Photoactive Electrospun Polymeric Meshes: Spatiotemporally Wetting of Textured 3-Dimensional Structures

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Supporting Information

General Procedure and Methods: All chemicals were purchased from Sigma Aldrich and used without further purification, unless otherwise noted. Solvents used during synthesis were dried and distilled prior to use. All reactions were done in dry conditions using nitrogen. All NMR spectra were recorded on a Varian Mercury spectrometer operating at 300 MHz. Chemical shifts are reported in parts per million as follows: chemical shift, multiplicity (s= singlet, d= doublet, t= triplet, q= quartet, m= multiplet, br= broad).

Polymer Synthesis: A Poly(glycerol-co-ε-caprolactone) (1:4) (PGC) backbone was synthesized using a previously published protocol.[1]

12-(1-(2-nitrophenyl)ethoxy)-12-oxododecanoic acid (C12-NPE) (01): Dodecanedioic acid (6 g, 26.1 mmol), cat. DMAP, and 1-(2-nitrophenyl)ethanol (1.45 g, 8.7 mmol) were dissolved in DMF (70 mL), and the solution was cooled to 0 °C. To the mixture was added 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (2.5 g, 13 mmol) and the reaction was stirred overnight and warmed to room temperature. The solution was taken up into EtOAc (500 mL) and washed with water, 1N HCl, and sat. NH_4Cl solutions. The organic layer was dried over Na_2SO_4. The volatiles were evaporated and the residue was purified on silica gel chromatography (gradient hexanes:EtOAc, 5:1 to 4:1 to 3:1 to 2:1), affording product **01** as a thick oil in 31% yield. ^{1}H NMR (300 MHz, $CDCl_3$, δ) (Figure S9): 1.21 (br.s, 12 H, CH_2), 1.49-1.66 (m, 7 H; CH_2, CH_3), 2.20-2.33 (m, 4 H; CH_2), 6.27 (q, 1 H, J= 6 Hz; CH), 7.30-7.43 (m, 1 H; CH), 7.53-7.65 (m, 2 H; CH), 7.87 (d, 1 H, J=8.2 Hz; CH). ^{13}C NMR (300 Hz, $CDCl_3$, δ) (Figure S10):

20.8, 21.9, 24.6, 24.7, 28.7, 29.0, 29.1, 29.3, 34.0, 34.2, 67.8, 124.3, 127.1, 128.3, 133.5, 138.0, 147.7, 172.7, 177.6, 180.3.

PGC-C12-NPE (02): Product **01** (1.02 g, 2.7 mmol), cat. DMAP, PGC (1.3 g, 2.2 mmol) were dissolved in DCM at room temperature. To the mixture was added N,N'-dicyclohexylcarbodiimide (DCC) (924 mg, 4.5 mmol) and the reaction was stirred overnight. The solution was filtered to remove the N,N'dicyclohexylurea, a byproduct of the reaction, and then the polymer was precipitated in 30 mL methanol overnight at -20 °C. The polymer was subsequently filtered and washed with methanol affording product **02** as a white solid in 69.3% yield. ^{1}H NMR (300 MHz, $CDCl_3$) (Figure S11): δ= 1.13 - 1.44 (m, 21 H; CH_2), 1.46 - 1.74 (m, 28 H; CH_2 and CH_3), 2.15 (s, 3 H; CH_3), 2.21 - 2.45 (m, 21 H; CH_2), 3.90 - 4.42 (m, 17 H; CH_2), 5.18 - 5.33 (m, 1 H; CH), 6.29 (q, J=6.48 Hz, 1 H; CH), 7.36 - 7.48 (m, 1 H; CH_2), 7.60 (d, J=4.12 Hz, 2 H; CH_2), 7.91 (d, J=8.24 Hz, 1 H; CH_2). ^{13}C NMR (300 Hz, $CDCl_3$) (Figure S12): δ= 22.0, 24.5, 25.5, 28.3, 29.2, 29.3, 33.8, 34.1, 34.3, 67.9, 68.2, 68.3, 124.4, 127.1, 128.2, 133.5, 138.1, 147.7, 154.8, 155.2, 172.6, 172.9, 173.6. Differential Scanning Calorimetry: Tg= -50.13 °C, Tc= -16.14 °C, Tm= 37.42 °C Figure S13. UV-vis absorbance Figure S14. M_n= 8243 g/mol, PDI = 2.23 as determined by gel permeation chromatography (GPC) compared to polystyrene standards.

Differential Scanning Calorimetry (DSC): A TA DSC Q100calorimeter was used to determine the glass transition temperature (Tg), crystallization temperature (Tc), and melting temperature (Tm) of the PGC-C12-NPE polymer. The sample was heated to 100 °C at 10°C/min and isothermed for 5 minutes, cooled to -70°C at 10°C/min and isothermed for 5 minutes, and this protocol was repeated 2 more times to eliminate any phase memory in the polymer sample. The data was stored on the 3rd cycle.

Fabrication of Photoactive Electrospun Meshes: The PGC-C12-NPE polymer was mixed with poly(ε-caprolactone) PCL (70,000-90,000 MW, Sigma) at a 3:7 ratio. A 10% wt. 5:1 chloroform:methanol solution was made using the 3:7 blend. The electrospinning parameters were modified from a previous publication based on PCL.[18] The procedure was modified to produce nano-fibers (~200 nm) using a 3 ml $hour^{-1}$ flow rate, a 10 kV source, a collector distance of 10 cm, and a 20 gauge needle. The thickness of the resulting mesh is directly related to the electrospinning time allowing for a dynamic range of mesh thicknesses (1 μm to 1 mm) using the same electrospinning parameters.

Gel Permeation Chromatography (GPC) analysis: PGC-C12-NPE molecular weights were determined by GPC versus polystyrene standards using a THF eluent at a 1.0 mL/min flow rate through a Styragel column (HR4E THF, 7.8 x 300 mm) with a refractive index detector.

UV degradation study: PGC-C12-NPE was dissolved in THF at a 10 mg/mL concentration and exposed to 21.6 J/cm^2 of 365 nm UV light. The molecular weight and dispersity of the polymer was determined by GPC analysis before and after UV exposure. No polymer degradation was observed; while, the expected weight loss of ≈ 10% associated with NPE deprotection was observed (Table S1).

UV Exposure: The photoactive meshes were fully wetted by washing first with ethanol and then water. The meshes were submerged under 35 ml (5.5 mm) of water. The samples were exposed to a 23 W spectroline long wavelength UV lamp (λ = 365 nm, Spectroline, Westbury, NY) for 0, 15, 30, 60, 90, and 120 minutes. (0.18 J cm^{-2} $minute^{-1}$) After UV exposure, the meshes were dried at room temperature before subsequent experiments.

NPE Deprotection on NMR: A Varian 400 MHz VNMRS NMR was used to analyze the polymer meshes after exposure to various doses of UV irradiation. The meshes were dissolved in 0.5 mL of deuterated chloroform. The integral of the 6.2 ppm peak, associated with the hydrogen on the carbon linking the 1-(2-nitrophenyl)ethyl protecting group to the dodecandioic acid was analyzed and compared to the integral of the 5.2 peak associated with the hydrogen on the central carbon in the glycerol part of the backbone (Figure S1).

Scanning Electron Microscopy: A Zeiss SUPRA 55VP field emission SEM was used to image the surfaces of the meshes before and after 120 minutes of UV exposure (Figure S3). The samples were affixed to an aluminum sample stub using copper tape and were coated with 5 nm of Au/Pd prior to imaging and imaged at an accelerating voltage of 2 kV.

Contact Angle Analysis: A Kruss DSA100 contact angle goniometer was used to quantify the contact angles of deionized water (4 µl) over time on the surface of the electrospun meshes. Each water droplet was recorded at 0.2 frames per second and the contact angle was analyzed frame-by-frame using the Drop Shape Analysis software provided by Kruss (Figure S4 and Table S2). The surface tensions of the liquids used in this study (Water, Visipaque, and I_2-BSA) were determined using the same instrument and a hanging drop method.

3D Water Imaging: A 1.59 mm in diameter circular photomask was used to UV irradiate a circular region of the meshes for 0, 30, and 60 minutes. A 4 µL drop of 80 mgI/mL Visipaque 320 (GE Healthcare, Lot: 10352690) in water solution was applied to the surface of the UV active meshes (~300 µm thick) (Visipaque surface tension measured in Figure S5). The water infiltration was measured using a µCT imaging system using an isotropic voxel resolution of 36 μm^3, 70 kVP tube voltage, 114 µAmp current and 300 ms integration time. The image slices were converted into the standard image format (DICOM) using proprietary software from Scanco

Medical. The data was then reconstructed and analyzed using a commercial image processing software (AnalyzeTM, BIR, Mayo Clinic, Rochester, MN, USA). Water infiltration was analyzed using ImageJ (Version 1.45, NIH)[2] where infiltration depth was measured as the length of pixels greater than 1500 arbitrary CT units from the mesh surface to the infiltration depth averaged across 3 measurements per sample (Figure S6).

3D Protein Adsorption Imaging: A 1.59 mm in diameter circular photomask was used to UV irradiate a circular region of the meshes for either 0 and 60 minutes. A 4 μL drop of 1 mgI/mL I_2-BSA (synthesized using a modification of a previously published protocol)[3] in water solution (~1.2% I_2-BSA) was applied to the surface of the UV active meshes (~300 μm thick) to simulate protein adsorption onto the mesh in 3D (Surface tension of I_2-BSA measured in Figure S7). The I_2-BSA adsorption was measured using a μCT imaging system using the same parameters and analysis as the 3D Water Imaging protocol described above.

Cell Culture: MCF7 cells were acquired from ATCC and cultured using full growth media (RPMI with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin-Gentamicin (P/S/G)) in a Thermo Electron Corporation Forma Water Jacketed CO_2 incubator (37°C, 5% CO_2, humidified). Cells were passaged a maximum of 20 times.

UV Light Controlled Cell-Patterning: 1.59 mm in diameter circular photomasks were placed over twelve photoactive meshes and each sample was pre-wetted in ethanol and then water. Half of the samples were exposed to UV irradiate for 60 minutes (365 nm, 10.8 J cm^{-2}) creating a small circular hydrophilic region in the center of these hydrophobic meshes. Each sample was then sterilized in 190 proof ethanol for one hour in a biological safety cabinet (Thermo Electron Corporation Forma Class II, A2). After sterilization, the meshes were allowed to dry in the biological safety cabinet for 2 days. Each sterile mesh was then pre-wet with 10 μL of full growth

media (RPMI with 10% FBS and 1% P/S/G) applied to the center of the mesh for 10 minutes to allow proteins within the media to adsorb onto the mesh. Any remaining volume was removed after 10 minutes. Four micro-liters of $1x10^6$ MCF7 cells/mL were applied to the pre-wetted spot. The meshes were immediately place in an incubator (Thermo Electron Corporation Forma Water Jacketed CO_2 incubator) (37°C, 5% CO_2, humidified) for 1 hour before 100 µL of full growth media was applied to the surface of each mesh. The meshes were incubated for 1 and 5 days (n=3 per UV exposure time and incubation time), where the media was replaced daily. A CellTracker™ green dye solution was prepared by dissolving 50 µg of lyophilized powder in 10.76 µL of DMSO to create a 10mM CellTracker™ solution. This solution was then diluted with 999 µL of serum free media (Phenol-red free Opti-mem® 1 (1X), Lot: 1125459) to 1 µL of the 10mM DMSO solution. After the appropriate incubation time, the media was aspirated from each mesh and replaced with 100 µL of the dilute CellTracker™ Green dye solution. The samples were placed in the incubator for 30 minutes to allow the dye to be internalized into the living cells on the meshes after which the solution was aspirated and replaced with 100 µL of full media. The samples were again incubated for 30 minutes to allow the dye to be metabolized into the fluorescent byproduct, resulting in living cells fluorescing at 529 nm when exposed to a 488 nm laser. After being washed twice with phosphate buffered saline (PBS), each mesh was fixed with 4% formaldehyde in water solution for 15 minutes at room temperature. The meshes were stored in PBS at room temperature prior to confocal imaging.

Confocal Imaging: Living cells on the meshes were examined and photographed using a Leica DMI6000 B confocal microscope equipped with a Nipkow (CSU-X1) spinning disk (Yokogawa) and a Hamamatsu ImagEM EMCCD camera imaging through a 10x objective. The samples were excited using a Coherent Sapphire laser at 488 nm and fluorescent images were captured using a

Chroma ET bandpass 525/50 filter to capture the 529 nm wavelength emission from the CellTracker™ live cell stain. An automated stage controlled via a μManager plugin for ImageJ (Version 1.45, NIH)[2] was used to create a montage of images to capture a grid spanning the entire mesh where each position consisted of 5 μm slices that spanned the entire depth (z direction) of the ~150 μm thick meshes. A 3-dimensional model was created from the montage of images using Imaris (Bitplane, version 7.2.3), which is a data visualization software for 3D data sets.

SEM of MCF7 Cells: After 24 hours of incubation on the photoactive meshes, the MCF7 cells were prepared for SEM imaging. The cells were washed twice in Hank's Balanced Salt Solution (HBSS) and then fixed in 3% glutaraldehyde in a sucrose-cacodylate buffer (0.1 M sodium cacodylate in a 0.1 M sucrose water solution) for 48 hours. The cells were then washed in sucrose-cacodylate buffer for 5 minutes (twice) and dehydrated by exposing the meshes to the following ethanol & sucrose-cacodylate buffer solutions: 35% ethanol, 50% ethanol, 70% ethanol, 95% ethanol, and 100% ethanol (twice). After dehydrating with the ethanol solutions, the meshes were exposed to 0.5 mL of hexamethyldisilazane (HMDS) for 10 minutes after which the cells were allowed to dry for 30 minutes. The meshes were then imaged according to the same protocol described above for scanning electron microscopy (Figure S8).

Supporting Figures:

Table S1. GPC analysis of PGC-C12-NPE before and after NPE deprotection due to UV exposure.

	Before UV exposure	After 21.6 J/cm^2 UV exposure
M_n (g/mol)	8243	7482
Dispersity	2.23	2.3
Average monomer:polymer ratio	42.5	45.5

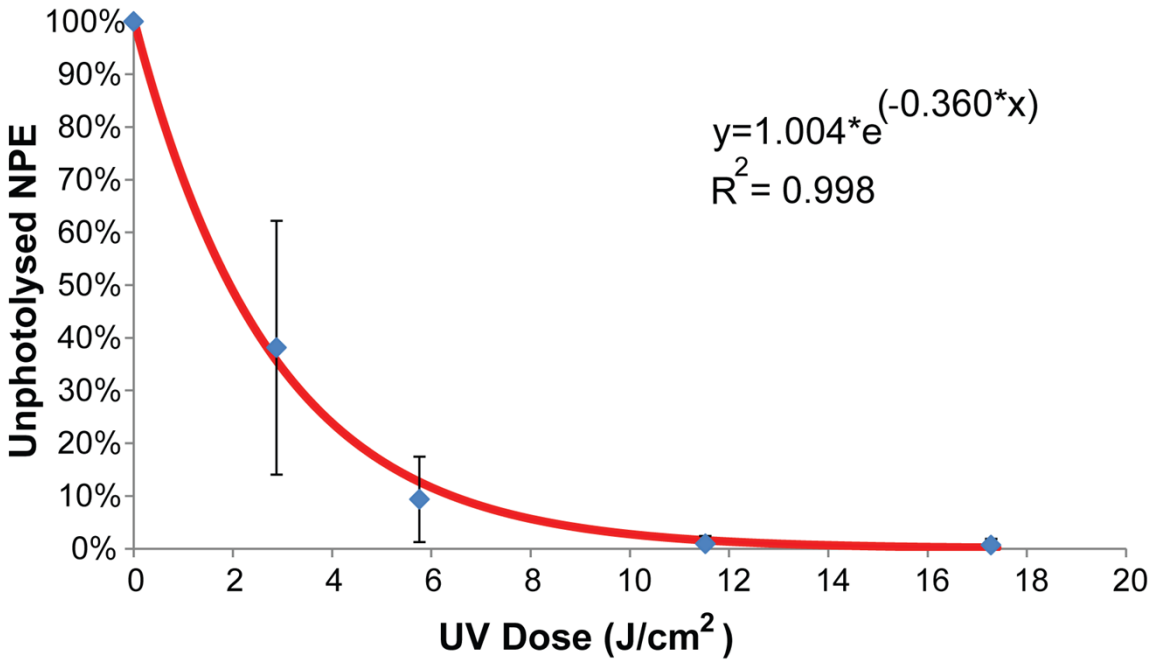

Figure S1. NMR evidence of NPE cleavage via diminishing peak integrals at ~6.2 ppm, corresponding to the lone hydrogen on the carbon linking the NPE group to the alkyl chain which is attached to the polymer backbone through the glycerol monomer. The data is fit to an exponential function (red).

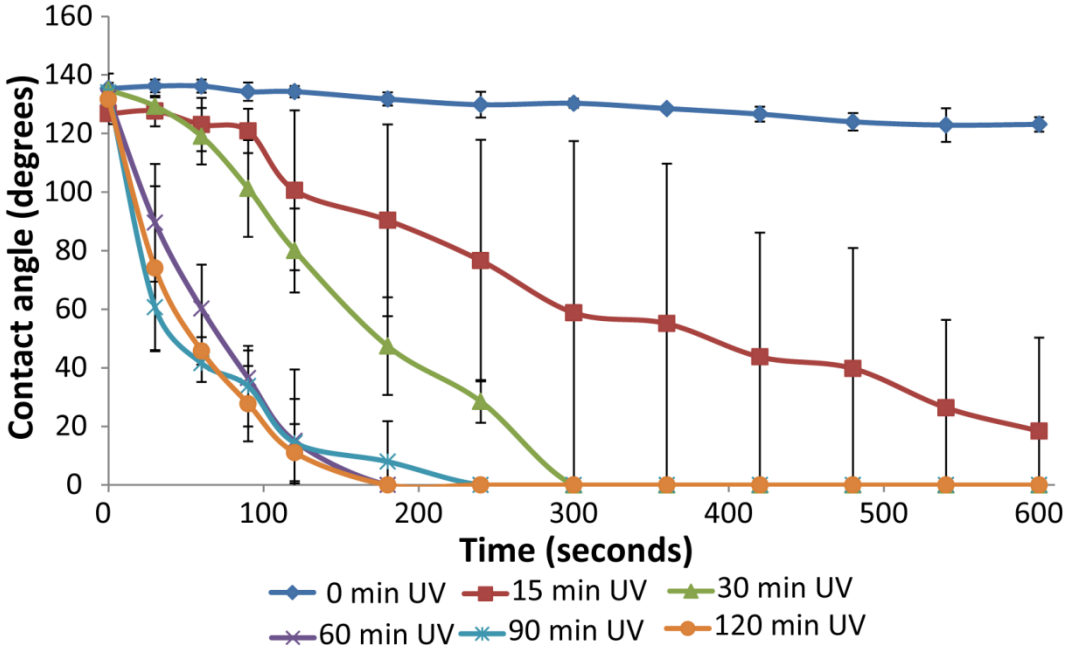

Figure S2. UV induced hydrophobicity change from hydrophobic (~135°) to hydrophilic (~0°) ACA after 0, 15, 30, 60, 90, or 120 minutes of UV exposure. The ACA of water (4 µl) on the electrospun polymeric mesh surface was measured over 600 seconds. (n=3; Avg ± SD)

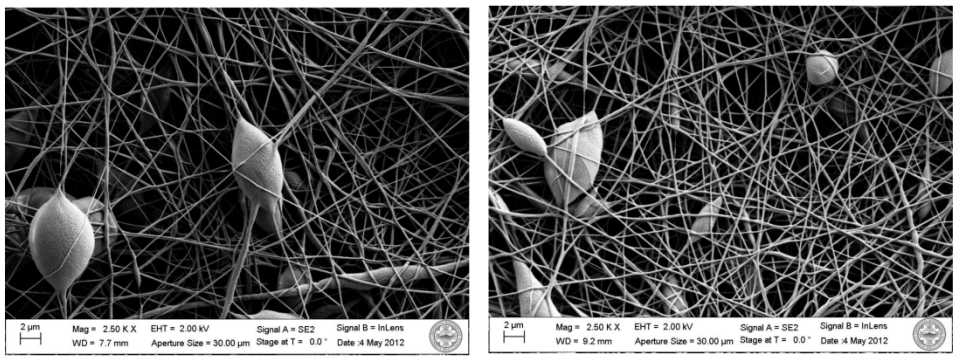

Figure S3. SEM images of 7:3 PCL:Photo mesh before UV irradiation (left) and after 120 minutes of UV irradiation (right). There are no observable changes to the fiber morphology or texture.

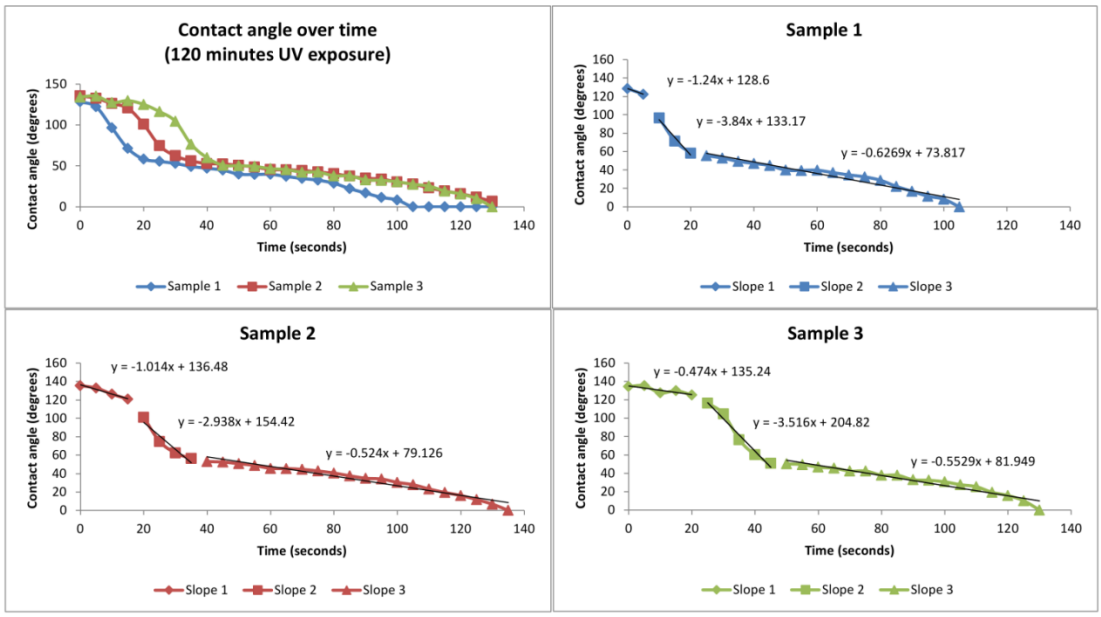

Figure S4. The wetting profile of three different meshes after 120 minutes of UV exposure. Illustrates three distinct wetting rates as the water droplet infiltrates the photoactive mesh (after 120 minutes (21.6 J/cm^2) of UV exposure) into each of the samples.

Table S2. The average ± standard deviation wetting rate data for the meshes described in Figure S4.

	Average Wetting Rate [Degrees Second^{-1}]	Standard Deviation
Hydrophobic wetting	-0.91	0.39
Rapid wetting	-3.43	0.46
Hydrophilic wetting	-0.57	0.05

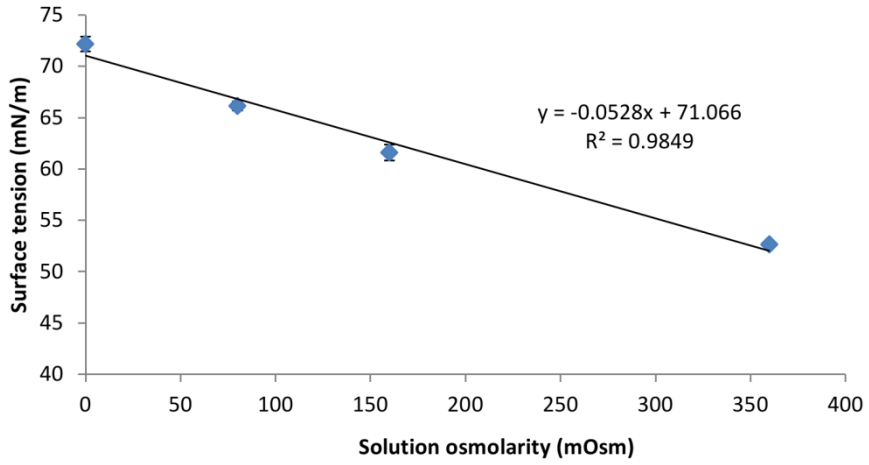

Figure S5. Illustrates the effect of dilution a 320 mOsm solution of Visipaque into water on the surface tension of the solution. (n=3, Avg ± SD)

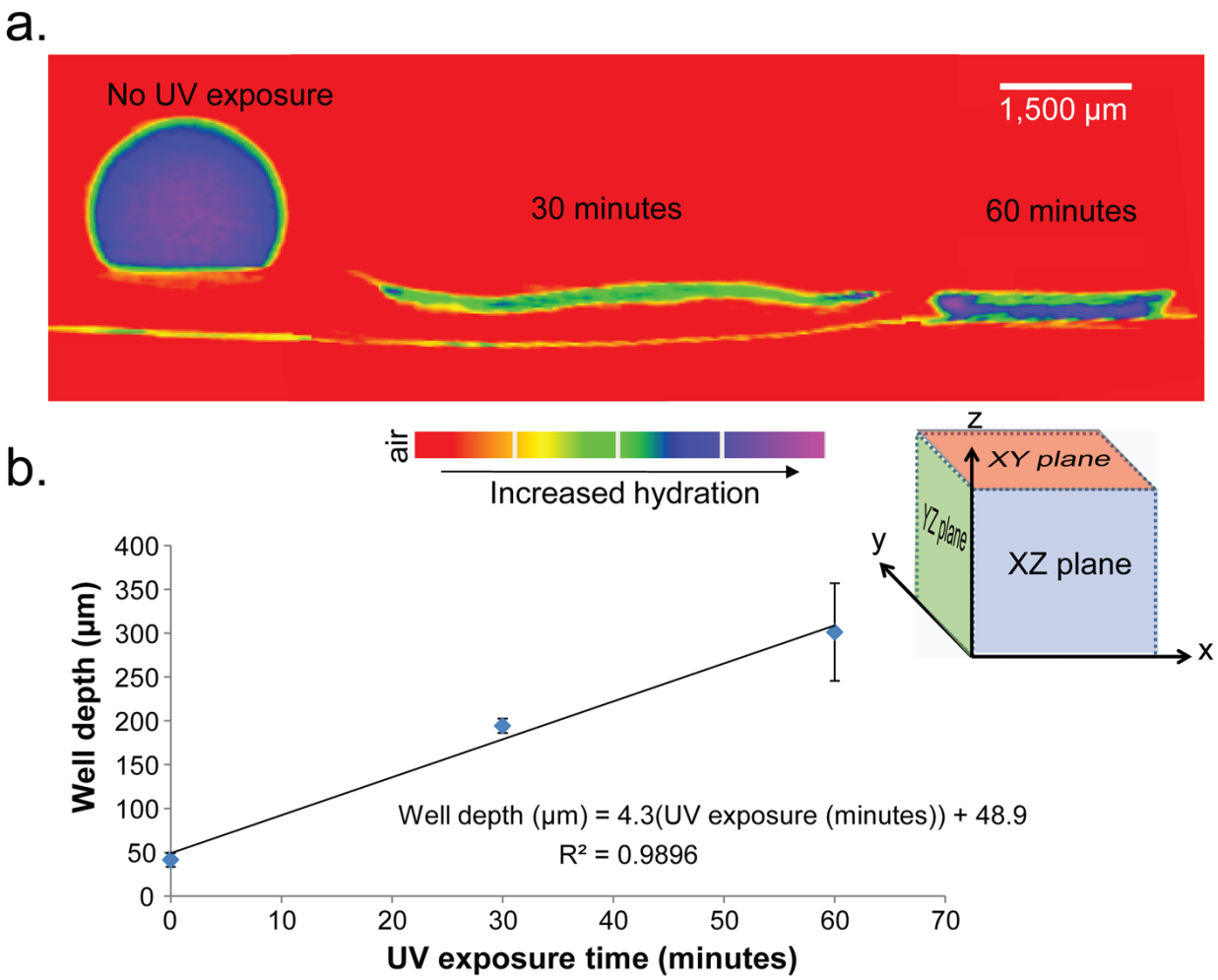

Figure S6. (a) µCT imaging of 3D hydrophilic regions within a hydrophobic bulk material using water soluble CT contrast agent penetration into the meshes. (b) CT contrast agent penetration versus UV exposure time where a linear relationship is observed. This trend is likely due to a gradient of UV irradiation intensity through the mesh causing reduced deprotection rates leading to the gradual deprotection of the mesh layer by layer. (n=3; Avg ± SD)

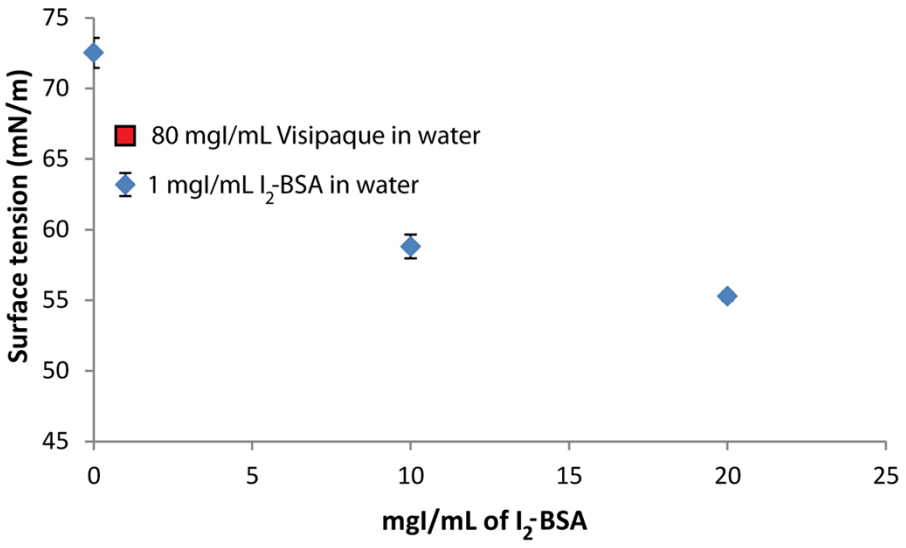

Figure S7. I_2-BSA in water solution surface tensions. A 1 mgI/mL I_2-BSA in water solution was chosen in Figure 3a to match the surface tension of the 80 mgI/mL Visipaque in water solution as close as possible while still maitaining a necessary contrast intensity. (n=3, Avg±SD)

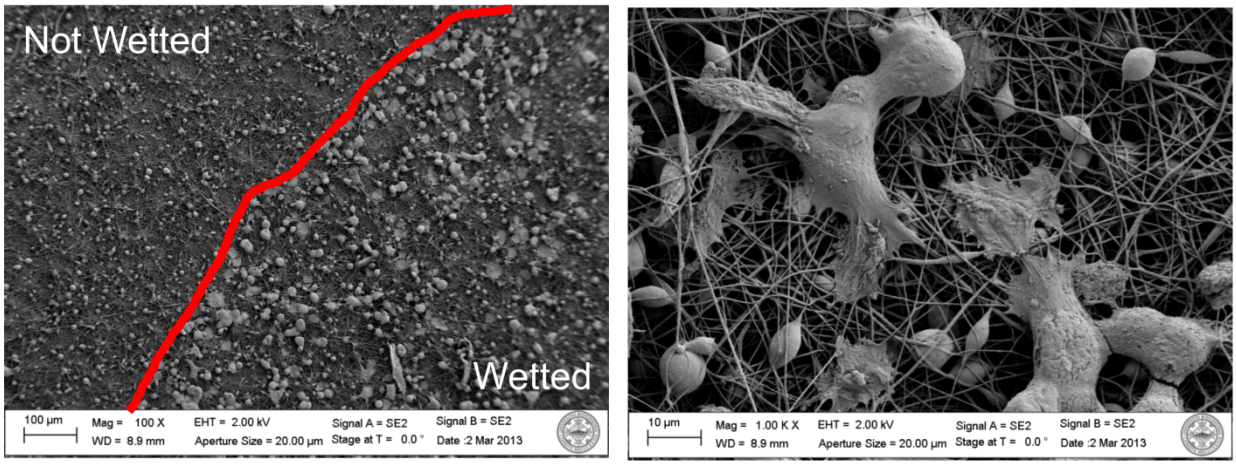

Figure S8. SEM images of dehydrated MCF7 cells on the surface of UV exposed mesh within the hydrophilic region. (Left) A clear boundary between wetted and non-wetted region is observable on these samples. (Right) At 1000x magnification, MCF7 cells can be observed interacting with both the fibers and beads of the electrospun mesh.

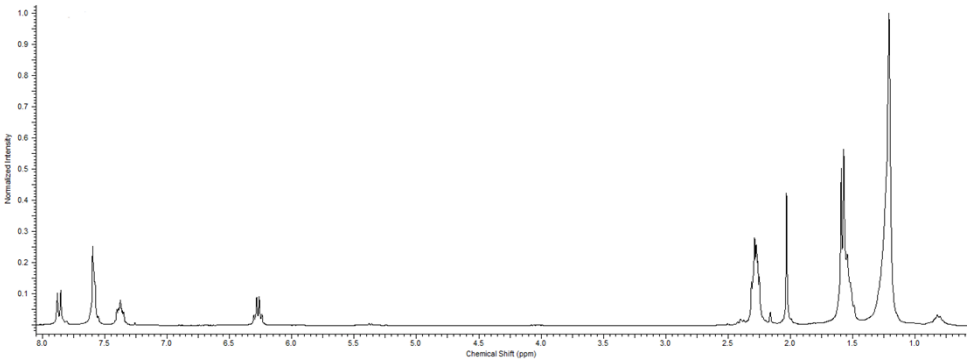

Figure S9. ^{1}H NMR for C12-NPE using a Varian 300 MHz VNMRS NMR

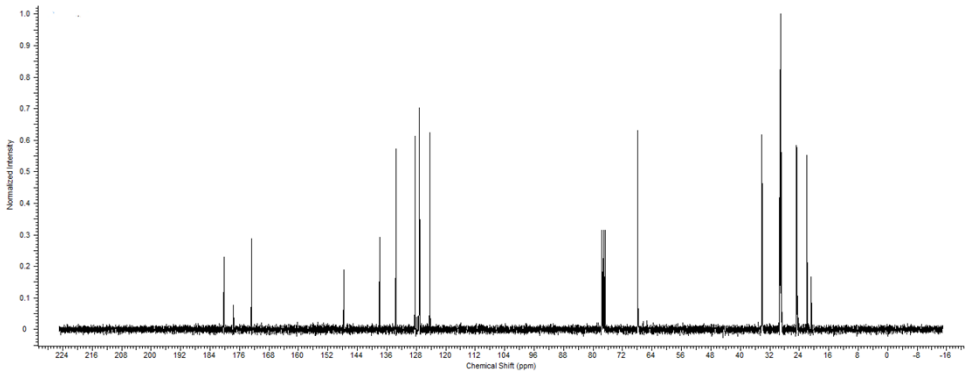

Figure S10. ^{13}C NMR of C12-NPE using a Varian 300 MHz VNMRS NMR

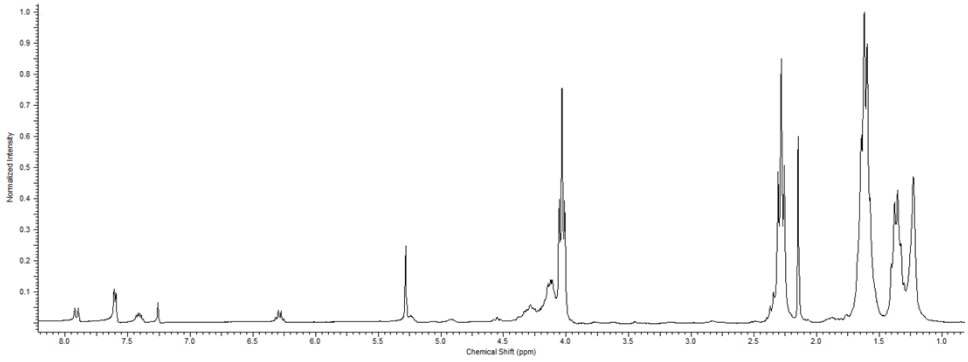

Figure S11. ^{1}H NMR of PGC-C12-NPE using a Varian 400 MHz VNMRS NMR

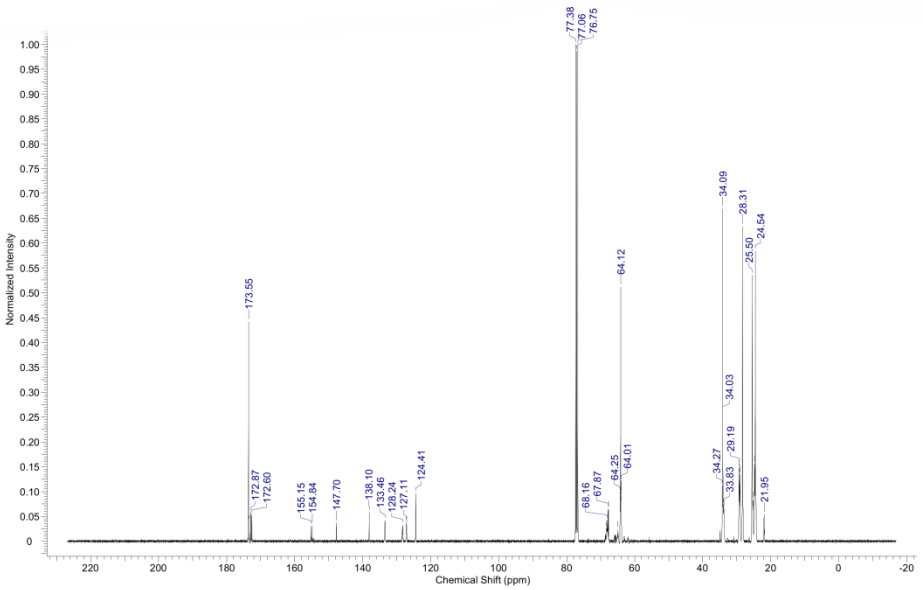

Figure S12. ^{13}C NMR of PGC-C12-NPE using a Varian 400 MHz VNMRS NMR

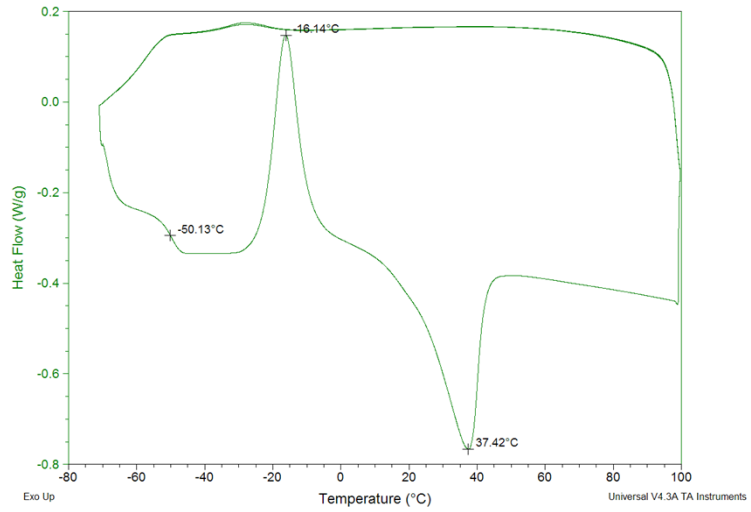

Figure S13. Differential Scanning Calorimetry of PGC-C12-NPE with a glass transition temperature at -50.13 °C, a crystallization temperature at -16.14 °C, and a melting temperature at 37.42 °C. The displayed scan is the 3rd heat/cool cycle to ensure a uniform phase throughout the polymer.

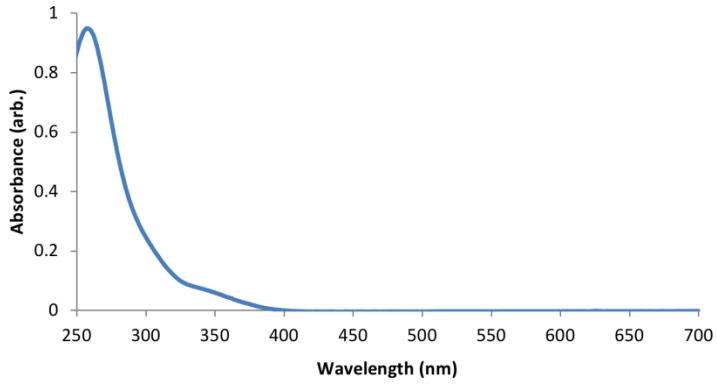

Figure S14. UV-Vis absorbance spectrum of PGC-C12-NPE in chloroform at 0.5 mg/ml.

References

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