Supporting Information for

Surface Modification of Melt Extruded Poly(ε-caprolactone) Nanofibers: Toward a New Scalable Biomaterial Scaffold

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Experimental Section

Materials. 4-hydroxyl benzophenone, *N,N*-dimethylformamide (DMF) (99%), dimethyl sulfoxide (DMSO), methanol (99.8%), acetone (99.8%), Azide-fluor 488, 5-bromovaleric acid, sodium azide, *N,N*-diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA) and triisopropylsilane (+99 %) were purchased from Sigma Aldrich. Propargyl bromide (80 wt% in toluene) was purchased from Acros Organics. Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) was a generous gift from the Finn lab. Rink Amide MBHA resin LL (100-200 mesh) was purchased from Novabiochem. Fmoc-protected amino acids and HCTU were obtained from Peptides International. 4',6-diamidino-2-phenylindole (DAPI) was purchased from EMD Biosciences, Inc. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and ActinGreen 488 ReadyProbes Reagent were purchased from Life technologies.

Instrumentation. Proton nuclear magnetic resonance (^{1}H NMR) and proton-decoupled carbon (^{13}C NMR) spectra were recorded on a Varian Inova 600 MHz NMR

spectrometer in deuterated solvents. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (δ 0.00) or residual protio solvent ($CDCl_3$, δ 7.26). A PerkinElmer Pyris Differential Scanning Calorimeter (DSC) was used to measure thermal properties of polymer films and nanofibers (TA Instruments Q100 DSC). A Micromeritics Tristar II BET instrument was used to measure the surface area of the PCL nanofibers. Multilayer co-extrusion was performed using the CLiPS two-component co-extrusion system with 12 multipliers. Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR) spectra were obtained using an FTS 7000 series DIGILAB, UMA 600. Gel Permeation Chromatography (GPC) was performed with a Varian Model 350 RI Detector with a Mesopore 50 x 7.5 mm column, flow rate was 1.0 ml/min with THF as the eluent. The mechanical properties were determined on a Zwick/Roell mechanical testing instrument with a rate of 50% per min at 37 °C.

Surface analysis of materials was investigated on a PHI Versaprobe 5000 Scanning X-Ray Photoelectron Spectrometer (XPS) with an Al Kα X-ray source (1486.6 eV photons). Scanning electron microscopy (SEM) was performed using a JEOL SEM under an emission voltage of 30kV. A high-intensity UV lamp (Bluepoint 4 Ecocure from Honle UV America Inc.) was used for surface modification of the PCL fibers with benzophenone. The molecular weight of the synthesized azido-peptide was measured on a Bruker AUTOFLEX III MALDI-TOF/TOF mass spectrometer using α-Cyano-4-hydroxycinnamic acid (CHCA) as a matrix. Fluorescent images were acquired via laser scanning fluorescence confocal microscopy using a Leica TCS SPE Confocal Microscope.

Synthesis of propargyl benzophenone. Propargyl benzophenone (Pr-Bz) was synthesized based on a procedure derived from Temel et al.[1] 4-hydroxybenzophenone (1 g, 5 mmol) and potassium carbonate (1.38 g, 10 mmol) were stirred in a round bottom flask in 30 mL of acetone for 6 hours at room temperature. After 6 hours, propargyl bromide (0.9 g, 7.5 mmol) was added and the reaction mixture was refluxed for 24 hours. The crude material was concentrated under reduced pressure and re-dissolved in CH_2Cl_2. The organic layer was washed with water (3x) and brine (3x) and dried over Na_2SO_4. Residual solvent was evaporated under reduced pressure and the crude material was recrystallized from n-hexane (yield 80%). ^{1}H NMR ($CDCl_3$ 600 MHz) δ 7.7 (d, 2H), 7.3 (m, 5H), 7.0 (d, 2H), 4.7 (s, 2H), 2.5 (s, 1H). ^{13}C NMR ($CDCl_3$ 600 MHz) δ 195.5, 161, 138.1, 132.4, 129.8, 128.2, 114.4, 78.8, 76.2, 55.6. ATR-FTIR 3236, 3059, 2113, 1635, 1600, 1572, 1307, 1264, cm^{-1}.

Coextrusion Processing of PEO/PCL multilayered strand and PCL fibrous scaffold. A multilayered film was extruded by the layer-multiplying co-extrusion system to produce polymeric nanofibers.[2] Polycaprolactone (PCL) CAPA 6800 pellets were co-extruded with poly(ethylene oxide) (PEO) to produce PCL nanofibers. In order to match the rheology of PCL and PEO melts during the extrusion, two grades of PEO (Dow POLYOX N80 (M_w = 200 kg/mol) and POLYOX N10 (M_w = 100 kg/mol)) with a weight ratio of 30:70 were pre-blended using a Haake Rheodrive 5000 twin screw extruder. The viscosities of the obtained PEO blend and PCL melt match at the extrusion temperature, 200 ºC. Ten vertical multipliers and two horizontal multipliers were used throughout the extrusion line to generate a 256 x 4 matrix architecture that contains 128 x 4 PCL nanodomains embedded in PEO. After removing PEO in stirring water at room

temperature for 24 hours or with a high pressure water jet (5 min), a PCL fibrous scaffold was obtained due to the high extent of entanglement among these PCL nanofibers. The PCL scaffold was washed with methanol 3 times and dried in a lyophilizer.

Surface Area Measurements. To measure the surface area of PCL fibers, BET measurements were conducted. The samples were degassed in an inert nitrogen gas atmosphere at 40 ºC for 6 hours prior to BET measurements. Krypton gas was deposited on the PCL fiber surface in the measurement, and 13 data points were collected with the relative pressure ranging from 0.06 to 0.30. The BET plot was generated with a linear region that has a slope of 1.31 ± 0.02 g/cm³ and an intercept of 0.059 ± 0.003 g/cm³. A similar multipoint BET plot was generated for electrospun PCL fibers, which has a slope of 8.57 g/cm^3 and an intercept of 0.91 g/cm^3 (R^2 = 0.998).

Thermal properties. The multilayered PCL/PEO strands and PCL fibers obtained after removing PEO were compared by DSC. The heating rate was 10 °C/min from -80 to 200 °C, 2nd run.

Surface modification of PCL fibers with benzophenone. The maximum absorbance (λ_{max}) of Pr-Bz was determined to be 337 nm, as expected for benzophenone derivatives. 3mg of PCL fibers were immersed in a 1 mg/mL solution of Pr-Bz in methanol (3 mL) which was allowed to dry through evaporation. The PCL fibers were UV irradiated and the light source was equipped with a 320-500 nm filter cube (33.2 mW/cm^2, 4 cm distance) for 30 minutes on each side. Samples were washed 3x in methanol and the solvent was completely removed under vacuum.

UV degradation study. In order to study UV degradation of the PCL fibers, the molecular weight of the as-extruded PCL fibers and PCL-Alkyne (post UV treatment) were determined by GPC using an RI detector with a flow rate of 1.0 ml/min (THF). Each sample was dissolved in THF (10 mg/ml).

Electrospun PCL fibers. Fabrication of electrospun fibers was performed as previously reported.[3] Briefly, conditions for electrospinning were 10 kV, 13 cm tip-to-collector distance, a flow rate of 0.7 mL/hr through a 20 gauge needle, with 13 wt% PCL solution in a mixed (90/10 v/v) DCM/DMF solvent. Samples were collected on a rotating mandrel.

RGD peptide synthesis. The following is a representative coupling cycle for one amino acid monomer – synthesis was carried out in a Torviq 50 mL filtered syringe, specifically designed for peptide synthesis. Peptide synthesis started from 1.0 g of Rink Amide MBHA resin (0.32 mmol/gram). The resin is first swelled in DMF for 10 minutes. The solvent is expelled through the syringe and a solution of 20% 4-methyl piperidine in DMF is added to the resin (10 mL), this is carried out twice for 5 minutes and 20 minutes, respectively. The resin is then washed with DMF and DCM for 5 minutes, 3 times each. A Kaiser test was performed to confirm Fmoc deprotection. After a positive Kaiser test, the amino acid (0.96 mmol, 3 equiv), HCTU (412 mg, 3 equiv) and DIPEA (353 µL, 6 equiv) were dissolved in minimal amount of DMF. This solution was added to the resin at room temperature on a rotary shaker and allowed to react for 1 hours. Following the coupling, the resin was again washed with DMF and DCM for 5 mins 3 times each and a qualitative Kaiser test was performed to ensure coupling. This synthetic process was repeated until the full peptide sequence was completed.

After synthesis of the full peptide (GRGDSPDG), the N-terminus of the peptide was conjugated with 5-Bromovaleric acid – as described above. After washing 3 times with DMF (10 mL) and DCM (10 mL), sodium azide (83 mmol, 4 equiv) was added in DMF and allowed to react overnight. Subsequently, the resin was washed with water to remove excess sodium azide and the resin was dried completely in a lyophilizer. To cleave the peptide, a solution of TFA/TIPS/H_2O (95/2.5/2.5, v/v) (5 mL) was incubated with the resin for 3 hours at room temperature and the resin was removed by filtration. The cleaved peptide solution was precipitated in cold ether, centrifuged (10,000 rpm for 10 minutes at 4 °C), and decanted to yield a white pellet. The final crude peptide was dried under vacuum to yield a white powder. The crude RGD peptides was purified by reverse phase-HPLC (LC-20AD pump, UV-VIS detector SPD-20A, Shimadzu HPLC System). Solution A is 0.05% TFA in water and solution B is 0.05% TFA in acetonitrile. Typical elution gradients were performed using a C18 column (column size: 21.2 × 250 mm, 7 µm particle size, Agilent ZORBAX 300SB-C18 PrepHT) using a linear gradient from 5 to 90% Solution B over 45 minutes. Collected fractions were pooled and lyophilized to generate a white powder. Azido-RGD peptide was confirmed via MALDI-TOF (positive ion mode) using α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix. Parent ion peak at 884 m/z corresponds to $[M+H^+]$ of the synthesized peptide.

Conjugation of Azide-Fluor 488/N_3-RGD peptide to PCL-alkyne. An azido-RGD of AF_{488} solution (40 µL, 2 mM RGD in phosphate-buffered saline (PBS) / 40 µL, 2 mM of AF_{488} in DMSO/PBS) was prepared in a 1.5 mL eppendorf tube and the PCL-alkyne fiber (6 mg) was submerged in the solution. The CuAAC reaction was carried out by combining 30 µL of a pre-mixed catalyst solution ($CuSO_4$ (5 µL, 50 mM) and THPTA (25

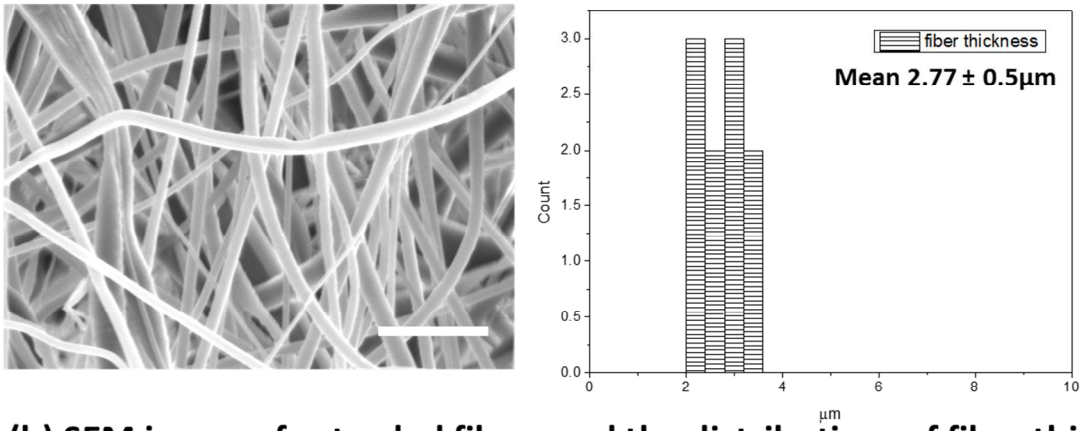
μL, 50 mM)) followed by a solution of sodium ascorbate (50 μL, 100 mM) to the fibers. After 24 hours, samples were washed in water (or DMSO for Azide-fluor 488) 3 times and dried for 24 hours.

Cell culture conditions. NIH3T3 fibroblast cells were obtained as a generous gift from Prof. Horst von Recum's lab. The cells were cultured and passaged in a T-75cm^2 flask with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% penicillin (Invitrogen), 10% newborn calf serum (NCS) and 1% GlutaMAX (Gmax) in a humidified atmosphere (37 °C, 5% CO_2). Media was changed every second day. At 80–90% confluency, the cells were detached with cell detachment solution (PBS/EDTA) at 37 °C for 10 minutes. The detached cells were collected by centrifugation at 500 g for 5 minutes.

Cell adhesion studies. PCL-RGD and PCL fibers were evaluated for cell adhesion using NIH3T3 fibroblast cells (passage 5). Fiber samples (1 mg) were seeded with 7 X 10^5 cells and incubated at 37 °C for 72 hours. Media was removed and the cells were washed 3 times with PBS. The cells were fixed with 4% (w/v) paraformaldehyde for 15 minutes at room temperature and washed 3 times with PBS. The cells were then permeabilized with 1 mL of 0.2% (w/v) Triton X-100 in PBS for 5 min and again washed 3 times with PBS. Fixed cells were incubated with Actin green 488 for 30 minutes using 2 drops/mL of media and washed 3 times with PBS. DAPI was used to stain the cell nuclei for 10 minutes 100 μl DAPI-solution (2 μL of DAPI stock solution (concentration of 5 μg/ml) suspended in 10 ml PBS) and cells were washed 3 times with PBS. Fibers were mounted on glass slides and images were taken via a laser scanning confocal microscope.

Cell viability assay. PCL and PCL-RGD fibers (1 mg per dish, in triplicate) were each placed in 60 mm petri dishes and $7x10^5$ NIH3T3 cells were seeded on top of the fibers in 6 mL of growth medium. Cells were incubated (37 °C, 5% CO_2, humidified) for 72 hours. After 72 hours, the media was removed and samples were washed with PBS (3x). Each fiber was transferred to a 24 well plate to reduce background signal from cells adhered to the bottom of the dish. A MTT solution (5 mg/mL, PBS) was sterilized through microfiltration, using a 0.2 μm filter. 500 μL of DMEM/MTT solution (15%/85%, v/v) was placed in each well in the absence of light and cells were incubated for 4 hours. After incubation, the solution was carefully removed and 1 mL of DMSO was added in each well to dissolve the formazan crystals, with 30 mins of shaking at 100 rpm. Relative viability was determined by measuring absorbance readings at 570 nm.

(a) SEM image of electrospun fibers and the distribution of fiber thickness



(b) SEM image of extruded fibers and the distributions of fiber thickness/width

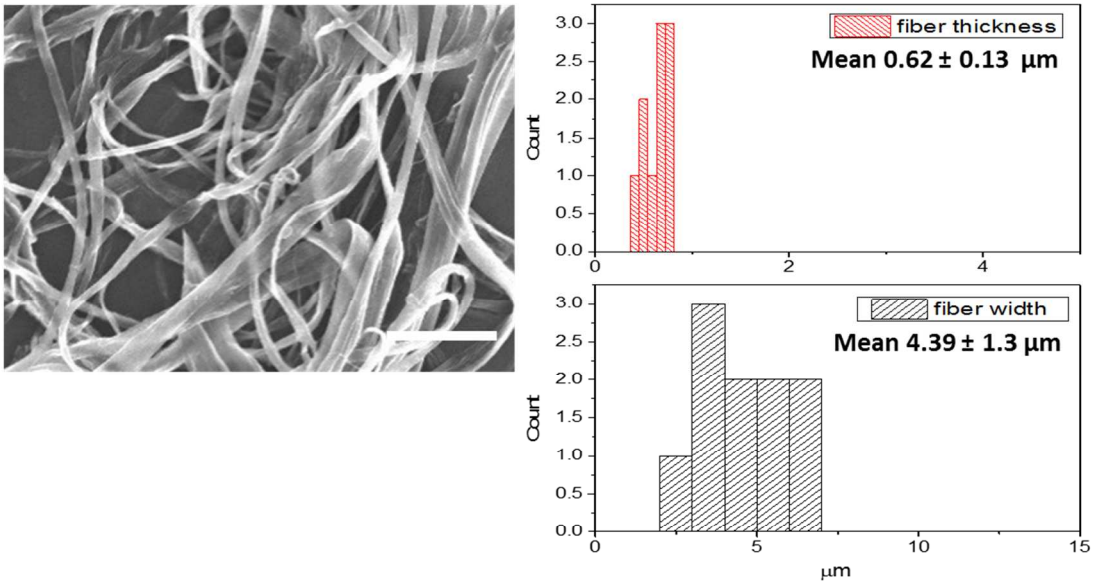


Figure S1. Size distribution of electrospun fibers (a) and extruded fibers (b). Scale bar is 20 µm.

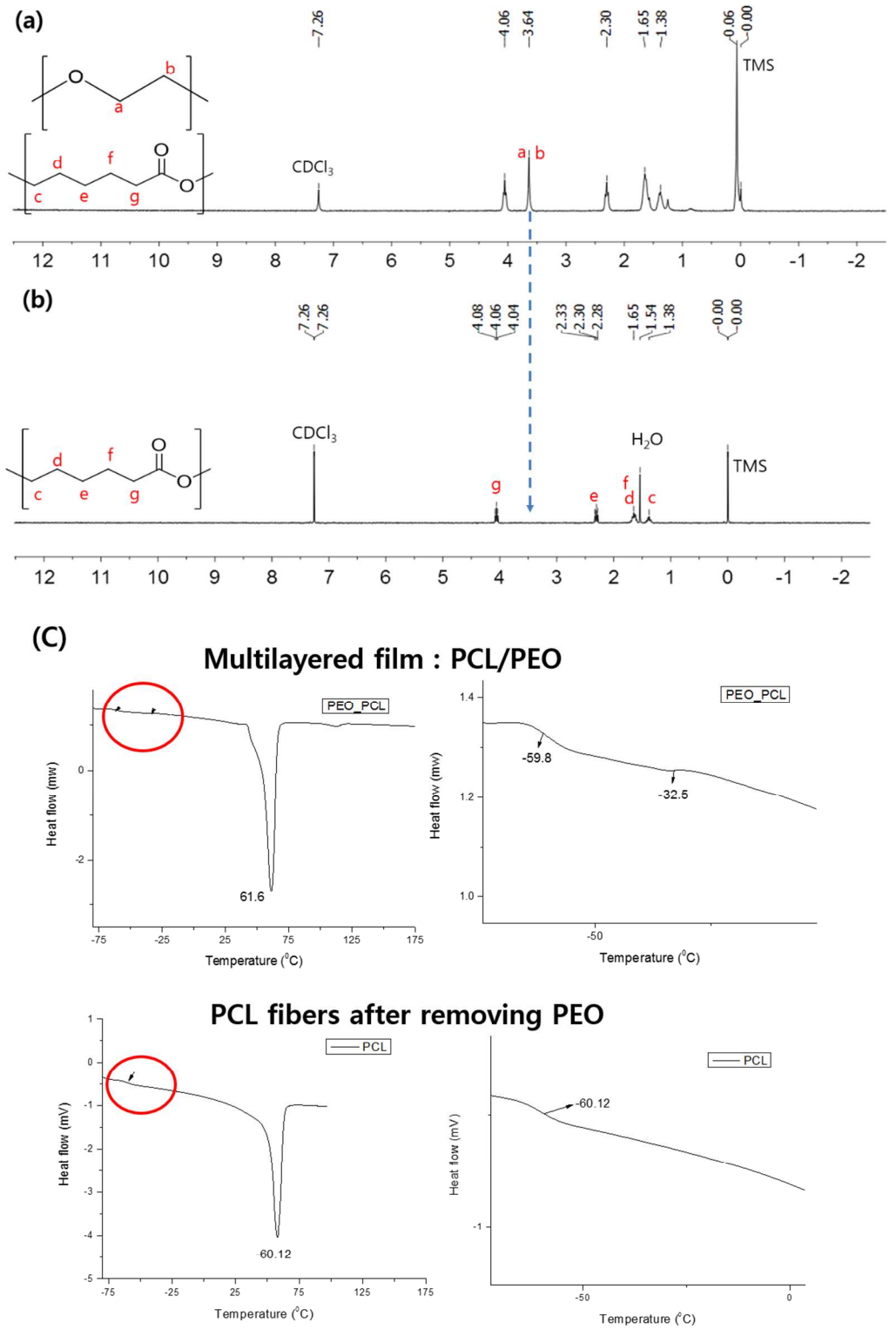


Figure S2. (a) ^{1}H NMR spectrum of PCL/PEO multilayered film. (b) PCL fibers after washing away PEO with a high pressure water jet. (c) DSC data shows two glass transition temperatures (T_g) in the case of PCL/PEO multilayered films (top) and one T_g for PCL nanofibers after removing PEO (bottom).

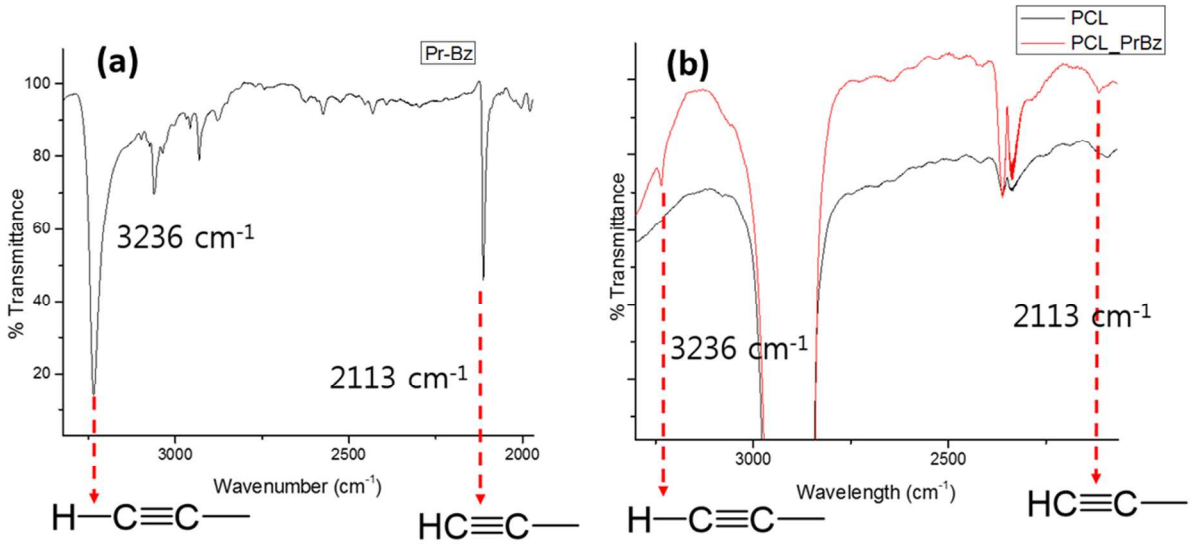


Figure S3. (a) ATR-FTIR spectrum of propargyl benzophenone (Pr-Bz). (b) Comparison of neat PCL fibers (red) and PCL-alkyne fibers (black). Peaks at 2133 cm^{-1} and 3236 cm^{-1} indicate alkyne attachment following photochemistry.

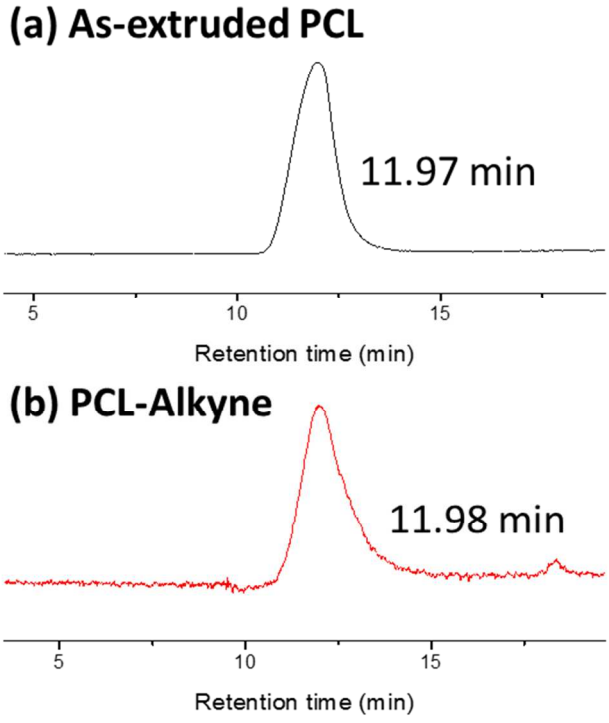
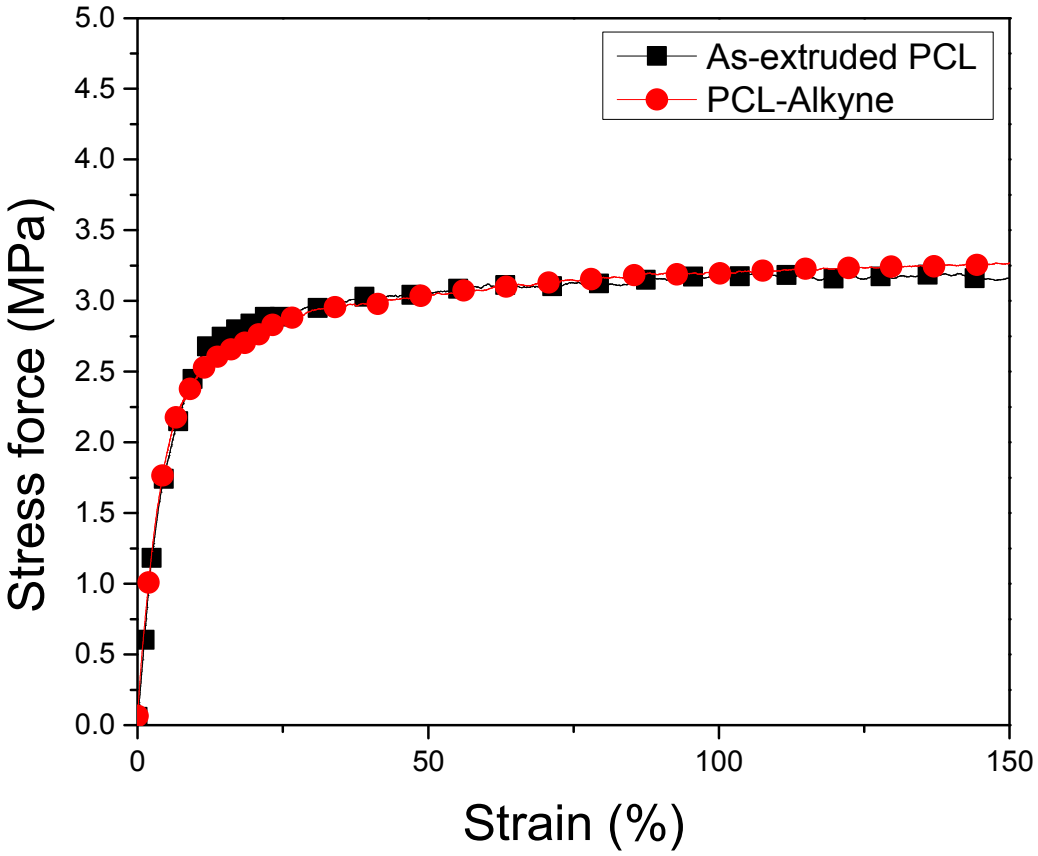


Figure S4. Gel permeation chromatograms before and after UV treatment. (a) as-extruded PCL fiber and (b) PCL-Alkyne. The retention times are matched with the molecular weight of CAPA 6800 using polystyrene standards (± 10%).



Samples	E (MPa)	Tensile strength (MPa)
As-extruded PCL	**46.5 ± 5.8**	**2.54 ± 0.94**
PCL-Alkyne	**50.6 ± 14.9**	**2.14 ± 0.92**

Figure S5. Tensile testing of the as-extruded PCL fibers (black) and PCL-Alkyne fibers after irradiation (red) (n=4).

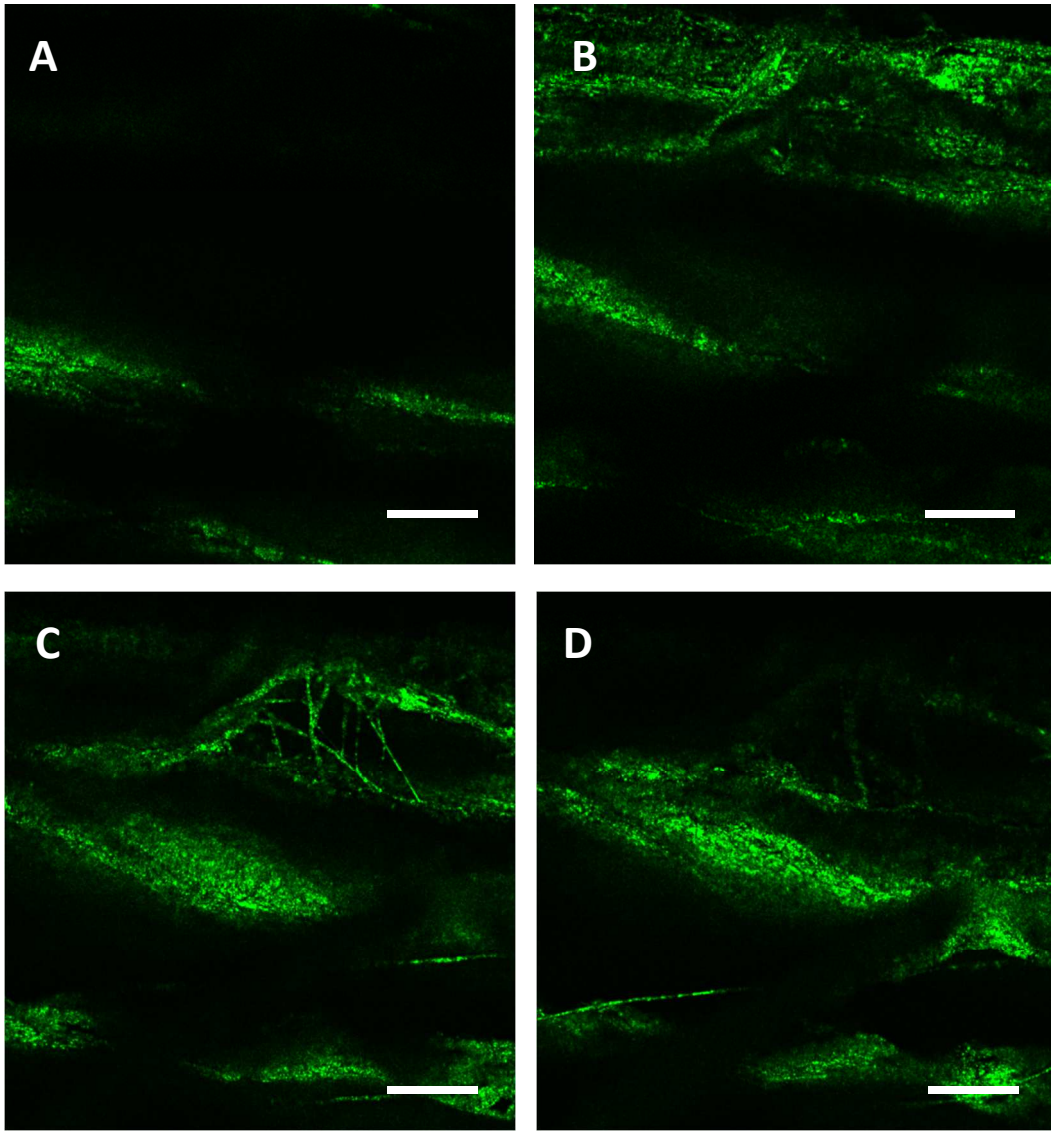
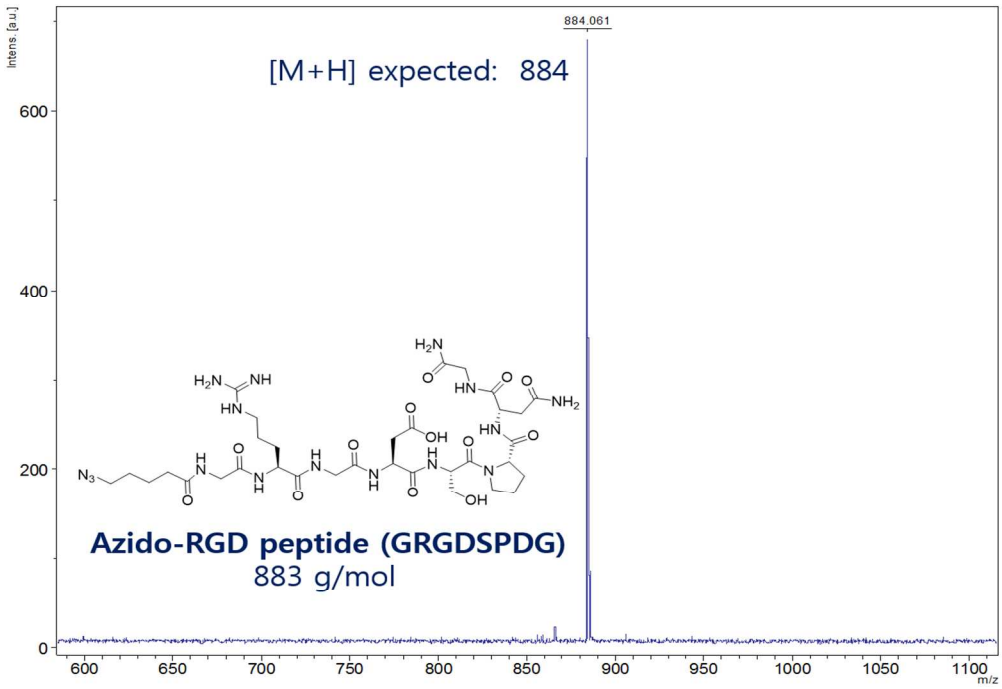


Figure S6. Z-stacked confocal micropgraphs of PCL-AF$_{488}$ indicating full distribution of chemical modification throughout the surface of the fibers. (A, B, C and D: z = 18.7, 64.1, 85.6 and 100.0 µm), scale bar is 50 µm.



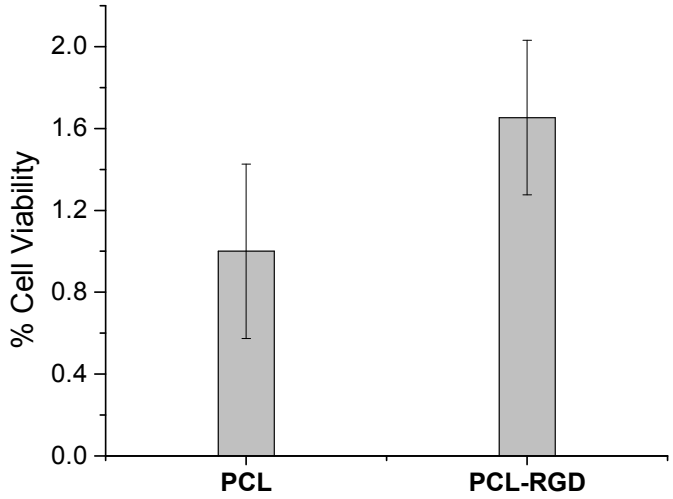


Figure S7. MALDI-TOF (positive) of the azido-RGD peptide: m/z 884 ([M]+H^{+1}).

Figure S8. Cell viability assay. Cells grown on PCL were normalized to 100% viability and PCL-RGD fibers were compared as a ratio thereof.

References

(1) Temel, G.; Aydogan, B.; Arsu, N.; Yagci, Y. *Macromolecules* **2009**, *42*, 6098.
(2) Wang, J.; Langhe, D.; Ponting, M.; Wnek, G. E.; Korley, L. T. J.; Baer, E. *Polymer* **2014**, *55*, 673.

(3) Stone, D.A.; Wanasekara, N.D.; Jones, D.; Wheeler, N.R.; Wilusz, E.; Zukas, W.; Wnek, G.E.; Korley, L. T. J. *ACS Macro Lett.*, **2012**, *1*, 80–83