Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Reinforcement of polymeric nanoassemblies for ultra-high drug loadings, modulation of stiffness and release kinetics, and sustained therapeutic efficacy

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Experimental procedures

PGC-PTX synthesis

Poly(1,2-glycerol carbonate)-*graft*-succinic acid-paclitaxel with 34 mol% (58 wt%) paclitaxel (PTX) conjugation (i.e., PGC-PTX) as well as PGC-PTX with 10 mol% rhodamine B conjugation were synthesized as previously described.[1, 2] Briefly, poly(benzyl 1,2-glycerol carbonate) (PGC-Bn) was synthesized via the alternating copolymerization of benzyl glycidyl ether and carbon dioxide. The benzyl group was then removed via high pressure hydrogenolysis to afford poly(1,2-glycerol carbonate) (PGC). PGC was subsequently reacted with succinic anhydride and 4-dimethylaminopyridine (DMAP) to obtain poly(1,2-glycerol carbonate)-*graft*-succinic acid (PGC-*g*-SA), which possesses a free hydroxy on each repeating unit. PTX was conjugated under standard coupling conditions using N,N'-dicyclohexylcarbodiimide (DCC) and DMAP to afford PGC-PTX (Fig. S1) with a molecular weight of 9393 Da and a dispersity index of 1.35.[2] To achieve 34 mol% PTX incorporation, PTX is coupled at a 0.4 equivalent ratio (85% incorporation efficiency). The PGC-PTX conjugate is isolated at 80% from PGC-*g*-SA. Rhodamine-conjugated PGC-PTX is synthesized by first reacting PGC with rhodamine B isothiocyanate, and following the same procedure to conjugate succinic acid and PTX.

NP synthesis

NPs were prepared by miniemulsion as previously described.[2] Briefly, PTX was added to 10 – 50 mg PGC-PTX polymer at 0, 10, 25, or 50 wt%. PGC-PTX and PTX were then dissolved in 0.5 mL dichloromethane, and added to a 2 mL solution of sodium dodecyl sulfate (SDS) in pH 7.4 10 mM phosphate buffer at a 1:5 SDS:polymer mass ratio. The mixture was emulsified under an argon blanket using a Sonics Vibra-Cell VCX-600 Ultrasonic Processor (Sonics & Materials; Newtown, CT). Following sonication, the NP suspension was subjected to stirring under argon for 2 hours, followed by stirring under air overnight to allow for the evaporation of remaining solvent. The resulting NP suspension was dialyzed for 24 hours against 1 L of 5 mM pH 7.4 phosphate buffer. The dialysis buffer was exchanged with fresh buffer after 10 hours of dialysis. Drug-free PGC-Bn NPs, PTX-loaded PGC-Bn NPs, and celecoxib-loaded PGC-PTX NPs were also prepared using the same procedure. To form PTX crystals, the same procedure was followed to emulsify free PTX into an SDS solution at a 1:5 SDS:PTX mass ratio.

NP diameter, polydispersity, and zeta potential

Dynamic light scattering (DLS) was used to assess NP diameter, polydispersity index (PDI), and zeta potential using a Brookhaven 90Plus Particle Size Analyzer (Brookhaven Instruments Corporation; Holtsville, NY). NPs were diluted in DI water in order to achieve a count rate of approximately 150 kilocounts per second. Zeta potentials were measured using the Brookhaven Instruments Corporation ZetaPALS zeta potential analyzer via the Smoluchowski method. Data

presented are the averages of number-weighted size distributions ± standard deviation (SD) of three independently prepared samples per NP formulation. Statistical analysis was performed with one-way analysis of variance (ANOVA).

NP visualization via scanning electron microscopy (SEM)

A Zeiss SUPRA 55VP field emission (FE)-SEM (Carl Zeiss Microscopy; Jena, Thuringia, Germany) was used to visualize NPs. Samples were prepared by diluting the NP suspension 1000x in DI water, and dropping a 10 µL aliquot on a silicon wafer. After samples were allowed to air-dry overnight, they were coated with Au/Pd using a Cressington 108 Manual Sputter Coater (Cressington Scientific Instruments; Watford, England, UK).

Quantitation of free PTX encapsulation

Free PTX encapsulation was determined using reversed-phase high performance liquid chromatography (HPLC) using a 7:3 acetonitrile:water solution as the eluent at a flow rate of 0.5 mL/min through a HxSil C18 4.6 x 250 mm column (Hamilton Robotics; Reno, NV) with a ProStar 335 Diode Array Detector (Varian, Inc; Palo Alto, CA). A set of free PTX standards was used to correlate the integrated area under the peak, at an absorbance of 228 nm, to concentration. Three linear standard curves were developed (0.1 - 5 µg/mL [R^2 = 0.989], 5 – 50 µg/mL [R^2 = 0.997], and 50 – 500 µg/mL [R^2 = 0.999]) and used to determine unknown PTX concentrations. To prepare the NP solutions for HPLC analysis, 30 µL of NP suspension was added to 970 µL acetonitrile, vortexed for 1-2 minutes, and filtered using a 0.2 µm Millex-GN nylon syringe filter (EMD Millipore; Billerica, MA).

Calculation and confirmation of total PTX loading

NPs were prepared using a conjugate with known PTX loading (34 mol%), as determined via ^{1}H NMR by integrating the peaks that correspond to the methine proton on the polymer backbone and the C-2' proton on the conjugated PTX.[2] PTX incorporation in molar ratio was then converted to mass ratio (58 wt%) using the following equation:

$$Conjugated\ drug\ loading\ (wt\%) = \frac{\nu * m_{PTX}}{([1-\nu] * m_{PGC-g-SA}) + (\nu *\ m_{PGC-g-SA-PTX})} * 100\%$$

Here, ν is the molar ratio of incorporated PTX, m_{PTX} is the molecular weight of PTX (854 Da), $m_{PGC-g-SA}$ is the molecular weight of a poly(1,2-glycerol carbonate)-*graft*-succinic acid (PGC-*g*-SA) monomer (218 Da), and $m_{PGC-g-SA-PTX}$ is the molecular weight of a poly(1,2-glycerol carbonate)-*graft*-succinic acid-paclitaxel (PGC-*g*-SA-PTX) monomer (1054 Da). The mass ratio was used to calculate NP PTX concentration from a known PGC-PTX concentration. Total PTX loading was determined by adding the conjugated PTX content and the physically entrapped PTX content (determined via HPLC). To confirm total PTX loading, NPs were diluted in

acetonitrile to achieve a concentration of 20 µg/mL PTX. The absorbance of the solution at 227 nm was then determined using a Hewlett Packard 8453 UV-Vis Spectrophotometer (The Hewlett-Packard Company; Palo Alto, CA). A set of PGC-PTX polymer standards was used to correlate absorbance to PTX concentration. A linear standard curve was developed (R^2 = 0.999) and used to confirm PTX content.

Nanomechanical characterization

Atomic force microscopy (AFM) was performed using a MFP-3D microscope (Asylum Research; Santa Barbara, CA). The contact mode was applied using a silicon nitride tip (MLCT, Bruker AFM Probes; Camarillo, CA) with a nominal spring constant of 40 pN/nm. Samples were prepared by diluting the suspensions 1000x in DI water, and dropping a 10 µL aliquot on a positively charged glass slide. NPs were immobilized by air drying overnight. Prior to imaging, samples were rehydrated by adding a 20 µL drop of DI water. An area scan was performed in tapping mode prior to indentation to confirm the presence and density of NPs (Fig. S2). Force spectroscopy was then obtained over a 250 nm extension length and a 500 nm/s approaching and retreating velocity. Force curves (N=100) were collected over an area of 500 x 500 nm^2. The set-point was at 400 pN. We distinguished force curves resulting from a tip-particle interaction from those resulting from a tip-substrate interaction by (1) the height difference and, (2) the presence of the extension peak(s) upon retraction. To determine indentation depth, the cantilever's inverse optical lever sensitivity (InvOLS; unit: m/V) was obtained by indenting the cantilever on a clean glass substrate. The glass substrate serves as an infinitely hard surface for determining the lever deflection response. Once an indentation was performed, the raw distance of the tip along the z-direction was converted into an indentation depth using the InvOLS. The cantilever stiffness was determined using the thermal tuning method, and compared with the fabrication specification. The indentation studies were performed on different particles from different locations to take NP variability into consideration. Only single, non-aggregated particles were probed in these experiments. To determine the elastic modulus, we assumed the contact of two elastic materials. The Hertz's model for non-adhesive elastic contact was first used to determine the reduced elastic modulus, $E_{reduced}$, as a result of the combined elasticities of the tip and sample by correlating the loading force, F, with indentation depth, δ:

$$F = \frac{2E_{reduced}}{\pi\ 1-\nu^2}\delta^2 \tan(\alpha)$$

Here, ν is the Poisson ratio of the sample (set to 0.33 for a polymer), and α is the half opening angle of the indenting tip (36°). Finally, the sample modulus was calculated using the following equation:

$$\left(-\frac{1-\nu_{Tip}^{2}}{E_{Tip}}+\frac{1}{E_{reduced}}\right)^{-1}=\frac{E_{sample}}{1-\nu_{Sample}^{2}}$$

The tip's Young's modulus was set to 290 GPa, with a Poisson ratio of 0.29 and a cone shape. Data were fitted for indentation depths of up to 50 nm, corresponding to approximately 50% of the total indentation needed to hit the infinitely hard glass substrate. This value was chosen empirically, based on both optimization of the goodness of fit (evaluated via the reduced chi-squared statistic) as well as the stability of the fitted parameter (elastic modulus). A representative set of data (from the PGC-PTX NP formulation; Fig. S3) shows that the elastic modulus is stable within the range of 40-60% indentation depth, and increases after 60% indentation depth due to the effect of the underlying stiff substrate. Meanwhile, the reduced chi-squared value is closest to 1 within the range of 40-60% indentation depth. All fitting was done using Igor Pro software (WaveMetrics Inc.; Portland, OR). Statistical analysis was performed with one-way ANOVA using GraphPad Prism.

Differential scanning calorimetry (DSC)

Calorimetry measurements were performed on PTX, PGC-PTX, PGC-PTX NPs, and PGC-PTX + 50 wt% PTX NPs using a Q100 DSC (TA Instruments; New Castle, DE). NP solutions were lyophilized in order to obtain dry samples. Samples were heated at rate of 10 °C/min to 150°C, cooled at a rate of 5 °C/min to -80 °C, and heated at a rate of 10 °C/min to 300 °C. An empty aluminum pan was used as a reference in each measurement.

X-ray powder diffraction (XRPD)

XRPD measurements were performed on PTX, PGC-PTX, lyophilized PGC-PTX NPs, and lyophilized PGC-PTX + 50 wt% PTX NPs using a Bruker D8 Discover X-ray diffractometer (Bruker Corporation; Billerica, MA). Measurements were carried out using Cu-Kα radiation at 40 kV and 40 mA, with samples continuously scanned between 5° and 90° (2θ), at a step size of 0.02° and a rate of 1s/step.

PTX release kinetics

PTX release kinetics were evaluated as previously described.[2] Briefly, release medium was prepared by adding 0.3% w/w SDS to 10 mM pH 7.4 phosphate buffer following a published procedure to ensure sink conditions.[3] PGC-PTX + PTX NPs (1.98 mg PTX equivalent) were diluted with release medium to achieve a total volume of 10 mL. The NP solution was then placed in 10,000 molecular weight cutoff dialysis tubing, and the tubing was placed into 290 mL of release buffer stirring at 37 °C. At given time-points, 400 µL samples were withdrawn from the release medium and replaced with fresh release buffer. The withdrawn samples were immediately quenched with 800 µL cold acetonitrile, vortexed for 20 seconds, and stored at -20

°C. PTX content was determined using a TSQ Triple Quadrupole mass spectrometer (Thermo Scientific; Waltham, MA) with an electrospray ionization source coupled to an Acquity UPLC system (Waters Corp.; Milford, MA). A 7.5 µL injection of each sample was eluted from an Acquity UPLC HSS PFP 2.1 x 50 mm column using a binary solvent gradient consisting of water with 10 mM ammonium acetate and acetonitrile. The mass spectrometer was operated in positive ionization mode monitoring ion 876.3 with a scan width of 0.4 da and a scan time of 0.05 sec. The mass spectrometer utilized the following settings: heated electrospray ionization source spray voltage set at 3500 V with a vaporizer temperature of 376 °C, capillary temperature set at 352 °C, sheath gas pressure set to 60, auxiliary gas pressure set to 50, and sweep gas set to 2. An external standard curve, prepared using two-fold dilutions of a PTX standard from 1 to 4096 nM in 1:3 release buffer:acetonitrile, was used to determine PTX concentration. Peak area of the extracted-ion chromatogram for m/z 877.3 was plotted against calculated concentrations and subjected to linear regression. A conservative approach was utilized to compensate for PTX degradation, as previously described.[2] Data represents the average ± SD of N=3/group. Statistical analysis was performed with two-way ANOVA.

Characterization of NPs following drug release

PGC-PTX NPs and PGC-PTX + 50 wt% PTX NPs (1.98 mg PTX equivalent) were each diluted in release medium to achieve a total volume of 10 mL. The NP suspensions were then transferred into 10,000 molecular weight cutoff dialysis tubing and placed in 290 mL of release buffer stirring at 37 °C. After 7 and 15 days of release, NP samples were withdrawn from the dialysis tubing and prepared for visualization and nanomechanical characterization via SEM and AFM, respectively.

Cell culture

MSTO-211H and MSTO-211H-luc (firefly luciferase gene transfected MSTO-211H cells, MSTO-211H/CMMPnlacZ/LucNeo [5/11/04T]) cells were cultured in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells were maintained at 37 °C with 5% CO_2 in a humidified environment.

In vitro cytotoxicity

MSTO-211H cells were seeded in 96-well plates at a density of 3000 cells/well, and allowed to adhere for 24 hours. On each plate, blank wells (media only) were defined as 0% viability, and wells with untreated cells were defined as 100% viability. Dilutions of PGC-PTX NPs, PGC-PTX + PTX NPs, or PTX formulated in Cremophor EL/ethanol (1:1 v/v; PTX-C/E) were prepared, and cells were treated with 5000, 1000, 500, 100, 50, 10, 5, 1, 0.5, or 0.1 ng/mL PTX for 5 days. Cells were also treated with PGC-Bn NPs as a drug free NP control (administered at equivalent PGC

backbone concentrations as PGC-PTX NPs; 2015.22, 403.04, 201.52, 40.30, 20.15, 4.03, 2.02, 0.40, 0.20, 0.04 ng/mL PGC backbone equivalent) for 5 days. Viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethonyphenol)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (CellTiter 96 Aqueous One, Promega; Madison, WI). The absorbance at 492 nm was determined using a Beckman Coulter AD 340 plate reader (Beckman Coulter Inc.; Brea, CA). The percent viability was determined by subtracting the average of media blanks and normalizing the absorbance of each well to the average absorbance of the untreated cells. The 50% inhibitory concentrations, or IC_{50} values, were determined by fitting the resultant data to sigmoidal four-parameter logistic curves using GraphPad Prism (GraphPad Software, Inc.; La Jolla, CA). Each curve represents the average ± SD of three experiments in which n=6-8/treatment group.

In vitro cellular uptake: flow cytometry

MSTO-211H cells were seeded in 6-well plates at a density of 300,000 cells/well, and allowed to adhere overnight. Cells were then incubated with rhodamine-labeled PGC-PTX NPs or rhodamine-labeled PGC-PTX + 50 wt% PTX NPs at a concentration of 100 ng/mL conjugated PTX. After 0, 1, 2, and 4 hours of incubation (N=3/group), wells were rinsed 3x with 1 mL/well Dulbecco's phosphate buffered saline (PBS) without calcium or magnesium and cells were detached using 0.5 mL/well trypsin-EDTA (0.25%). The cells were then collected using 2 mL media/well, pelleted via centrifugation at 1000 RPM for 5 minutes, and the supernatant was removed. Cells were subsequently fixed via resuspension in 3 mL 4% formaldehyde for 15 minutes. After fixation, cells were washed with 5 mL cold fluorescence-activated cell sorting (FACS) buffer (0.1% sodium azide, 1% BSA in PBS), pelleted via centrifugation, and resuspended in 0.75 mL FACS buffer. The fluorescence of the cell population was evaluated using a BD LSRII flow cytometer (BD Biosciences; Franklin Lakes, NJ), with 10,000 events (single cells) recorded per sample. Data analysis was performed using the FACSDiva (Version 6.2; BD Biosciences) software. Cells exhibiting greater fluorescence than 99% of the untreated cell population were considered positive for NP internalization. Data represents the average ± SD. Statistical analysis was performed with two-way ANOVA.

In vitro cellular uptake: confocal microscopy

MSTO-211H cells were seeded in 6-well plates at a density of 200,000 cells/well and allowed to adhere for 24 hours onto 25 mm diameter, # 1.5 thickness, sterile, poly(L-lysine) coated coverslips (Neuvitro Corporation; Vancouver, WA). Cells were then incubated with rhodamine-labeled PGC-PTX NPs at a dose of 100 ng/mL PTX for 1, 4, or 24 hours. After incubation, wells were rinsed 2x with 1 mL/well PBS and cells were fixed in 4% formaldehyde for 20 minutes. Coverslips were then rinsed 2x with 1 mL/well PBS, and incubated for 8 minutes with 100 μg/mL Concanavalin A-fluorescein conjugate (ThermoFisher Scientific; Waltham, MA) for

membrane staining and 3 μg/mL Hoechst 33342 trihydrochloride trihydrate (ThermoFisher Scientific) for nuclear staining. Wells were washed 2x with 1 mL/well PBS, and coverslips were mounted onto glass slides using ProLong Gold Antifade Mountant (ThermoFisher Scientific). Slides were visualized using a Zeiss LSM 710 confocal microscope (Carl Zeiss Microscopy; Jena, Thuringia, Germany). Images were analyzed using ImageJ software (NIH; Bethesda, MD).

Animals used in in vivo studies

Athymic female mice (6-8 week old NU/J; The Jackson Laboratory; Bar Harbor, ME) were housed at the animal facility of Dana Farber Cancer Institute (Boston, MA) under sterile conditions. Animal care and procedures were conducted with Institutional Animal Care and Use Committee approval, in strict compliance with all federal and institutional guidelines for the care and use of laboratory animals.

In vivo NP safety

Three healthy NU/J mice received a 200 mg/kg PTX dose as PGC-PTX + 25 wt% PTX NPs via IP injection. Mice were housed with free access to food and water, and were euthanized 14 days after IP injection. Two healthy NU/J mice of the same age served as untreated controls, and were housed for more than two weeks in separate cages at the same animal facility.[2] Tissues including heart, lung, liver, spleen, kidney, and small intestine were harvested, fixed with 10% formalin, paraffin embedded, and stained with hematoxylin and eosin (H&E). Pathological evaluation was performed by a licensed pathologist (R.F.P.).

In vivo treatment of established tumor

PGC-PTX + PTX NP efficacy was evaluated in a model of established peritoneal mesothelioma as described previously.[2] Briefly, one week after IP tumor inoculation ($5x10^6$ MSTO-211H-luc cells), animals received one of the following IP treatments: 200 mg/kg PTX as PGC-PTX + 25 wt% PTX NPs, saline, or 20 mg/kg PTX-C/E per week for up to 10 weeks (permitting animal survival) (n=8/group). Tumor burden was evaluated via bioluminescence imaging (BLI) at 4 and 6 weeks after tumor inoculation (3 of the 8 mice per group being randomly selected at the beginning of the study). Images of mice were taken 10 minutes after subcutaneous injections of 2.25 mg firefly luciferin, using a Xenogen IVIS-50 bioluminescence camera (Caliper Life Sciences; Hopkinton, MA). The exposure setting was 60 s exposure, F4, small bin, and distance of 25 cm. All animals were monitored for tumor burden three times a week and were euthanized upon evidence of morbid disease including weight loss more than 15%, large palpable abdominal solid tumor, slow movement, and/or difficulty obtaining food and water. Overall survival was described by the Kaplan-Meier method and compared via log-rank test.

Statistics

All data are reported as mean ± SD. Differences between groups were evaluated using one-way ANOVA, two-way ANOVA, or log-rank test using GraphPad Prism (GraphPad Software, Inc.; La Jolla, CA). Animals were randomly assigned to treatment groups. No blinding was used in the animal studies.

Supplementary tables and figures

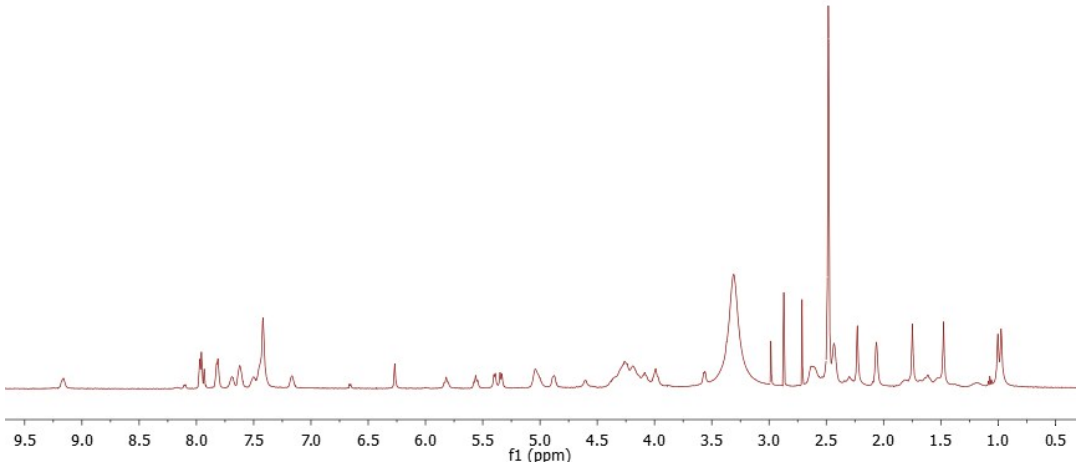

Fig. S1 Characterization of PGC-PTX. Sample proton NMR spectrum of PGC-PTX acquired in DMSO-d_6 at 500 MHz.

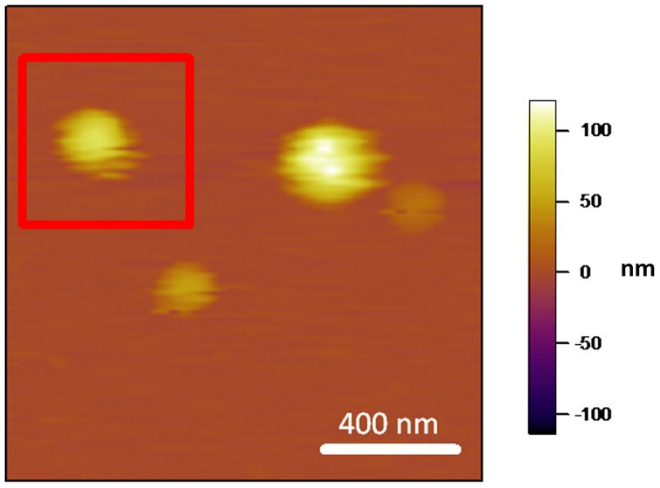

Fig. S2 Representative AFM image of NPs. After NPs are identified, force curves are obtained (red square).

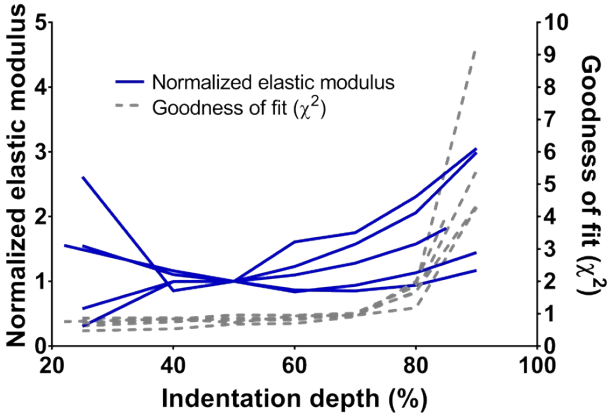

Fig. S3 Normalized elastic modulus and goodness of fit for force spectroscopy data as a function of cumulative indentation depth used for fitting. Elastic moduli are normalized by the elastic modulus derived from fitting the force-indentation depth data for up to 50% indentation depth. The elastic modulus is stable within the range of 40-60% indentation depth, and increases after 60% indentation depth due to the effect of the underlying stiff substrate. Meanwhile, the reduced chi-squared value is closest to 1 within the range of 40-60% indentation depth.

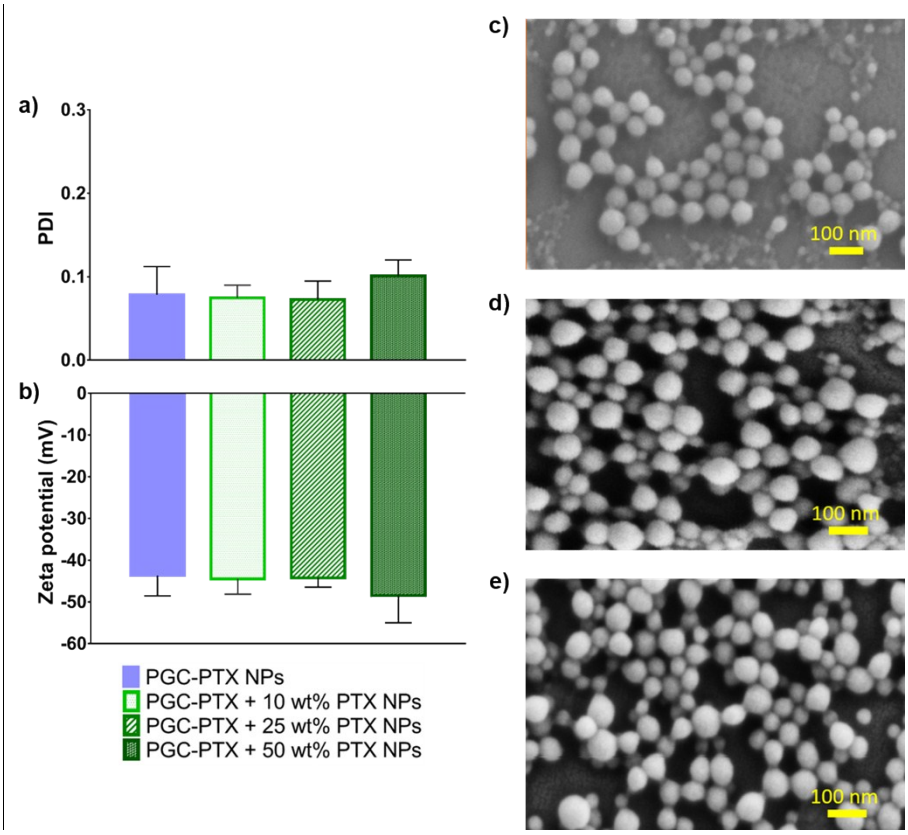

Fig. S4 Characterization of PGC-PTX + PTX NPs. (a) Polydispersity indices and (b) zeta potentials of NPs as measured by DLS. Scanning electron micrographs of (c) PGC-PTX NPs, (d) PGC-PTX + 10 wt% PTX NPs, and (e) PGC-PTX + 25 wt% PTX NPs.

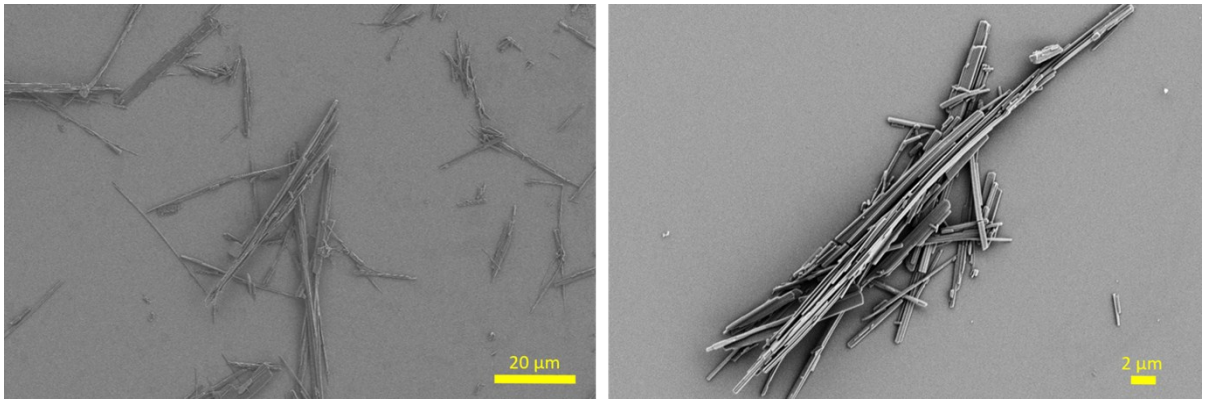

Fig. S5 Scanning electron micrographs of PTX crystals formed by emulsifying a solution of the free drug in an aqueous solution of SDS.

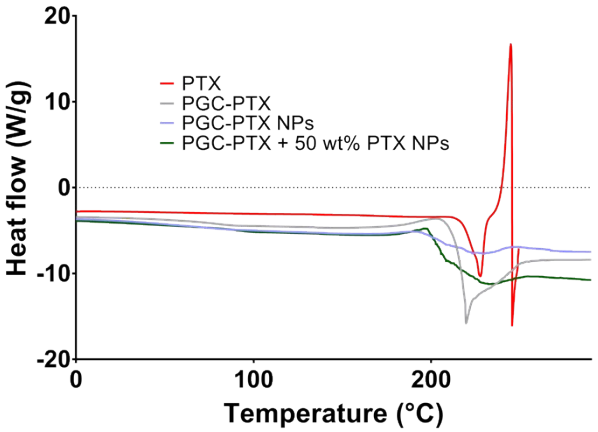

Fig. S6 Representative DSC thermograms of PTX, PGC-PTX, PGC-PTX NPs, and PGC-PTX + 50 wt% PTX NPs.

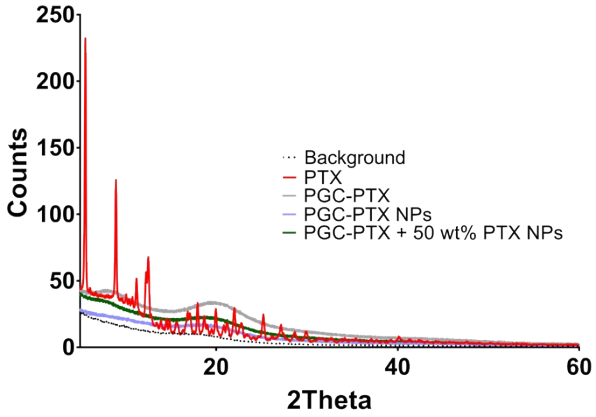

Fig. S7 Representative XRPD patterns of PTX, PGC-PTX, PGC-PTX NPs, and PGC-PTX + 50 wt% PTX NPs.

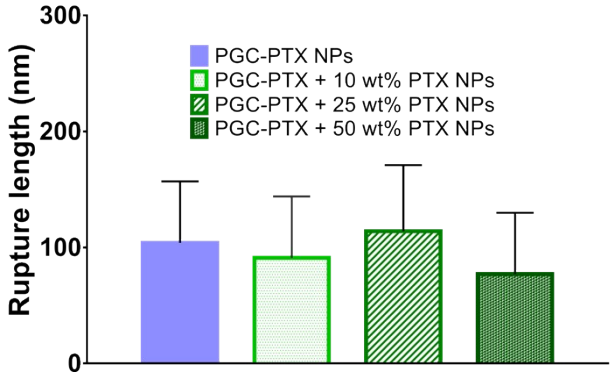

Fig. S8 Force spectroscopy analysis of rupture length.

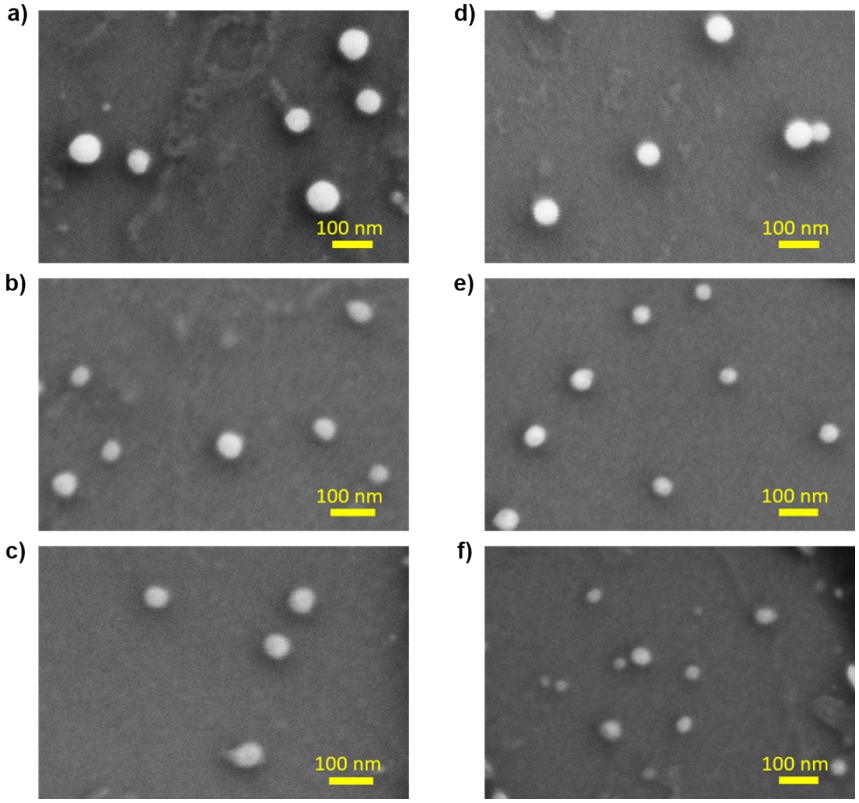

Fig. S9 Visualization of PGC-PTX + PTX NPs following drug release. Scanning electron micrographs of (a-c) PGC-PTX NPs and (d-f) PGC-PTX + 50 wt% PTX NPs (a,d) before and after (b,e) 7 and (c,f) 15 days of release.

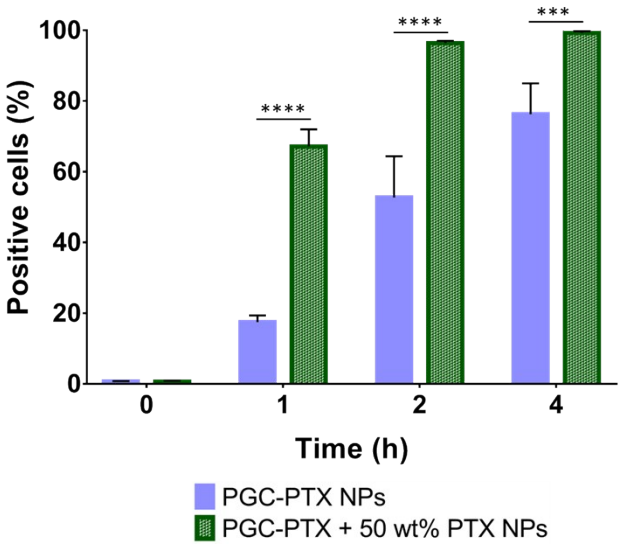

Fig. S10 Cellular internalization of fluorescent, rhodamine-labeled NPs in MSTO-211H cells. Cells exhibiting greater fluorescence than 99% of the untreated population were defined as positive for NP internalization (***$p < 0.001$, ****$p < 0.0001$).

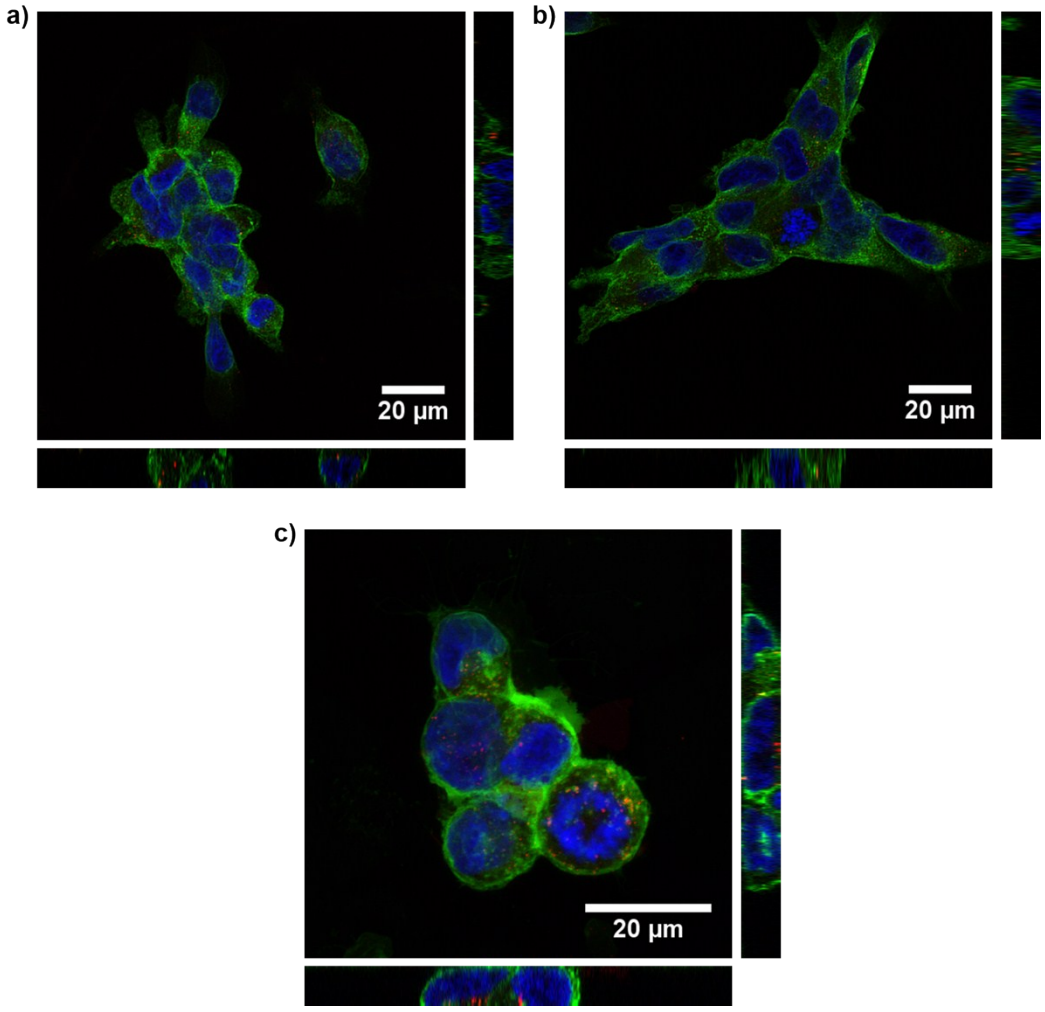

Fig. S11 Laser-scanning confocal microscope images of MSTO-211H cells after (a) 1, (b) 4, and (c) 24 hours of incubation with rhodamine-labeled PGC-PTX NPs. Images are the 2-D projections of 3-D, 4-7 µm z-stacks. Cross-sectional images shown on the periphery confirm the presence of NPs in the cell cytoplasm. Cell nuclei are visualized in blue, cell membranes in green, and NPs in red.

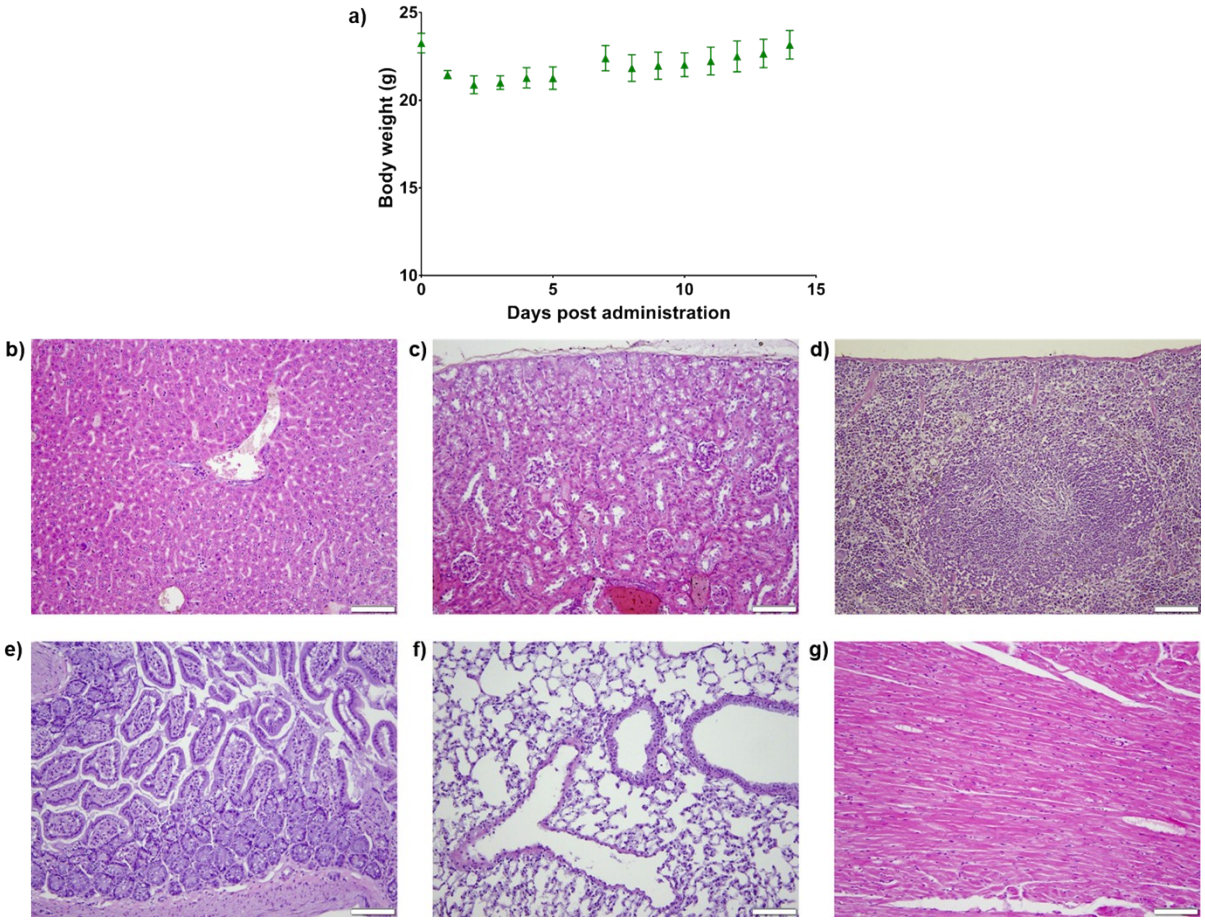

Fig. S12 Evaluation of PGC-PTX + 25 wt% PTX NP safety. Healthy mice received PGC-PTX + 25 wt% PTX NPs at a dose of 200 mg/kg PTX via IP administration. (a) Animal weight was monitored for 14 days post-treatment. (b) Liver, (c) kidney, (d) spleen, (e) small intestine, (f) lung, and (g) heart tissue sections were harvested from treated animals and stained with H&E. Images at 200x original magnification (scale bar = 50 µm). No pathologic changes were observed relative to untreated controls.[2]

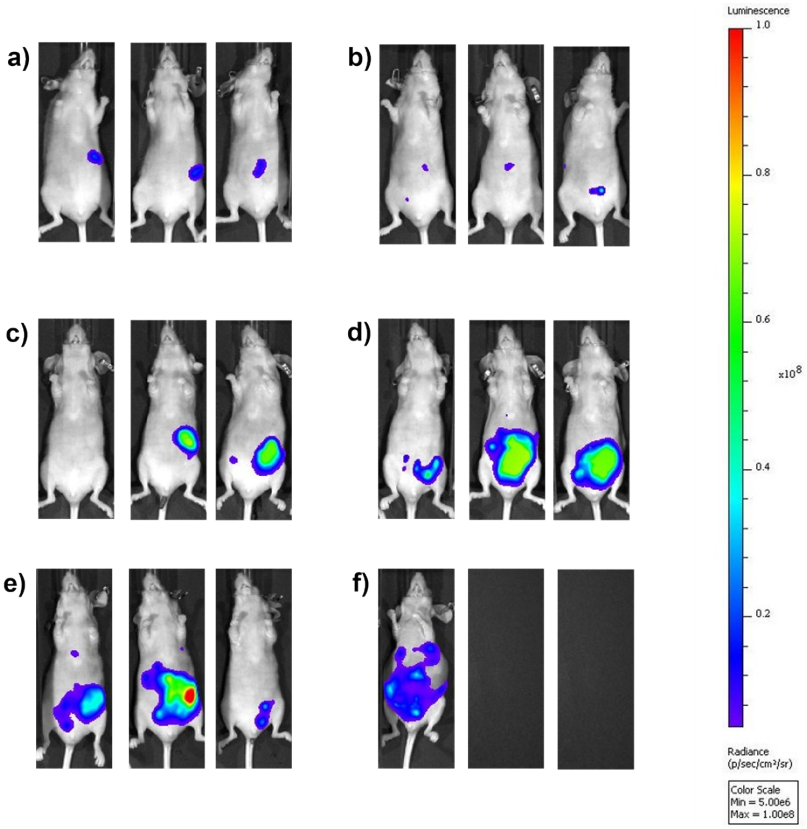

Fig. S13 Efficacy of PGC-PTX + 25 wt% PTX NPs in the treatment of peritoneal mesothelioma. Tumor burden was evaluated for 3 randomly selected animals per group via BLI. BLI images are shown for (a,b) PGC-PTX + 25 wt% PTX NP, (c,d) 20 mg/kg weekly PTX-C/E, and (e,f) saline treated animals at (a,c,e) 4 and (b,d,f) 6 weeks after tumor inoculation.

Table S1 Representative drug loadings by physical entrapment.

Polymer	Drug	Loading (wt%)	Reference
Poly(methyl methacrylate)	Paclitaxel	1	4
Poly(lactic acid)	Docetaxel	≤ 1	5
Poly(lactide-co-glycolide)	Paclitaxel	< 3	6
Poly(ε-caprolactone)	4’-demethylepipodophyllotoxin	< 5	7
Poly(phosphoester)	Paclitaxel	10	8

Table S2 *In vitro* 50% inhibitory concentrations (IC_{50}s) of PTX-C/E, PGC-PTX NPs, and PGC-PTX + PTX NPs after 5 days of treatment in MSTO-211H mesothelioma cancer cells. IC_{50} values are derived from fitting the cell viability data (Fig. 4d) to sigmoidal four-parameter logistic curves. Concentrations are noted in ng/mL PTX.

Treatment	**IC_{50} (ng/mL)**
PTX-C/E	9.4
PGC-PTX NPs	107.4
PGC-PTX + 10 wt% PTX NPs	68.3
PGC-PTX + 25 wt% PTX NPs	41.8
PGC-PTX + 50 wt% PTX NPs	36.7

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