Bioresorbable Vesicles Formed through Spontaneous Self-Assembly of Amphiphilic Polyethyleneoxide-Block-Polycaprolactone

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Supporting Information

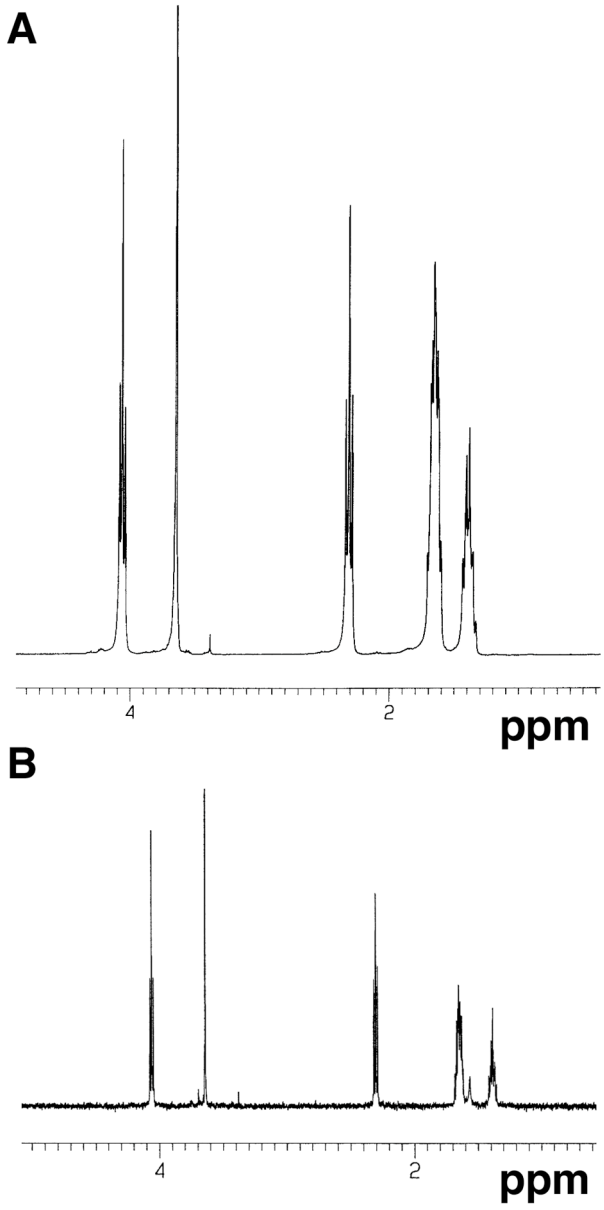
MATERIALS AND METHODS:

Loading and Release of Doxorubicin in PEO-b-PCL-based Polymersomes: Self-assembly via thin-film hydration was employed in order to form PEO(2k)-b-PCL(12k)-based vesicles. Film hydration has been extensively utilized for preparing non-degradable polymersomes comprised of PEO-b-PBD and PEO-b-PEE diblock copolymers[3, 21]. Briefly, 200 microliters of 7 mg/mL PEO(2k)-b-PCL(12k) copolymer solution in methylene chloride were uniformly deposited on the surface of a roughened Teflon plate followed by evaporation of the solvent for > 12h. Addition of aqueous solution (290 milliosmolar ammonium sulfate, pH 5.5) and sonication led to spontaneous budding of biodegradable polymersomes, off the Teflon-deposited thin-film, into the aqueous solution. The sonication procedure involved placing the sample vial containing the aqueous based solution and dried thin-film formulation (of polymer uniformly deposited on Teflon) into a sonicator bath (Branson; Model 3510) with constant agitation for 60 minutes at 65°C. Five cycles of freeze-thaw extraction followed by placing the sample vials in liquid N_2 and subsequently thawing them in a 65 °C water bath. Extrusion using a pressure

driven Lipex Thermobarrel Extruder (1.5 mL capacity) at 65°C was performed to yield small (~ 200 nm diameter) unilamellar polymersomes that possess appropriately narrow size distributions. The size distributions of vesicles were determined by dynamic light scattering (see Supplemental Figure 2).

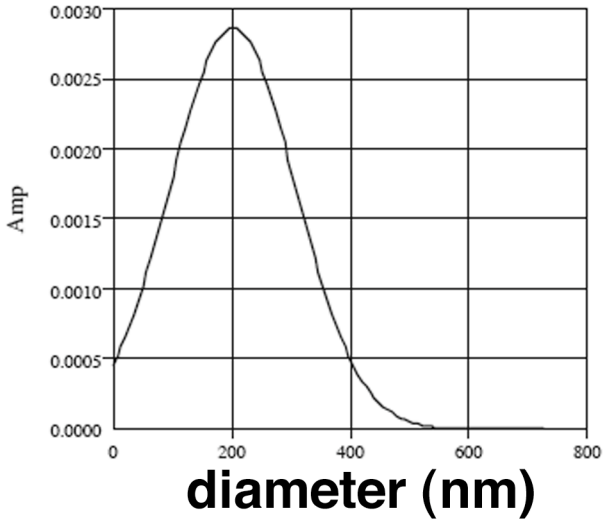
Extruded samples were dialyzed in iso-osmotic sodium acetate solution (50 mM sodium acetate, 100 mM sodium chloride, pH ~ 7.4). Dialysis solutions were changed 3 times over approximately 30 hours. Post-dialysis, doxorubicin was loaded into the polymersomes via an ammonium sulfate gradient[24-26]. The polymersomes were incubated with doxorubicin in a ratio of 1:0.2 polymer:drug (w/w) for 7 hours at a temperature above their main gel to liquid-crystalline phase transition temperature (65 ^{o}C). Aggregation of DOX within the polymersome core led to quenching of its fluorescence emission. Non-entrapped DOX was removed using an Acta Basic 10 HPLC with Frac 950; the solution was passed through a C-1640 column with Sephacryl S500-HR media. The collected DOX-loaded polymersome suspension was centrifuged and concentrated into an approximately 1 mL volume. The vesicles were then aliquoted into various (290 mOsM) solutions buffered at pH ~5.5 (50 mM sodium acetate and 100 mM sodium chloride) and pH ~ 7.4 (PBS), with N = 4 samples for each buffer. Release studies of DOX from the loaded polymersomes were initiated immediately following aliquoting; DOX fluorescence was measured fluorometrically (using a SPEX Fluorolog-3 fluorimeter; λ_{ex} = 480nm, λ_{em} = 590nm) at various intervals up to fourteen days. As DOX was released from the polymersome core, and diluted into the surrounding solution, its fluorescence emission increased over time. At the culmination of the study, the samples were solubilized using Triton X-100. The percent release over time was calculated by comparing the measured fluorescence at each time point to final DOX fluorescence, as determined upon solubilization of remaining intact polymersomes with TritonX-100, at the completion of the study.

Supplemental Figure 1:



Supplemental Figure 1: H^1-NMR spectra of PEO(2K)-B-PCL(12K) diblock copolymer (A) before and (B) after generation of 200 nm diameter polymersomes via thin-film self assembly (65 ºC for 1 hr) and subsequent sizing via 5 cycles of freeze-thaw extraction and extrusion. Comparison of relative peak intensities demonstrates that Mn values of the diblock copolymer decrease slightly during the processing involved in nanovesicle formation (from 14k to 13.7k); results were further corroborated via GPC: Mn values were 12.43k (before) and 12.11k (after), respectively, polymersome formation.

Supplemental Figure 2:



Supplemental Figure 2: Cumulative histogram of the size distribution of PEO(2k)-PCL(12k)-based polymersomes as obtained via dynamic light scattering (DLS) at 25 ºC. Vesicles were formed via thin-film self- assembly upon aqueous hydration and heating at 65 ºC for 1 hr. A mono-dispersed distribution of 200 nm diameter polymersomes was subsequently obtained upon 5 cycles of freeze-thaw extraction followed by extrusion through a thermo-barrel supported (5 passes at 65 ºC) 200 nm pore-cutoff membrane.