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Bioresorbable Vesicles Formed through Spontaneous Self-Assembly of Amphiphilic Poly(ethylene oxide)-*block*-polycaprolactone

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Liposomes (nontoxic/nonantigenic vesicles derived from phospholipids) have long been utilized in numerous biotechnology and pharmaceutical applications to improve therapeutic indices and enhance cellular uptake.1 Their structural stability, however, is dependent upon many intrinsic and environmental parameters that often serve to compromise their efficacy.2 Polymersomes (polymer vesicles formed from a wide variety of fully synthetic amphiphiles) 3−5 have similar utility to their lipid counterparts but possess several advantageous properties including vastly superior stability6 and diverse functionality afforded by tuning material chemistries through polymer synthesis. Recently, there has been considerable interest in the development of degradable polymersomes suitable for in vivo drug delivery. Here, we present the generation of self-assembled vesicles comprised entirely of an amphiphilic diblock copolymer of poly(ethylene oxide) (PEO) and polycaprolactone (PCL), two previously FDA-approved polymers. Unlike degradable polymersomes formed from blending bioinert and hydrolyzable components,7,8 PEO-*b*-PCL-based vesicles promise to be fully bioresorbable,[9] leaving no potentially toxic byproducts upon their degradation. Moreover, unlike published reports of other degradable (peptide-, polyester-, or polyanhydride-based) polymersomes,[10–12] these bioresorbable vesicles are formed through spontaneous self-assembly of their pure component amphiphile.

Poly(ethylene oxide) was chosen as the hydrophilic block as it imparts to the vesicle's surface biocompatibility and prolonged blood circulation times.13−15 Polycaprolactone constitutes the vesicles' hydrophobic membrane portion. PCL is degraded by hydrolysis of its ester linkages in physiological conditions (such as in the human body) and has therefore received a great deal of attention for use as an implantable biomaterial in drug delivery devices, bioresorbable sutures, adhesion barriers, and scaffolds for injury repair via tissue engineering.16−19 Compared to other biodegradable aliphatic polyesters, PCL has several advantageous properties, including (1) high permeability to small drug molecules, (2) maintenance of a

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Supporting Information Available: Materials and methods, NMR, and DLS data. This material is available free of charge via the Internet at http://pubs.acs.org.

neutral pH environment upon degradation, (3) facility in forming blends with other polymers, and (4) suitability for long-term delivery afforded by slow erosion kinetics as compared to polylactide (PLA), polyglycolide (PGA), and polylactic-*co*-glycolic acid (PLGA).[17] Utilization of PCL as the hydrophobic block in our formulations promises that the resultant polymersomes should have safe and complete in vivo degradation.

Amphiphilic poly(ethylene oxide)-*b*-polycaprolactone was generated via ring-opening polymerization of cyclic ∈-caprolactone (CL) in the presence of stannous(II) octoate (SnOct) and monocyano- or monomethoxypoly(ethylene oxide) (PEO, 0.75K, 1.1K, 1.5K, 2K, 5K, 5.5K, 5.8K; Polymer Source, Dorval, Canada).[20] The reactions yielded PEO-*b*-PCL copolymers with varying PCL block size (between 2K and 30K); pure PEO-*b*-PCL diblock copolymers were isolated via gel permeation chromatography (GPC; PDI 1.1–1.3). The molecular weight values and PCL/PEO ratios of the various copolymers were evaluated from their ^{1}H NMR spectra (supplemental Figure 1 in Supporting Information) and corresponded to those obtained by GPC. The polymers were dissolved in methylene chloride, with and without 1 wt % Nile Red (for facile visualization), and deposited on the surface of roughened Teflon. The polymer films were then dried for >12 h under vacuum and rehydrated in aqueous solution. The samples were subsequently sealed and heated at 65 °C (above the melting point of the diblock) for 24–48 h. Each aqueous sample was then plated on a microscope slide and the resultant polymer morphology visualized.

Figure 1 illustrates 10–20 μm diameter polymersomes formed from self-assembly of PEO(2K)-*b*-PCL(12K) (PDI = 1.2) upon heating in aqueous solution for 48 h. The micrographs were taken via fluorescence scanning confocal microscopy (BioRad, Hercules, CA) and illustrate that the polymer exclusively forms vesicles with a continuous membrane structure (Nile Red dissolved in bilayer, Figure 1A) surrounding an entrapped aqueous volume (Calcein, Figure 1B). Calcein from the external solution was removed by dialysis or membrane ultracentrifugation to yield stable vesicles that entrap their aqueous encapsulants (at 4 °C and pH = 7.4) as measured for greater than 1 month. The size distributions of the polymersomes can be controlled with standard techniques such as sonication, freeze/thaw extraction, and extrusion above T = 65 °C to yield monodispersed vesicle diameters ranging anywhere from tens of microns (confocal micrographs, Figure 1A,B) to hundred nanometer sized structures (cryo-TEM micrograph, Figure 1C) useful for in vivo applications.[13,21] Of the numerous PEO-*b*-PCL formulations screened, including PEO(5.8K)-*b*-PCL(24K) that has been previously shown to form vesicles via a solvent injection technique,[11] only PEO(2K)-*b*-PCL(12K) afforded a robust yield of polymersomes via spontaneous self-assembly. In this regard, it is important to underscore that once these PEO-(2K)-*b*-PCL(12K)-based polymersomes are formed (at $T > T_m$), their vesicular morphology is fixed at lower temperatures ranging from 37 °C to cryogenic. While all previously known vesicle-generating, self-assembled, amphiphilic diblock polymers possess a hydrophilic volume fraction of 0.3–0.4,[4,6] notably PEO(2K)-*b*-PCL(12K) possesses a calculated fraction[22] which is significantly lower (~0.15). A complete study of PEO-*b*-PCL phase behavior and morphology in aqueous solution will be presented elsewhere.

Differential scanning calorimetry (DSC; TA Instruments Q100, New Castle, DE) was utilized to elucidate the thermal transitions of PEO(2K)-*b*-PCL(12K) in bulk and within aqueous vesicle solutions (Figure 2). Figure 2A shows two distinct first-order transitions in bulk PEO(2K)-*b*-PCL(12K) consistent with a diblock copolymer comprised of two crystallizable homopolymers[20,23] (onset of melting = 52.6 °C; total heat of transition = 90.2 J/g). Immediately upon dispersion of the dry polymer in water (at 30 mg/mL), the double melting peaks were transformed to a single first-order transition with a peak melting temperature at 52.3 °C upon second heating (onset of melting = 48.8 °C; heat of transition = 33.2 J/g; Figure 2B). The polymer showed no further changes in its thermal transitions upon aqueous dissolution

and isothermal heating at 65 °C for 48 h. DSC of PEO(2K)-*b*-PCL(12K)-based polymersomes, whose structure was observed by cryogenic transmission electron microscopy (cryo-TEM) and size distributions determined by dynamic light scattering (DLS; see supplemental Figure 2 in Supporting Information), displayed the same first-order transition upon heating irrespective of vesicle size (onset of melting = 48 °C; peak = 52 °C; heat of transition = 43 J/g). The isothermal crystallization and melting behavior of bulk PEO-*b*-PCL has been previously studied by WAXD, SAXS, and DSC, which demonstrated that despite strong crystallizablility in the PEO homopolymer, only the PCL block in the PEO-*b*-PCL copolymer is crystallizable when the PEO weight fraction is less than 20%.[23] As such, it is strongly suggestive that in PEO-(2K)-*b*-PCL(12K)-based vesicles the membrane consists entirely of a PCL lamella with a PEO corona facing the external solution and internal aqueous milieu.

Aliphatic polyesters, including PLA, PGA, and PCL, and their copolymers, have been extensively studied for controlled release and targeted drug delivery. We assessed the mechanism by which PEO(2K)-*b*-PCL(12K)-based polymersomes release a physiologically relevant encapsulated component. As a model system, doxorubicin (DOX), a weakly basic anti-neoplastic agent, was incorporated into the internal aqueous milieu of 200 nm diameter PEO(2K)-*b*-PCL(12K)-based vesicles via an ammonia sulfate gradient[24–26] (see Materials and Methods in Supporting Information); in situ DOX release in various physiological conditions (pH 5.5 and 7.4; T = 37 °C) was subsequently monitored fluorometrically (λ_{ex} = 480 nm, λ_{em} = 590 nm) over 14 days (Figure 3A).

Under both pH conditions (5.5 and 7.4, at 37 °C), we observed an immediate burst release phase (~20% of initial vesicle load from 0 to 8 h), followed by controlled release. The dynamics of release were different at the two pHs. At pH 7.4, release was observed in two distinct phases, denoted α and β, which were well fit by exponential regression analysis (R^2 = 0.99). The α phase (days 1–5, dotted line Figure 3B) corresponds to a regime where DOX release is predominantly dependent upon the rate of drug permeation through the PCL membrane, dominating the slower rate of matrix erosion (see schematic Figure 3C). The β phase (days 5–14, solid line Figure 3B) is consistent with DOX release facilitated predominantly by significant hydrolytic membrane degradation. At pH 5.4, a single phase (β′) is observed. The rate constant for release in this phase is similar to that observed during the β phase of pH 7.4, indicating that the mechanism of release is similar. DOX release at pH 5.4, however, is more rapid since acid-catalyzed hydrolysis of the PCL membrane is the dominant mechanism at short times.

As such, in vivo drug release from these bioresorbable polymersomes will likely depend on both PCL matrix erosion and a drug's intrinsic permeability from the aqueous core through the membrane. Notably, when compared to degradable polymersomes formed from blending "bioinert" and hydrolyzable components (where $\tau_{1/2}$ release[7,8] ≈ $\tau_{1/2}$ circulation[15] ~ tens of hours), PEO(2K)-*b*-PCL(12K)-based vesicles possess much slower release kinetics ($\tau_{1/2}$ release ~ days), offering potential advantages for future intravascular drug delivery applications. Moreover, their large membrane core thickness (22.5 ± 2.3 nm) affords the opportunity for facile incorporation of both hydrophobic (membrane sequestered) and hydrophilic (internal aqueous core) compounds within a single complex delivery vehicle. Finally, the self-assembled vesicular architecture allows for the economic generation of mesoscopic (nanometer to micron) colloidal devices, enabling large-scale production while eliminating the need for costly removal of organic cosolvents postassembly.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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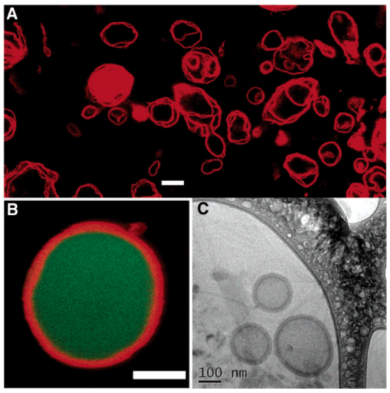

Figure 1.

(A) Scanning fluorescence confocal micrograph of PEO(2K)-*b*-PCL(12K)-based vesicles, containing membrane-encapsulated Nile Red (peak emission = 603 nm) in DI water at 25 °C, that display continuous spherical morphology but jagged edges supportive of solid vesicle membranes. Scale bar = 5 μm. (B) Confocal micrograph of PEO(2K)-*b*-PCL(12K)-based vesicles containing membrane-encapsulated Nile Red (2 mol % loading, peak emission = 603 nm) and aqueous entrapped Calcein dyes (250 μm, peak emission = 520 nm). Temperature = 25 °C; scale bar = 5 μm. (C) Cryogenic transmission electron micrograph of PEO(2K)-*b*-PCL (12K)-based vesicles in DI water (5 mg/mL). The membrane core thickness of the vesicles is 22.5 ± 2.3 nm. Scale bar = 100 nm.

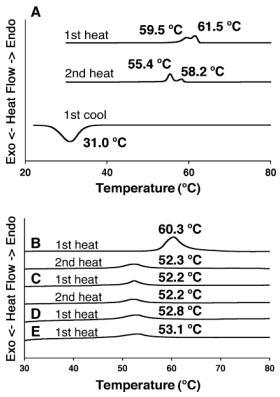

Figure 2.

Differential scanning calorimetry (DSC) of amphiphilic PEO(2K)-*b*-PCL(12K) diblock copolymers in (A) bulk, (B) immediately after dissolution in DI water at 30 mg/mL, and (C) after dissolution in DI water (30 mg/mL) and isothermal heating at 65 °C for 48 h. (D) DSC of aqueous solutions of 400 nm diameter PEO(2K)-*b*-PCL(12K)-based polymersomes (~30 mg/mL). (E) DSC of aqueous solutions of 200 nm diameter PEO(2K)-*b*-PCL(12K)-based polymersomes (~30 mg/mL). Scan rate = 10 °C/min using hermetically sealed aluminum pans (typical sample weight = 12 mg).

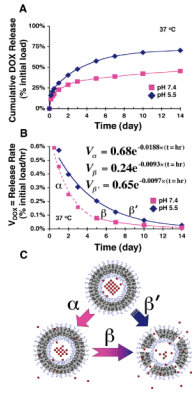

Figure 3.

(A) Cumulative in situ release of doxorubicin, loaded within 200 nm diameter PEO(2K)-*b*-PCL(12K)-based polymersomes, under various physiological conditions (pH 5.5 and 7.4; T = 37 °C) as measured fluorometrically over 14 days. N = 4 samples at each data point; individual data points for each sample varied by less than 10% of the value displayed at each time interval. (B) Release rates of DOX (V_{dox}) from 200 nm diameter PEO(2K)-*b*-PCL(12K)-based polymersomes vs time. Dotted and solid lines represent exponential fits obtained by regression analysis (R^2 = 0.99 for each curve), and the displayed equations correspond to the respective release regimes (α, β, β′). (C) Schematic illustrating differing regimes of DOX release via (α) intrinsic drug permeation through intact vesicle membranes vs (β and β′) release predominantly by PCL matrix degradation.