Superhydrophobic Materials for Tunable Drug Release: Using Displacement of Air to Control Delivery Rates (Supplementary Information)

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Materials and Methods

Materials

All solvents were purchased from Sigma without further purification. Stannous 2-ethylhexanoate, ε-caprolactone, stearic acid, N,N′-dicyclohexylcarbodiimide, and 4-(dimethylamino)pyridine were purchased from Sigma. Palladium on carbon was purchased from Strem Chemicals. Poly(ε-caprolactone) (70,000-90,000 MW) was purchased from Sigma. 7-ethyl-10-hydroxycamptothecin was purchased from Sigma. 5-benzyloxy-1,3-dioxan-2-one was prepared as previously reported. All reactions were performed under nitrogen atmosphere unless otherwise noted. NMR spectra were recorded on a Varian INOVA spectrometer (1H at 400 MHz). Chemical shifts were referenced to residual solvent peaks ($CHCl_3$ peak at 7.24 ppm). DCM=dichloromethane, THF=tetrahydrofuran, DCC=N,N′-dicyclohexylcarbodiimide, DMAP=4-(dimethylamino) pyridine, SN-38 =7-ethyl-10-hydroxycamptothecin, PCL= poly(ε-caprolactone), PGC-C18=poly(glycerol monostearate-co-ε-caprolactone), Pd/C=10% palladium on activated carbon, and PBS=phosphate buffered solution.

Synthesis of poly(glycerol monostearate-co-ε-caprolactone) (PGC-C18)

Poly(glycerol monostearate-co-ε-caprolactone), or PGC-C18, was prepared as reported previously.[1] Briefly, ε-caprolactone and 5-benzyloxy-1,3-dioxan-2-one monomers were mixed at a 4:1 molar ratio in a schlenk flask and subsequently evacuated and flushed with N_2 three times. $Sn(Oct)_2$ was used (M/I=500) to catalyze the ring-opening polymerization of the co-monomers at 140 °C for 24 h, and the resulting copolymer was isolated by precipitation in cold methanol (99% yield). The benzyl-protecting group was removed via palladium-catalyzed hydrogenation overnight in THF, and filtered through Celite (99% yield). The deprotected polymer, stearic acid, DCC, and DMAP were dissolved in DCM and stirred at room temperature for 18 hours. DCU was filtered and the solvent evaporated. The product, PGC-C18, was dissolved in DCM and precipitated in cold methanol. The polymer was filtered and dried by evaporation (93% yield).

Size exclusion chromatography (SEC)

Molecular weight determinations were performed via size exclusion chromatography using THF as the eluent on a Polymer Laboratories PLgel 3 μm MIXED-E column (3 μm bead size) and a Rainin HPLC system (temp=25 °C; flow rate=1.0 mL/min). Polystyrene standards (Polysciences, Inc.) were used for calibration. PGC-C18 was shown to have a Mn=21,100 and a polydispersity of Mw/Mn=1.73.

Formation of doped and undoped poly(ε-caprolactone) meshes

All electrospinning solutions were 20 w/v% and prepared in a solvent mixture of 5:1 chloroform:methanol. Undoped PCL electrospinning solutions were prepared by dissolving PCL in chloroform and allowing full dissolution. This was followed by adding methanol with rigorous vortexing. Doped PCL electrospinning solutions were prepared in a similar fashion, where 1 – 50 wt% of PCL was replaced with PGC-C18 to maintain a 20 wt/v% solution. Solutions were loaded into a glass syringe and placed into a syringe pump set at a flow rate of 25 mL/hr. A 12-15 kV high voltage lead was applied at the base of the syringe needle. A grounded rotating collector was covered in aluminum foil and placed 15 - 30 cm away from the needle. Meshes were

fabricated such that the total thickness was 300 um. Drug loaded meshes were made in the same way, where SN-38 were added to the electrospinning solution and allowed to completely dissolve.

Differential scanning calorimetry (DSC) to measure drug dissolution

Thermal analysis of electrospun meshes was performed using differential scanning calorimetry. Meshes between 2 and 3 mg were hermetically sealed in an aluminum crucible with an empty crucible used as a reference. Samples were analyzed from -50-300 °C heated at a rate of 5 °C/min with a N_2 flow rate of 20 mL/min. Melting peaks for substituent polymers and encapsulated SN-38 were looked at to examine phase separation and drug dissolution.

Electrospun mesh and electrospun melt morphology and characterization

Samples for scanning electron microscopy were prepared by mounting meshes on an aluminum sample stub and then sputter-coating with a 5 nm layer of gold–palladium alloy. Samples were then imaged on a Zeiss SUPRA 40VP field emission scanning electron microscope using an accelerating voltage of 2 kV. Fiber size analysis for electrospun samples was done using Image J, where all fibers in a representative SEM image were sized to characterize the mesh.

Surface area measurements by Kr BET

Surface area Kr BET measurements were performed by Micrometrical Analytical Services (Norcross, GA).

Atomic Force Microscopy (AFM)

A Digital Instruments Dimension 3000 AFM was used to determine surface roughness on a single electrospun fiber and on a larger portion of melted mesh. Measurements were taken using a silicon tip in tapping mode. A 5 um x 5 um area was examined on a single electrospun fiber, and a larger 30 um x 30 um was examined on melted meshes.

Contact angle measurements

Solvent-cast PCL films were prepared containing 0 – 100 wt % PGC-C18. The polymers were co-dissolved in dichloromethane (1 w/v %) and films were cast onto glass substrates. Electrospun meshes for contact angle measurements were prepared using the above procedures. Melted electrospun meshes were melted for 1 minute at 80 °C. Static contact angle measurements of solvent cast, electrospun, and melt electrospun films were measured as a measure of hydrophobicity with each doping concentration of PGC-C18. Contact angle measurements using 10% fetal bovine serum (FBS) were performed in the same way, where a serum solution was used as the applied droplet. Meshes were also incubated in 10% serum containing PBS for 24-hours, after which they were dried and probed with water to determine the importance of kinetics on protein adsorption.

A drop test was also performed on electrospun meshes by dropping water droplets from 2 feet and measuring the contact angle in the same way as above. This was done to determine if meshes were in a stable or metastable Cassie state, where a significant decrease in contact angle indicated wetting of a mesh had occurred.

***In vitro* drug release and encapsulation efficiency determined by HPLC**

In vitro release and encapsulation efficiency of SN-38 were determined using a HP 1090 192 HPLC system with a fluorescence detector, and a Phenomenex Prodigy 5 ODS reverse-phase column (150×4.6 mm, 5 μm). For SN-38 detection, the mobile phase was composed of 40% acetonitrile and 60% 0.075 M ammonium acetate buffer and delivered at 0.8 mL/min (λex=380 nm, λem=550 nm). Calibration curves were constructed for both lactone (rt=3.8 min) and carboxylate (rt=2.7 min) forms with sensitivities of 1 ng/mL.

Encapsulation efficiency of SN-38 within meshes was determined by dissolving meshes in dichloromethane, followed by the addition of a large quantity of water. Nitrogen was bubbled through the dichloromethane phase to completely remove it, which allowed precipitation of polymer, and the once encapsulated drug to stay in the water phase. Concentration of the drug in the water phase was then measured using HPLC. Encapsulation efficiency for all mesh chemistries was found to be quantitative.

Electrospun and melt electrospun release samples were prepared using the above mentioned procedures. The release kinetics from SN-38 was assessed in PBS buffer at 37 °C. Each mesh was 10 mg (1x1x0.03cm) and loaded with 1% SN-38 (wt. drug/wt. polymer). Release was done in 50 mL of PBS. At specific time points, an aliquot of release media was removed and the concentration of drug was measured using HPLC. The release medium was changed at regular intervals to maintain the pH and ensure sink conditions. Meshes were degassed using a probe ultrasonicator for 3 seconds to 5 minutes, with longer times used for more hydrophobic meshes.

Measuring contrast agent infiltration using µCT

Water infiltration into undoped PCL and 10% PGC-C18 doped PCL native electrospun, melted electrospun, and sonicated electrospun meshes was assessed using the contrast agent Hexabrix. Mesh samples were incubated in an 80 mgI/mL solution of Hexabrix for 2 hours, after which meshes were allowed to drip dry and scanned using a µCT imaging system at an isotropic voxel resolution of 36 mm3, 70 kVP tube voltage, 113 µAmp current and 300 ms integration time. The sequential slices obtained using the mCT system were then converted into the standard image format (DICOM) using the proprietary software from Scanco Medical. This data was then analyzed using commercial image processing software (Analyze!, BIR, Mayo Clinic, Rochester, MN, USA). The 3D µCT data sets were imported into Analyze! where a representative section of the meshes was analyzed to compare intensity through the mesh.

Cell care and maintenance

Lewis Lung Carcinoma (LLC) cells were maintained in Dulbecco's Modified Eagle's Media containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were maintained in a humidified atmosphere at 37°C and 5% CO_2. At designated time points for use *in vitro* cytotoxicity study, subconfluent cells were harvested and seeded on 12-well plates at 30,000 cells/well.

***In vitro* cytotoxicity study**

Undoped and doped drug loaded PCL and PCL with 10% PGC-C18 meshes were prepared with 1 wt% and 0.1 wt% SN-38. Meshes were placed in permeable transwells (Polyester membrane insert, 3.0 µm pore size; Corning Incorporated, Corning, NY) and maintained in serum positive media (SPM) at 37 °C, where media was changed daily to ensure sink conditions for SN-38. At day 5 in the released study, the transwells and meshes were transferred to wells containing tumor cells in 3 mL of fresh SPM. After 24 hours of incubation with LLC cells, transwells and meshes were removed and replaced in fresh SPM at 37 °C. Five days after treatment, tumor cell viability was tested using a colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell proliferation assay (Sigma, St. Louis, MO). Cell viability in each well was calculated as the percentage of the positive control absorbance. This procedure was repeated every 5 days in order to determine long term anti-proliferation effects of SN-38 loaded electrospun meshes.

References

(1) Wolinsky, J. B.; Ray III, W. C.; Colson, Y. L.; Grinstaff, M. W. *Macromolecules* **2007**, *40*, 7065.

Table S1. Electrospinning parameters used for undoped PCL and 10% PGC-C18 doped PCL meshes. Fiber sizes and macroscopic mesh surface characterization are shown.

		PCL + 0% PGC-C18	PCL + 10% PGC-C18
Electrospinning conditions	Concentration	20 wt/v%	20 wt/v%
	Solvent	5:1 $CHCl_3$	5:1 $CHCl_3$
	Flow rate	25 mL/hr	25 mL/hr
	Voltage	15 kV	12 kV
	Collector distance	25 cm	15 cm
Characterization	Fiber size	7.7 ± 1.2 µm	7.2 ± 1.4 µm
	Thickness (spun)	300 µm	300 µm
	(melt)	80 µm	80 µm
	Surface area (spun)	0.5700 m^2/g	0.5633 m^2/g
	(melt)	> 0.02 m^2/g	> 0.02 m^2/g

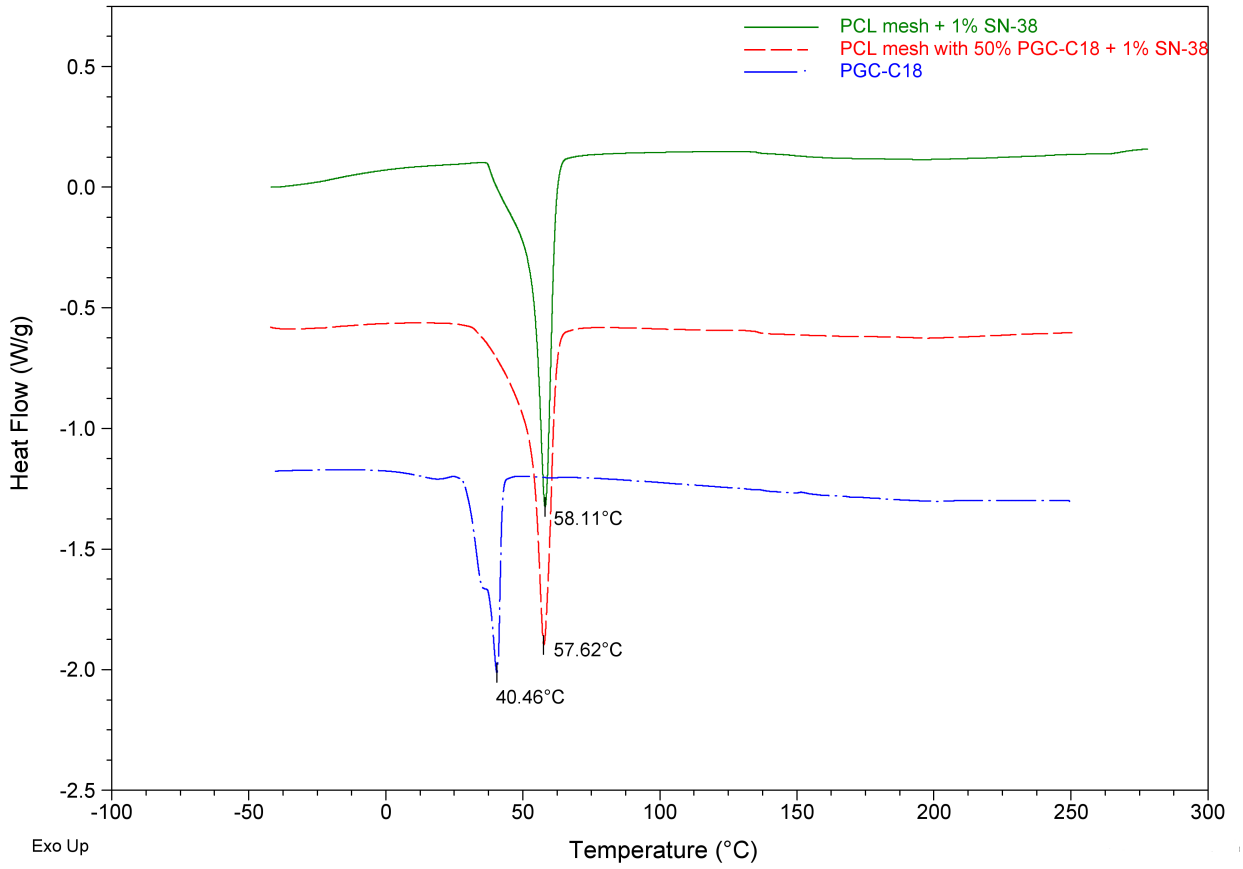

Figure S1. Representative Differential Scanning Calorimetry (DSC) traces for PCL electrospun meshes, PCL electrospun meshes with 50% PGC-C18, and non-electrospun PGC-C18. Both electrospun meshes were loaded with 1 wt% SN-38, but no melting peak was observed. Traces have been offset for clarity.

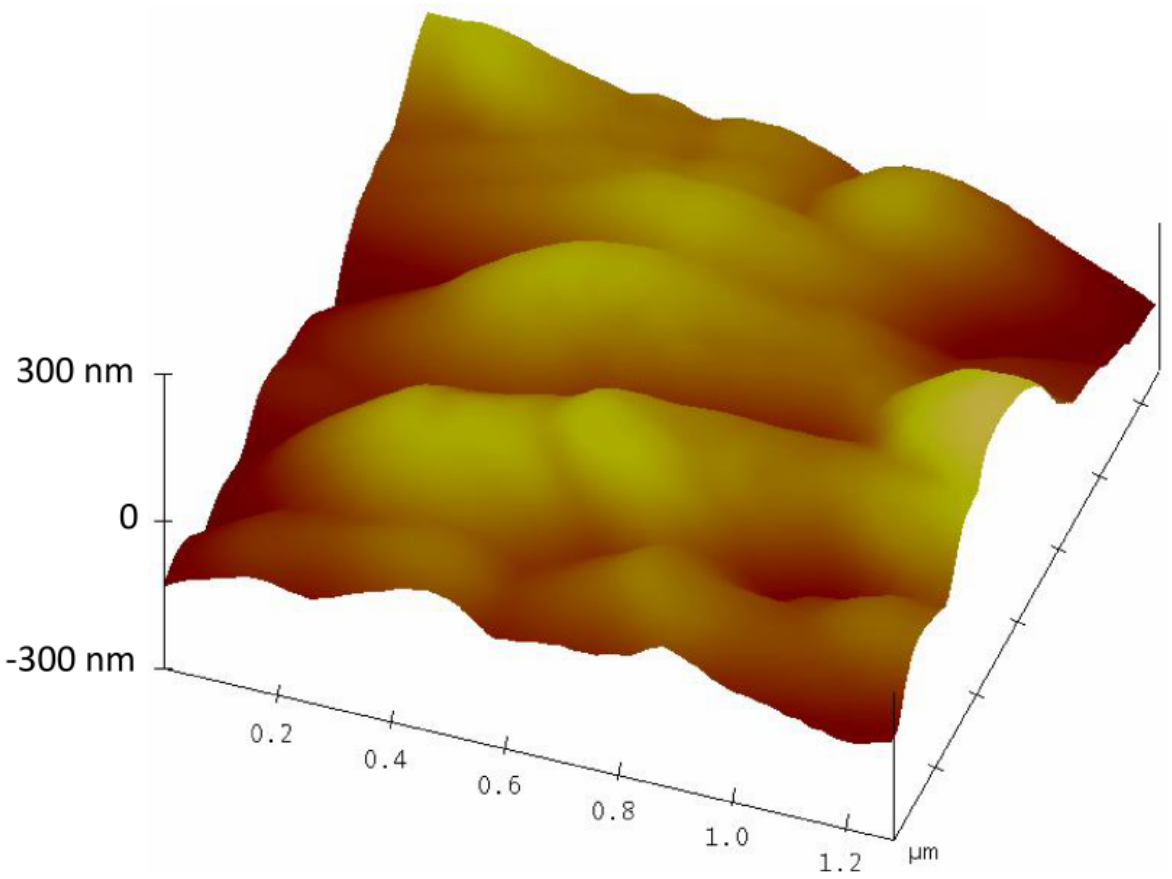

Figure S2. Sample AFM image of the surface of a single electrospun fiber (RMS ≈ 50 nm).

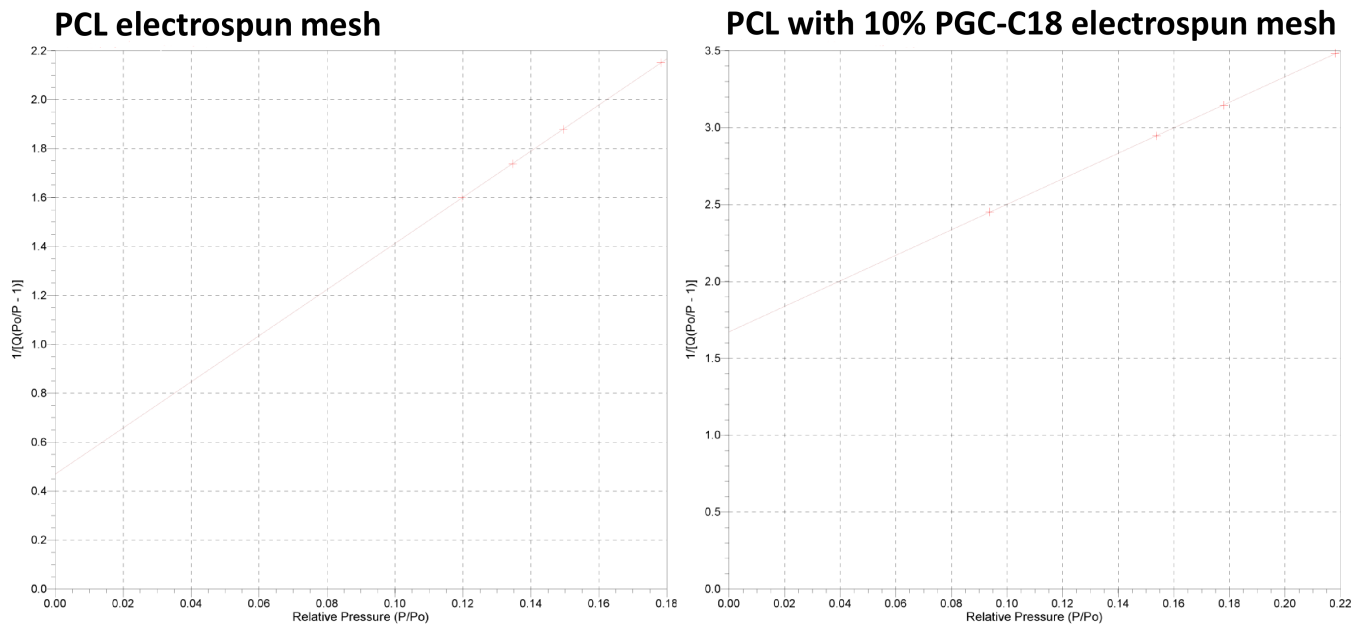

Figure S3. Raw Kr BET surface area plots for PCL and PCL with 10% PGC-C18 electrospun meshes. Surfaces areas of melted PCL and PCL with 10% PGC-C18 were below the detection limit and are not shown.

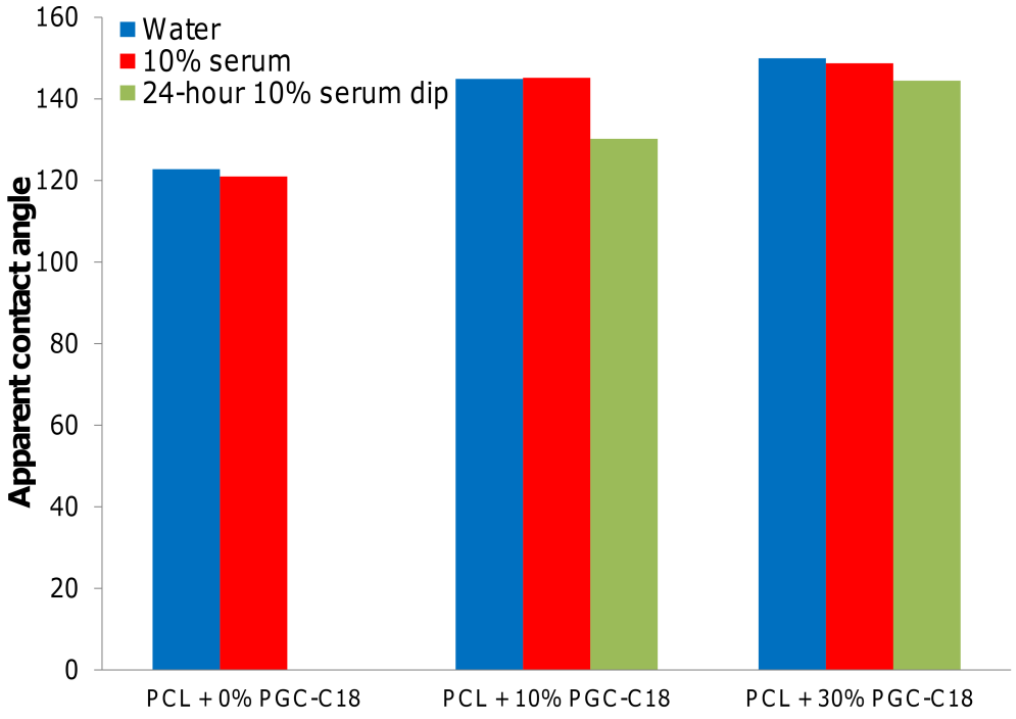

Figure S4. Apparent contact angle measurements of superhydrophobic electrospun meshes with and without serum. Meshes were either probed with serum in the applied droplet for contact angle measurements, or were incubated with serum containing solutions for 24 hours, dried, and probed with pure water. A larger decrease in apparent contact angle was seen after incubation in serum for 24 hours, and increasing PGC-C18 reduced the decrease in apparent contact angle.