Supporting Information for

Multifunctional and Spatially Controlled Bioconjugation to Melt

Coextruded Nanofibers

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Materials

Amine-PEG-Thiol (NH₂-PEG-SH) (MW 1,000) was purchased from Laysan Bio, Inc. Fluorescein isothiocyanate (FITC) was purchased from Chem. Impex Int'l. Inc. Rink Amide MBHA resin LL (100-200 mesh, 0.52 meq) was purchased from Novabiochem.

Experimental

Thiol FITC Synthesis. The thiol terminated FITC dye (SH-PEG-FITC) was synthesized by combining FITC (9.4 mg, 24 μ mol) SH-PEG-NH₂ (16 mg, 16 μ mol), TEA (3.4 μ L, 24 μ mol) and 2 mL of DMF to a round bottomed flask purged with N₂ gas and protected from light. The reaction proceeded overnight at room temperature. After 18 hours, the reaction was diluted with MeOH, then subsequently dried under reduced pressure. Crude synthesized SH-PEG-FITC was purified by reverse phase-HPLC (LC-20AD pump, UV-VIS detector SPD-20A, Shimadzu HPLC System), monitoring at 494 nm. Purification via HPLC was carried out on a C18 prep-column (column size: 21.2 \times 250 mm, 7 μ m particle size, Agilent ZORBAX 300SB-C18 PrepHT) using a

water/acetonitrile gradient from 5 to 95% acetonitrile over 45 minutes. After collecting the purified material, SH-PEG-FITC was dried in a lyophilizer overnight. The molecular weight of SH-PEG-FITC was confirmed via MALDI-TOF (positive ion mode) using α -cyano-4-hydroxycinnamic acid (CHCA) as a matrix ([M+Na]⁺ = 1435 Da).

Surface Area Measurements. To measure the surface area of PCL fibers, BET measurements were conducted. The samples were degassed with nitrogen gas to remove impurities from the fibers at 40 °C overnight. Krypton gas was absorbed on the surface of PCL fibers 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 2.773 ± 0.048 g/cm³ and an intercept of 0.228 ± 0.010 g/cm³ (R² = 0.998). Based on BET measurement, coextruded PCL fibers have 1.88 ± 0.0306 m²/g of surface area.



Figure S1. Synthetic scheme for modified benzophenones.



Figure S2. Synthetic scheme of SH-PEG-FITC



Figure S3. A) Synthetic scheme of Thiol-ene chemistry on alkene decorated PCL fibers with SH-PEG-FITC or CYGFGG. B) MALDI-TOF of SH-PEG-FITC, and C) ATR-FTIR spectrum of 4-hydroxyl benzophenone decorated PCL fibers and propenyl benzophenone decorated PCL fibers.



Figure S4. ESI-MS/MS spectrum of Azido-GRGDSP ([M+H]⁺ = 712 Da)



Figure S5. MALDI TOF of CYGFGG ([M] = 601 g/mol).



Figure S6. MTT Assay. Relative cell viability of MC3T3 cells on the three different scaffolds. OGP is normalized to 1 and dually modified and RGD modified substrates are reflected relative to OGP.