**Figure 1.** Schematic procedure to prepare block copolymer/paclitaxel supermolecular structures using membrane diaysis (a), and AFM and transmission electron microscopy (TEM) images showing morphologies formed from PEG-*b*-PLLA+PEG-*b*-PDLA (b), and (PEG-*b*-PLLA+PEG-*b*-PDLA)/paclitaxel mixture (c,d).

solvent and a nonsolvent, respectively. Atomic force microscopy (AFM) images show that the air-dried stereoblock copolymer/paclitaxel mixture forms a long fiber-like assembled structure, while complexes formed from the block copolymer mixture without paclitaxel have a spherical shape of tens of nanometers as observed by others (Figure 1b–d).[21] We have found no reports on the formation of elongated fiber-like hierarchical structures through the coassembly of PEG-*b*-PLA with paclitaxel. Paclitaxel molecule contains a unique stereochemistry, that is, four *R*- and seven *S*-chiral carbons. It was shown that paclitaxel molecules alone assembled into long ribbon-like or hollow fibrous structures from the same membrane dialysis process (see Supporting Information, Figure S1) as reported in previous works.[23,24] The paclitaxel-only fiber structure floats on the water surface, indicative of low density, well-contrasted with those from the block copolymer/paclitaxel complex.

Figure 2 shows morphologies of block copolymer/paclitaxel assemblies when varying the initial paclitaxel content from 1 to 2 and 5 mg. Addition of more paclitaxel in PEG-*b*-PLLA and PEG-*b*-PDLA block copolymers led to a drastic change in their resulting morphologies from dumbbell-shaped fibers to bundles of straight fibers to individual fibers. The dimensions of the dumbbell-shaped fibers were ≈200 nm in diameter and ≈40 μm in length, while the bundle of straight fibers was ≈200 nm in diameter and ≈200 μm in length, which was similar to that of the individual fibers. These supermolecular structures had a high density and easily settled down to the bottom of the dialysis bag. Nanoparticles were also formed in the supernatant. The mass of settled supermolecular structures increased with paclitaxel content. At 1 mg paclitaxel, only a small amount of supermolecular structures were formed. In sharp contrast, at the initial paclitaxel loading of 5 mg, the majority of PLA and paclitaxel complexes were in supermolecular structures (more than 83% in weight). In addition, the majority of paclitaxel was incorporated into the supermolecular structures. For example, the final loading levels of paclitaxel in the supernatant and the sendiment were measured to be ca. 0.89% and 40.6% in weight, respectively.

The effect of the molecular weight of PLA block copolymers on the resulting morphology of paclitaxel-induced assemblies could be inferred from the scanning electron microscopy (SEM) images in Figure 3. We can also see the formation of highly elongated, fiber-like supermolecular structures depending on the paclitaxel loading. As similarly demonstrated in a study with various molecular weights of block copolymers,[21,25] the diameters of paclitaxel-loaded stereocomplex block copolymers with the greatest proportion of lactide units ranging from 2k- to 5k- and 10k-block length PLA were greater. Structures prepared from 10k-PLA block copolymer mixtures were more evenly shaped as compared to those prepared from 2k and 5k PLA block copolymers. X-ray diffraction (XRD) patterns of paclitaxel stereocomplexes and paclitaxel-loaded stereocomplexes were tested to further study the physical state of paclitaxel in these systems. As shown in Figure 3d, pure paclitaxel shows three intense peaks at $2\theta$ of 5.74°, 9.05°, and 12.74°. The D-block/L-block mixture of 10k PLA shows both the peaks of PEG at 18.3° and 23.5° and those due to PLA stereocomplex formation at 12.0° and 20.8°. These stereocomplex peaks are still found in the paclitaxel-loaded block copolymers, evidencing the absence of crystalline paclitaxel. Differential scanning calorimetry (DSC) was performed to investigate further details on the physical state

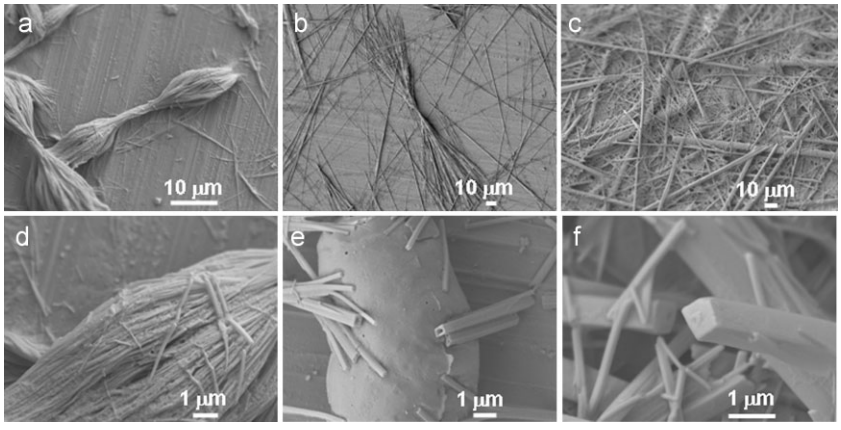


**Figure 2.** Field-emission SEM images for block copolymer/paclitaxel assemblies after adding 1 (a,d), 2 (b,e), and 5 mg (c,f) paclitaxel in 15 mg of PEG-*b*-PLLA (5k–5k) + PEG-*b*-PDLA (5k–5k) solution.

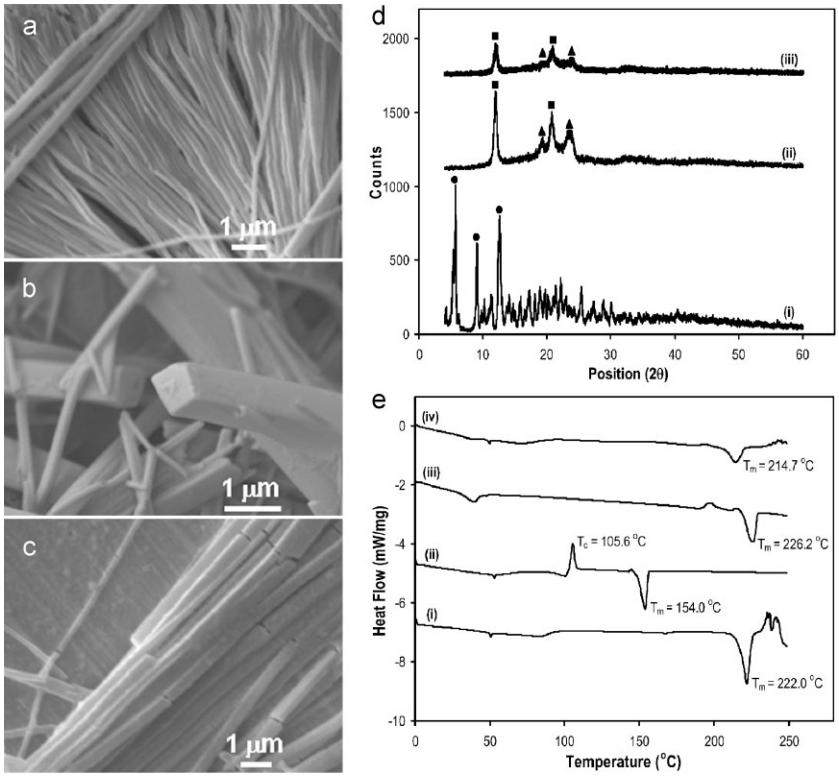


**Figure 3.** SEM images of self-assembled structures from block copolymer/paclitaxel (15 mg/5 mg) mixtures with different PLA block lengths of 2k (a), 5k (b), and 10k (c), and their XRD patterns (d) as well as DSC thermograms (e). For the XRD results, i) paclitaxel, ii) 10k sterecomplex, and iii) 10k stereocomplex with 5 mg paclitaxel, where (●), (■), and (▲) represent those from paclitaxel, stereocomplex formation (with a 10k-PLA block length), and PEG, respectively. For the DSC results, i) paclitaxel, ii) 10k PDLA, iii) 10k stereocomplex, and iv) 10k stereocomplex with 5 mg paclitaxel.

of the block copolymer/paclitaxel assemblies. The mixture of L- and D-block copolymers prepared from our catalyst show a crystalline melting temperature, $T_m$, of ≈226 °C, significantly higher than that of PLA homocrystal (≈154 °C).[26,27] The complex of D-block/L-block mixture and paclitaxel displays the melting point at ≈215 °C. From the combined consideration of this DSC results with the XRD results, we can see the formation of stable stereocomplex PLA block copolymers even after coassembly of block copolymers with paclitaxel. Full details of XRD and DSC results from a different molecular weight of block copolymer are described in the Supporting Information (Figure S2).

Finally, the effect of PLA chirality on drug loading and micellar stability was further explored using 10k D-block, L-block, and their D/L stereomixture with 5 mg paclitaxel. No paclitaxel melting point peak was observed in any of the three types of supermolecular structures, suggesting that paclitaxel molecules were encapsulated within the assemblies at the molecular level. The final loading content of paclitaxel was determined to be about 30%, 41%, and 60% in weight for D-block, L-block, and D/L stereomixture, respectively (see Supporting Information, Figure S3). The mixture of D/L stereopair block copolymers offers the most favorable environment to encapsulate paclitaxel molecules into PEG-*b*-PLA micelles. Furthermore, the chemical composition of the surface of paclitaxel-loaded stereoblock copolymer assemblies (PEG-*b*-PLA of 5–10k, 5 mg of initial paclitaxel loading) was studied via X-ray photoelectron spectroscopy (XPS). The O/C mass ratio of the surface of the paclitaxel- loaded stereoblock copolymer assemblies was 37:63, which was close to that of PEG (40:60), but very different from those of PLA and paclitaxel (47:53 and 29:71, respectively), indicating that the surfaces of paclitaxel-loaded stereoblock copolymer assemblies consist mainly of PEG. The in vitro drug release profile of the paclitaxel-loaded stereoblock copolymer assembly (10k, 5 mg of initial paclitaxel loading) was also tested in phosphate-buffered saline (PBS, pH 7.4) at 37 °C (Figure 4). A sustained release over 30 days was achieved without significant initial burst. A high loading level and sustained release of paclitaxel over more than 30 days without significant initial burst were achieved after the formation of a new interesting supermolecular morphology of fiber-like stereoblock copolymer/paclitaxel complexes, as similarly observed in the results of electrospun fibers.[16] In addition, the small size of paclitaxel-loaded supermolecular structures and hydrophilic PEG shells formed on the surfaces is expected to prevent protein deposition and cell/tissue adhesion[28,29] and make them suitable for injection. Localized delivery may result in a reduced systemic toxicity of paclitaxel, and sustained release improves the therapeutic efficacy of paclitaxel,[5,17] which could increase cell exposure time to prevent aggravation of multidrug resistant (MDR) cancer cells, cancer regrowth, and neoangiogenesis.[30]

In summary, tens of micrometers-long fiber-like hierarchical supermolecular structures with a radius of ≈200 nm were formed from coassembly of a stereocontrolled block copoly-

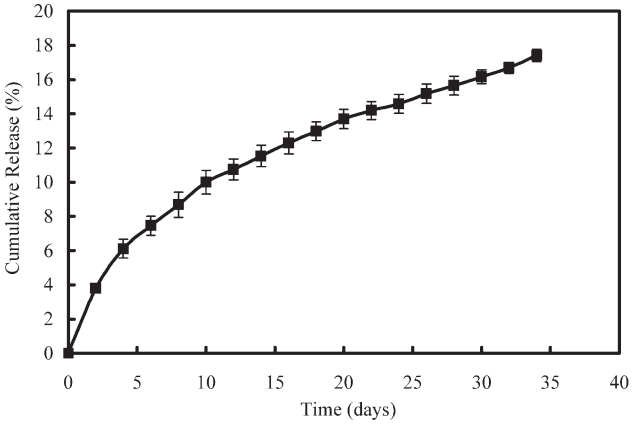


**Figure 4.** In vitro drug release profile of paclitaxel-loaded stereoblock copolymer assemblies (PEG-*b*-PLAs (5k-b-10k) at pH 7.4 and 37.0 ± 0.1 °C.

mer mixture with paclitaxel using a membrane dialysis technique. The PEG-*b*-PDLA/PEG-*b*-PLLA stereoblock copolymer mixture with a 10k-PLA block length offered the most favorable environment to encapsulate paclitaxel at the molecular level. Importantly, the paclitaxel-loaded block copolymer complexes possess a PEG shell, show a stable sustained release of paclitaxel under the simulated physiological conditions, and have a great potential in clinical applications for localized drug delivery.

### Experimental Section

PEG-*b*-PLA block copolymers were synthesized by the ring-opening polymerization (ROP) of lactide monomers using monomethoxy hydroxyl PEG ($PEG_{5k}$-OH) as a macrointiator, in which the stereosequence of the resulting block copolymer was governed by the optical purity of lactide monomers. The ring-opening of enantiomeric lactides was performed in a glovebox using thiourea and tertiary amine catalysts designed for bifunctional activation of both monomer and alcohol through hydrogen bonding. Coassembly of paclitaxel with block copolymer was carried out using a membrane dialysis technique. Block copolymer (i.e., PEG-*b*-PDLA, PEG-*b*-PLLA, or their equimolar mixture, 15 mg) and paclitaxel (1, 2, or 5 mg) were dissolved in 5 mL of DMF, and the solution was dialyzed with water. The loading level of paclitaxel in block copolymer/paclitaxel complexes was determined with high performance liquid chromatography (HPLC). In vitro release experiments of paclitaxel-loaded assemblies were done by dissolving freeze-dried samples in PBS buffer (pH 7.4), followed by extraction of released medium with dichloromethane (DCM) and HPLC measurement (full experimental details given in the Supporting Information).

**Keywords:**
block copolymers · paclitaxel · self-assembly · stereocomplexes · supermolecular structures

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