Supporting Information

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Triggered Drug Release from Superhydrophobic Meshes using High-Intensity Focused Ultrasound

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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. 5-benzyloxy-1,3-dioxan-2-one was prepared as previously reported[29]. 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, DCU = dicyclohexylurea, DMAP=4-(dimethylamino) pyridine, 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[29]. 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 18 h, and the resulting copolymer was isolated by precipitation in cold methanol (99% yield). The benzyl-protecting groups on the copolymer were removed via palladium-catalyzed hydrogenation overnight in THF, and filtered through Celite (95% yield). The deprotected polymer, stearic acid, DCC, and DMAP were dissolved in DCM and stirred at room temperature for 18 hours. Precipitated 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 M_n=21,100 Da and a polydispersity of M_w/M_n=1.73.

Fabrication of doped and undoped poly(ε-caprolactone) superhydrophobic 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 10% or 30% PCL was replaced with PGC-C18. Solutions were loaded into a glass syringe and placed into a syringe pump set at a flow rate of 10 mL/hr. A 12-18 kV high voltage lead was applied at the base of the syringe needle. A grounded rotating collector was covered in aluminum foil and placed 9 - 24 cm away from the needle. Meshes were produced

such that the total thickness was 300 μm. SN-38 loaded meshes were fabricated by adding 0.1 wt% or 1 wt% relative to total polymer into the electrospinning solution. Previous work has demonstrated that SN-38 partitions to the surface of PCL-PGC-C18 electrospun meshes when present in small concentrations, and that the SN-38 is located at the core and surface of the fibers when present in larger concentrations.[42] Layer-by-layer (LbL) constructs were produced where two non-drug loaded layers sandwiched a 1% SN-38 containing layer, where iterative electrospinning solutions were run on the same aluminum foil. All layers contained 30% PGC-C18. Each of the outer layers was 120 μm thick, while the drug-containing layer was 100 μm thick.

Electrospun mesh 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. Thickness of electrospun meshes was measured using a micrometer, and confirmed using SEM.

Apparent contact angle measurements

Hydrophobicity and surface energy of electrospun meshes were assessed by apparent contact angle measurements **(Kruss DSA100 Goniometer).** A 3 μL water droplet was placed on the surface for measurement and fit using Young-Laplace mode.

Application of acoustic pressure using high intensity focused ultrasound (HIFU)

A 1.1-MHz HIFU transducer (64 mm diameter, 63 mm radius of curvature, Sonic Concepts, Bothell, WA) was used to insonify the meshes. The ultrasound signals were generated with an arbitrary waveform generator (33250A, Agilent, Santa Clara, CA, USA) and amplified with an RF amplifier (A150, ENI, Rochester, NY, USA). An impedance matching network (Sonic Concepts) was connected between the amplifier and the transducer in order to optimize the efficiency of the acoustic output. A custom-built plastic cone was placed over the transducer and filled with degassed, deionized water to couple the transducer to the sample. A hole was drilled through the tip of the cone to allow ultrasound transmission to the sample, and the hole was covered with Tegaderm (3M, St. Paul, MN, USA) to prevent the water from draining out of the cone. Peak rarefaction pressures of 0.71-4.25 MPa were used to insonify electrospun meshes.

Direct visualization of water infiltration into superhydrophobic meshes

The wetting of meshes was directly visualized by acquiring video to determine changes in transparency. Native and degassed electrospun meshes were also visualized using a Vevo 770 imaging setup (VisualSonics, Inc, Toronto, Canada) and a 55-MHz scanhead. Native meshes were visualized with no prior treatment. Degassed PCL electrospun meshes received a 10 second HIFU treatment with a 4.25 MPa peak rarefaction pressure.

Quantification of water infiltration by total wetted area

Videos were transferred to a computer and analyzed using MATLAB (Mathworks, Natick, MA, USA). A reference frame was obtained before sonication and this was subtracted from each subsequent frame to remove the background. The resulting change in image intensity was used to calculate the area of the wetted mesh. Significance in the difference between

water infiltration into PCL, PCL with 10% PGC-C18, and PCL with 30% PGC-C18 at 3 rarefaction pressures was tested using a Student's t-test.

In vitro SN-38 release and encapsulation efficiency determined by HPLC in PBS

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 (λ_{ex}=380 nm, λ_{em}=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.

PCL meshes with 30% PGC-C18 were prepared using the above mentioned procedures with 0.1% or 1% SN-38. The release kinetics from SN-38 was assessed in 50 mL of PBS buffer at 37 °C. Each mesh had a mass of 10 mg (1x1x0.03 cm). 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. For ultrasound-triggered release, meshes were sonicated with HIFU in CW mode using 3.54 MPa of power over the entirety of the mesh to promote release. As a positive control, SN-38 loaded meshes for the ethanol dip study were dipped in ethanol for <1 second, and were immediately placed in PBS buffer. The amount of SN-38 released was measured as described above.

SN-38 release in PBS supplemented with fetal bovine serum

A second SN-38 release study was performed using 10% fetal bovine serum (FBS) in PBS. A 10% serum supplementation was selected to allow consistency and comparison between the

drug release and cell culture studies. Higher serum concentrations may lead to quicker air removal and drug release, but were not tested in this study, and will be the focus of future studies. Both non-layered and LbL constructs were evaluated. Measurements for this study were made using a fluorimeter (λ_{ex}=380 nm, λ_{em}=550 nm, slit size = 1 mm) with a sensitivity of 10 ng/mL in disposable polystyrene cuvettes. At specific time points, an aliquot of release media was removed and the concentration of drug was measured. The aliquots were diluted 9:1 in pH 9 borate buffer so all SN-38 in solution will be in the lactone form, as the lactone and carboxylate form cannot be distinguished without chromatography and have different extinction coefficients. For ultrasound-triggered release, meshes were sonicated with HIFU in CW mode using 3.54 MPa of power over the entirety of the mesh to promote release. Statistical significance between SN-38 release from layered meshes before and after ultrasound was analyzed using analysis of covariance (ANCOVA) with Prism 5.0d (GraphPad, La Jolla, CA) ($F=23.9$, DOF=1,8, $p=0.0012$).

Cell care and maintenance

Michigan Cancer Foundation-7 (MCF-7) cells were maintained in Eagle's Minimum Essential Media containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were incubated 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 24-well plates at 15,000 cells/well.

In vitro cytotoxicity study

Two sets of LbL meshes (with and without ultrasound treatment, 1% SN-38) and unloaded meshes were prepared. 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 1 in the released study, the transwells and meshes were transferred to wells containing tumor cells in 1.5 mL of fresh SPM. After 24 hours of incubation with MCF-7 cells, transwells and meshes were removed and replaced in fresh SPM at 37 °C. Five days after initial 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). Prior to the MTS assay, the cells were washed to remove any remaining SN-38. Relative cell viability was determined by dividing the absorbances of sample wells by the untreated control wells, after the blank (5:1 media:MTS solution) was subtracted from each. This procedure was repeated every 5 days in order to determine long-term anti-proliferation effects of SN-38 loaded electrospun meshes. One set of LbL meshes received a HIFU treatment at day 10 of the study to induce drug release.