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A modular approach to easily processable supramolecular bilayered scaffolds with tailorable properties

Supporting information

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1. Synthesis of UPy-polymers and UPy-peptide

1A. Materials

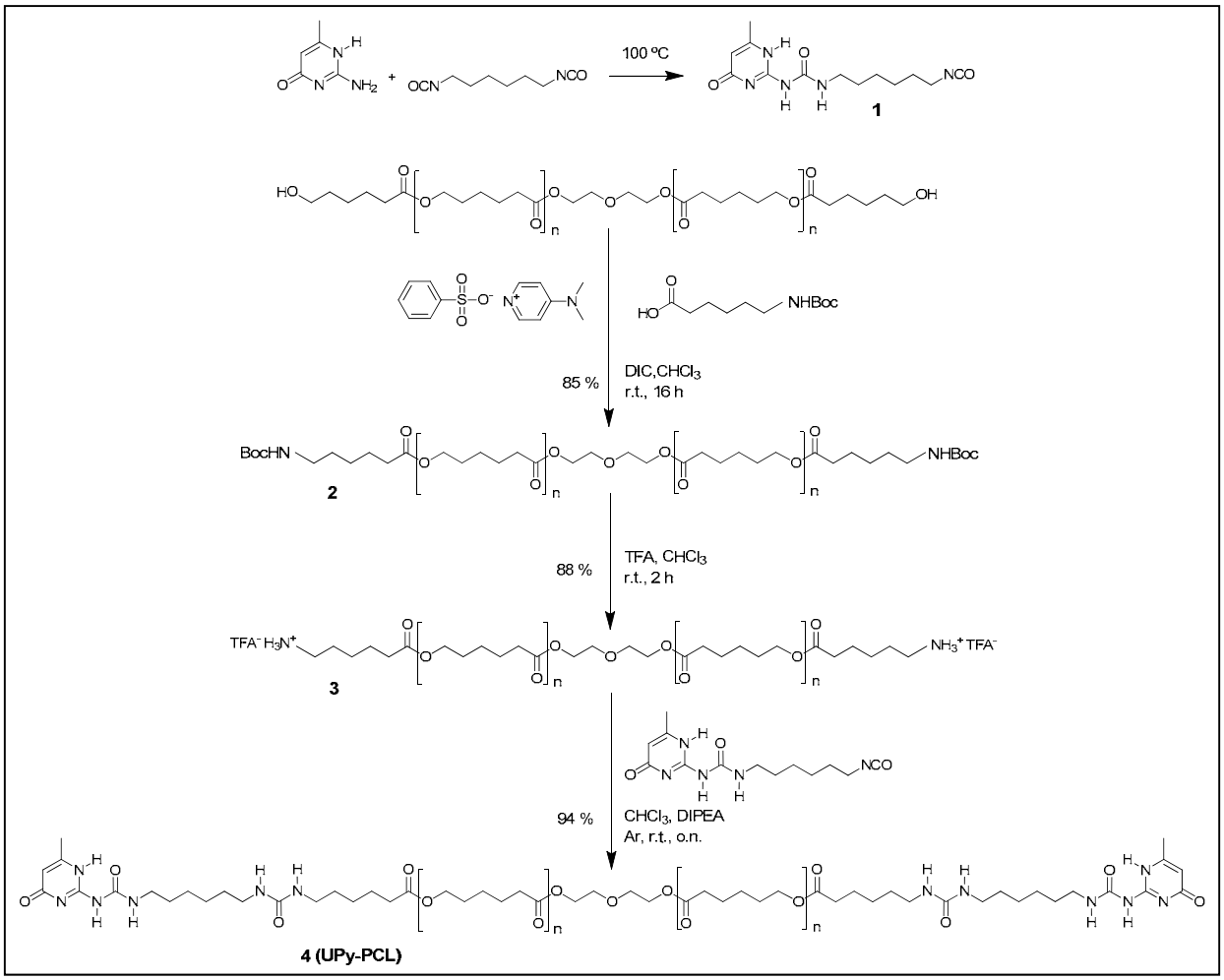
Poly(ethylene glycol)diol (M_n = 2000 g/mol; PEG), polycaprolactonediol (M_n = 2000 g/mol; PCL), hexa(ethylene glycol) (OEG_6), N-(3-dimethyla-minopropyl)-N-ethylcarbodiimide (EDC), triethylsilane (Et_3SiH), p-toluenesulfonyl chloride (TsCl), succinic anhydride, dibutyltin dilaurate (DBTDL), diisopropylcarbodiimide (DIC), Boc-6-aminohexanoic acid and di-*tert*-butyldicarbonaat (BOC_2O) were purchased from Sigma Aldrich and used as received, unless stated otherwise. Pd/C was purchased from Merck and Triethylamine (Et_3N) was purchased from Acros. PyBOP and amino acids for solid phase peptide synthesis were purchased from Novabiochem. The solvents chloroform ($CHCl_3$), dichloromethane (DCM), tetrahydrofuran (THF), dimethylformamide (DMF), methanol (MeOH), hexane, and ethyl acetate (EtOAc), N-methylpyrrolidone (NMP), N,N-diisopropylethylamine (DIPEA) were purchased from Biosolve and used as received, unless stated otherwise. The compounds 4-(dimethylamino)pyridinium-4-toluenesulfonate (DPTS),[1] and 2(6-Isocyanatohexylaminocarbony-lamino)-6-methyl-4[1H]pyrimidinone (UPy-synthon **1**) (See Scheme SI-1),[2] were synthesized as described.

1B. General methods

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Mercury (400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR) spectrometer at 298 K. Chemical shifts (δ) are reported in in ppm downfield from tetramethylsilane (TMS) or relative to the solvent residual peak. Abbreviations used for splitting patterns are s = singlet, d = doublet, t = triplet,

q = quartet, m = multiplet and br = broad. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer with a Universal ATR Sampling Accessory for solids. SEC was performed on a Shimadzu LC–10ADVP system with a Shimadzu RID–10A refractive index detector, a Shimadzu SPD-M10AVP UV-Vis detector at 254 nm, and a serial configuration of a PLgel 5-μm mixed-C column and a PLgel 5-μm mixed-D column, using chloroform as eluent. The apparent number averaged molecular weights (M_n) and polydispersity indices (PDI) are reported relative to polystyrene standards. Preparative reversed phase liquid chromatography (prep-RPLC) was performed on a system consisting of the following components: Shimadzu LC-8A preperative liquid chromatography pumps (with an Alltima C18 5u (125 x 20 mm) preparative reversed phase column and gradients of water-acetonitrile, supplemented with 0.1% trifluoro acetic acid), a Shimadzu CBM-20A prominence communications bus module and Shimadzu DGU 20A3 prominence degasser, Thermo Finnigan Surveyor PDA detector, Finigan LCQ Deca XP and Thermo Finnigan surveyor autosampler. Reversed phase liquid chromatography–mass spectrometry (RPLC-MS) was performed on a system consisting of the following components: Shimadzu SCL-10A VP system controller with Shimadzu LC-10AD VP liquid chromatography pumps (with an Alltima C18 3u (50 mm x 2.1 mm) reversed phase column and gradients of water-acetonitrile supplemented with 0.1% formic acid, a Shimadzu DGU 20A3 prominence degasser, a Thermo Finnigan surveyor autosampler, a Thermo Finnigan surveyor PDA detector and a Thermo Scientific LCQ Fleet.

1C. Synthesis of UPy-PCL polymer (4)



Scheme SI-1: *The synthesis of UPy-PCL, starting from commercial polymer* PCL_{2000}*diol.*

Synthesis of compound ***2***

Boc-6-aminohexanoic acid (25 g, 110 mmol), DPTS (0.7 g, 2.5 mmol) and DIC (16.3 g, 125 mmol) at 0 ºC was added to a stirring solution of PCL_{2000}diol (100 g, 50 mmol) in chloroform (150 mL). For 16 h the reaction mixture was stirred while it was allowed to heat to r.t. Then the reaction mixture was filtered over celite and concentrated resulting in a viscous, yellowish oil. This oil was subsequently stirred with 200 mL diisopropyl ether, cooled to 4 ºC and decanted, giving a waxy solid. This solid was dried *in vacuo*, and was subsequently dissolved in 200 mL toluene leaving undissolved precipitate. The suspension was filtered over celite, and concentrated to again obtain a viscous, yellowish oil. This oil was precipitated with 200 mL diisopropyl ether, yielding a white to off-white waxy solid, which was collected by filtration and dried *in vacuo* to obtain product **2** (yield = 103 g, 85%). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.55 (br.s, 2H), 4.20 (t, 4H), 4.05 (t, 2(2n)H), 3.85 (t, 4H), 3.05 (br.t, 4H), 2.25 (m, 2(2n+2)H), 1.7–1.2

(br.m, 6(2n+2)H + 2*9H) ppm. IR (ATR): ν = 3396, 2939, 2865, 1730, 1515, 1459, 1419, 1390, 1364, 1236, 1161 cm^{-1}.

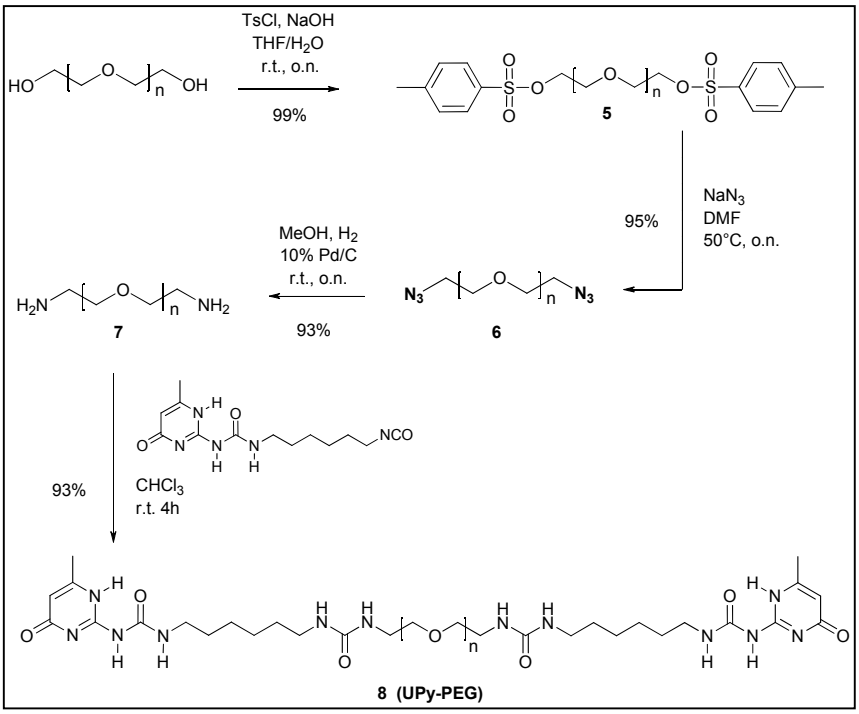
*Synthesis of compound **3***

TFA (70 mL) was added to a solution of compound **2** (50 g) in chloroform (70 mL). The reaction mixture was stirred for 2 h, after which the complete deprotection of the terminal amine group was confirmed by both IR and ^{1}H-NMR. Concentration *in vacuo* to remove most of the solvent resulted in a viscous oil that was stirred with diethyl ether and decanted (3x200 mL) to extract residual TFA and chloroform. During the last extraction the product precipitated as an off-white solid. This solid was stirred with diethyl ether and was collected by filtration, yielding product **3** as the TFA salt, as a white, waxy solid (44 g, 88%). ^{1}H-NMR ($CDCl_3$): δ = 7.95 (br.s, 3H), 4.20 (t, 4H), 4.05 (t, 2(2n)H), 3.85 (t, 4H), 2.95 (br.t, 4H), 2.25 (m, 2(2n+2)H), 1.7–1.2 (br.m, 6(2n+2)H) ppm. IR (ATR): ν = 2944, 2865, 1722, 1471, 1419, 1397, 1366, 1294, 1240, 1172 cm^{-1}.

*Synthesis of compound **4***

The TFA-salt of compound **3** (100.7 gram, 28.9 mmol) was mixed with UPy-synthon **1** (18.3 g, 62.4 mmol) in dry chloroform (300 mL). The mixture was kept under argon at r.t., and a mechanical stirrer was used to stir the viscous, thick white suspension. DIPEA N,N-diisopropylethylamine (29 mL, 0.17 mol) was added dropwise to the stirred reaction mixture over a 30 min period. After overnight stirring, IR spectroscopy indicated the absence of isocyanate reactant, and a ninhydrin test showed the presence of amines. So additional portions of UPy-synthon **1** (3 portions of in total 4.8 g, 16.4 mmol) were added, and stirring was continued until IR spectroscopy showed the presence of isocyanates in the reaction mixture. This indicated that all amines were converted into urea. The viscosity of the reaction mixture increased during reaction. The mixture was transferred to a 1 L flask and the solvent was removed by using a rotary evaporator yielding a white residue. This crude product was stirred in methanol (0.5 L), filtered and washed with two portions of methanol (100 mL). This procedure was repeated two times. Then, the residue was mixed with methanol (0.5 L), refluxed for 1.5 h, allowed to reach r.t., filtered and washed with methanol (100 mL). This procedure was repeated also two times. The white residue was finally dissolved by stirring in chloroform (600 mL) and methanol (50 ml) and the beige, hazy solution was filtered over celite in a glass filter yielding a clear filtrate. Concentration of the filtrate *in vacuo* gave a white solid that was dried *in vacuo* at r.t. overnight (yield = 108 g, 94%). ^{1}H-NMR ($CDCl_3$): δ = 13.16 (s, 2H), 11.83 (s, 2H), 10.08 (s, 2H), 5.83 (s, 2H), 4.73 (bs, 2H), 4.57 (bs, 2H), 4.23 (t, 4H), 4.06 (t, 2(2n)H), 3.69 (t, 4H), 3.24 (q, 4H), 3.19-3.13 (m, 8H), 2.37-2.27 (m, 2(2n)H+4H), 2.24 (s, 6H), 1.71-1.59 (2n*4H + 4H), 1.53-1.46 (12H), 1.42-1.31 (2(2n)H + 12H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 173.7, 173.6, 173.4, 173.3,158.4, 156.5, 154.8, 148.5, 106.6, 69.1, 64.4, 64.2, 63.3, 40.2, 40.2, 39.6, 34.1, 34.0, 30.0, 29.8, 28.4, 26.4, 26.2, 26.1, 25.5, 25.5, 24.6, 24.5, 19.0 ppm. FT-IR (ATR): ν = 3333, 2938, 2863, 1728, 1701, 1668, 1620, 1585, 1527, 1462, 1417, 1391, 1361, 1257, 1236, 1160, 1100, 1066, 1043, 1016, 994, 964, 942, 879, 814, 799, 785, 768, 742 cm^{-1}. SEC ($CHCl_3$, UV at 254 nm): M_n = 1.05 kg/mol and PDI = 3.6. SEC ($CHCl_3$, RI): M_n = 3.5 kg/mol, PDI = 1.4.

1D. Synthesis of UPy-PEG polymer (8)



Scheme SI-2: *The synthesis of UPy-PEG, starting from commercial polymer PEG_{2000}diol.*

Synthesis of compound ***5***

Poly(ethylene glycol)diol (M_n = 2000 g/mol; 20 g, 10 mmol) was dissolved in 100 mL THF. NaOH (1.6 g, 40 mmol) dissolved in 10 mL H_2O was added. The mixture was cooled to 0 °C, and p-toluenesulfonyl chloride (TsCl, 4.96 g, 26 mmol) dissolved in 20 mL THF was added dropwise. The reaction mixture was allowed to warm up to r.t., and was stirred overnight. The reaction was checked with IR and ^{1}H NMR showing complete conversion. The mixture was supplemented with 100 mL H_2O, extracted twice with $CHCl_3$ and washed once with brine. The resulting product **5** in chloroform was dried over $MgSO_4$, and the chloroform was evaporated to yield a white solid. The product was immediately used for the subsequent reaction (yield = 26 g, quantitatively, with residual THF). M_n = ~2310 g/mol (calculated from ^{1}H-NMR). ^{1}H-NMR ($CDCl_3$): δ = 7.80 (d, 2H), 7.35 (d, 4H), 4.16 (q, 4H), 3.69-3.60 (2t, 4nH) 2.45 (s, 6H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 162.4, 77.3, 77.2, 77.0, 76.7, 70.6, 70.6, 70.5, 70.0, 50.6, 36.4, 31.4 ppm.

Synthesis of compound ***6***

Compound **5** was dissolved in 100 mL DMF and NaN_3 (2.6 g, 40 mmol) was added. The reaction mixture was stirred overnight at 50 °C. The reaction was checked with ^{1}H NMR, showing complete conversion. Then 1 L water was added, followed by 6 extractions with 100 mL DCM. The resulting product in DCM was dried over $MgSO_4$, and DCM was evaporated. The product was obtained as white solid. Apart from traces of solvent the product was

pure as detected with ^{1}H NMR. The total amount of product was immediately used for the subsequent reaction (yield = 20.1 g, ~98%). M_n = ~2242 g/mol, n=49, (calculated from ^{1}H-NMR). ^{1}H-NMR ($CDCl_3$): δ = 3.69-3.64 (2t, 4nH), 3.39 (t, 4H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 162.4, 77.3, 77.2, 77.0, 76.7, 70.6, 70.5, 70.0, 50.6, 36.4, 31.4 ppm.

*Synthesis of compound **7***

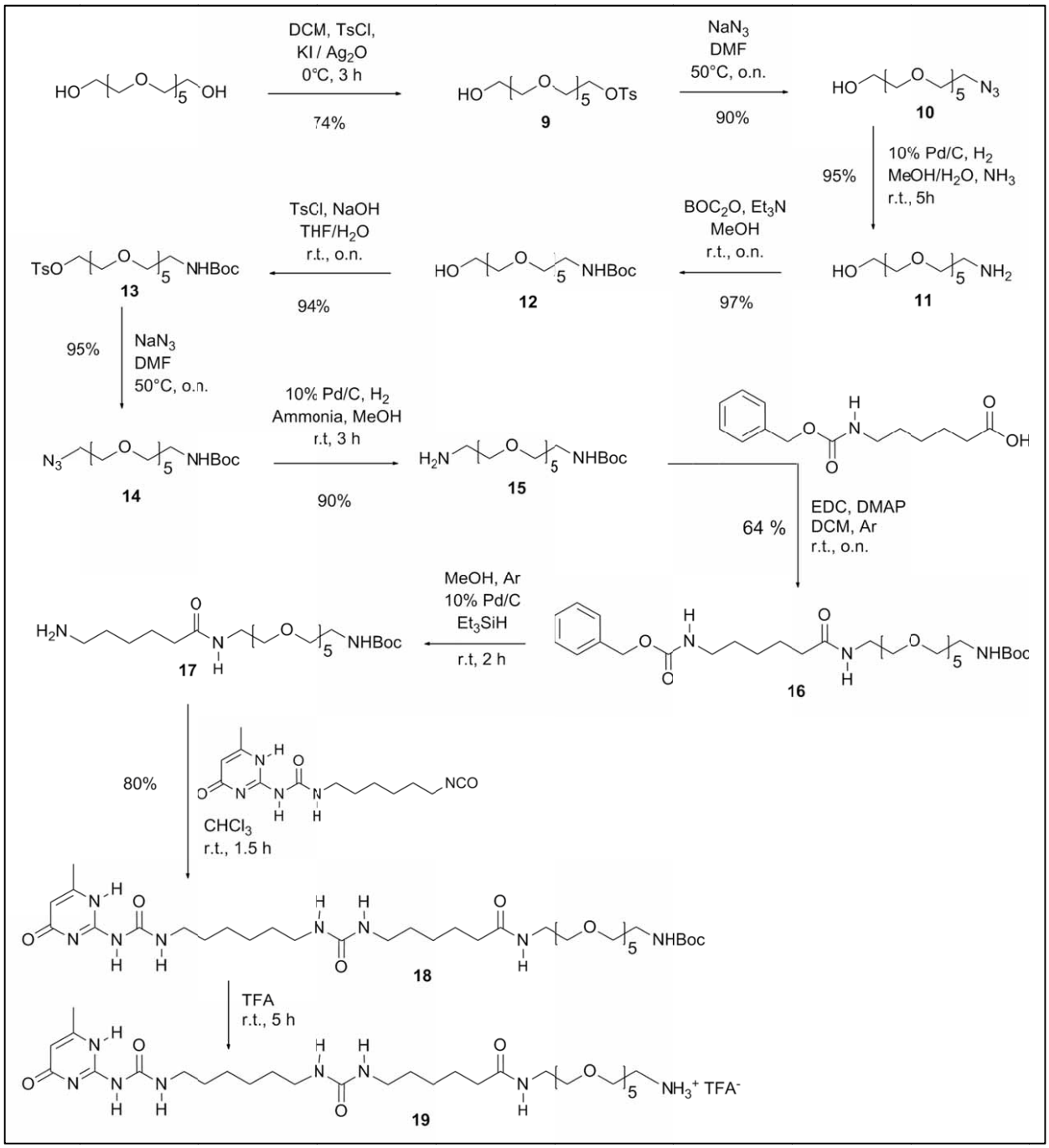
Compound **6** (20.1 g, 9.8 mmol) was dissolved in 100 mL MeOH and hydrogenated in a Parr reactor with H_2 in the presence of Pd/C (10%, 2 g) overnight. Disappearance of the peak at 3.39 ppm ($C\underline{H}_2N_3$) in ^{1}H-NMR indicated complete conversion. The product was filtered over hyflo, and the solvent was evaporated. The resulting light gray solid was immediately used for the subsequent reaction (yield = 18.4 g, 94%). ^{1}H-NMR ($CDCl_3$): δ = 3.65 (t, 4nH), 3.56 (t, 4H), 2.90 (t, 4H), 2.33 (s, br, 4H) ppm.

*Synthesis of compound **8 (UPy-PEG)***

Compound **7** (18.4 g, 9.2 mmol) was dissolved in 300 mL dry $CHCl_3$ (molsieves). UPy-synthon **1** (6.4 g, 21.8 mmol) was added and stirred at r.t. under argon for 4 h. Unreacted **1** was removed by reaction with an amine-resin (Sigma Aldrich) and stirred at r.t. for 2 h. The mixture was filtered over hyflo and 15 mL MeOH was added. UPy-PEG was precipitated in 2.5 L ether, filtered and dried in vacuo. The product was obtained as white powder (yield = 22.25 g, 93%). M_n = 2585 g/mol, M_n = 2763 g/mol, with n = ~49, calculated from ^{1}H-NMR). ^{1}H-NMR ($CDCl_3$): δ = 13.12 (s, 2H), 11.84 (s, 2H), 10.11 (s, 2H), 5.83 (s, 2H), 5.32 (s, 2H), 5.14 (s, 2H), 3.64 (t, 4nH), 3.54 (t, 4H), 3.35 (q, 4H), 3.24 (q, 4H), 3.14 (q, 4H), 2.24 (s, 6H), 1.59-1.35 (3m, 16H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 173.0, 158.6, 156.4, 154.6, 148.3, 106.6, 70.6, 70.5, 70.4, 70.2, 70.0, 69.9, 42.8, 40.1, 40.0, 39.7, 31.1, 30.1, 29.3, 29.2, 26.5, 26.4, 26.1, 18.9 ppm. IR (ATR): ν= 3320, 2876, 1701 (UPy), 1667 (UPy), 1620 (urea), 1585 (UPy), 1527 (UPy), 1412, 1348, 1281, 1257, 1091, 1039, 943, 844, 810, 768, 742 cm^{-1}

1E. Synthesis of UPy-RGD peptide (24)

1Ei. Synthesis of UPy-OEG-linker (19)



Scheme SI-3: *Synthesis of UPy-OEG-linker*

Synthesis of compound ***9***

Hexa(ethylene glycol) (10.0 g, 35.4 mmol) was dissolved in 120 mL DCM and the solution was cooled to 0 °C and stirred before addition of Ag_2O (12.4 g, 53.5 mmol), TsCl (7.4 g, 38.8 mmol), and KI (1.2 g, 7.23 mmol). After

stirring under argon at r.t. for 3 h, the silver salt precipitates were removed by filtration over hyflo. The hyflo was thoroughly washed with EtOAc. The combined filtrates were evaporated, and the residue was purified using SiO_2 column chromatography with 4% isopropanol in DCM as eluent. The pure product was obtained as clear oil (yield = 11.5 g, 74%). RPLC-MS: In the chromatogram one peak with m/z = 437.25 $[M+H]^+$, 459.33 $[M+Na]^+$ was measured. Calculated MW = 436.53 g/mol. ^{1}H-NMR ($CDCl_3$): δ = 7.80 (d, 2H), 7.34 (d, 2H), 4.16 (t, 2H), 3.74-3.59 (m, 22H), 2.45 (s, 3H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 144.8, 133.0, 129.8, 128.0, 77.3, 77.2, 77.0, 76.7, 72.4, 70.7, 70.6, 70.5, 70.3, 69.2, 68.7, 64.4, 61.7, 25.3, 21.6 ppm.

*Synthesis of compound **10***

Compound **9** (10 g, 23 mmol) was dissolved in 50 mL DMF. Subsequently NaN_3 (2.24 g, 34.5 mmol) was added. The reaction mixture was stirred at 50 °C overnight. Then 1 L water was added, followed by 6 extractions with 100 mL DCM. The product was dried over $NaSO_4$ and solvent was evaporated under reduced pressure. The product was obtained as clear oil (yield = 6.38 g, 91%). RPLC-MS: In the chromatogram the product peak m/z = 308.08 $[M+H]^+$, 330.25 $[M+Na]^+$ was present. Calculated MW = 306.39 g/mol. Calculated MW = 306.39 g/mol. ^{1}H-NMR ($CDCl_3$): δ = 3.70 (q, 2H), 3.67 (t, 18H), 3.61 (t, 2H), 3.39 (t, 2H), 2.51 (t, 1H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 77.3, 77.0, 76.7, 72.5, 70.7, 70.6, 70.5, 70.3, 70.0, 61.7, 50.7 ppm.

*Synthesis of compound **11***

Compound **10** (6 g, 19.6 mmol) was dissolved in 80 mL MeOH and 20 mL 25% ammonia solution, and reacted with H_2 in presence of Pd/C (10%, 0.6 g) in a Parr reactor for 5 h. The reaction mixture was filtered over hyflo and solvent was evaporated. The product was dissolved in THF/EtOAc, dried over Na_2SO_4, filtered, and the solvent was evaporated. The product was obtained as clear oil (yield = 5.26 g, 96%). RPLC-MS: In the chromatogram one peak was observed with m/z = 282.33 $[M+H]^+$. Calculated MW = 281.35 g/mol. ^{1}H-NMR ($CDCl_3$): δ = 3.73-3.52 (m, 22H), 2.87 (t, 2H), 2.36 (s, broad, 3H) ppm. ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 77.3, 77.2, 77.0, 7607, 73.1, 72.8, 70.5, 70.2, 61.4, 41.6 ppm.

*Synthesis of compound **12***

Compound **11** (5.0 g, 17.8 mmol) was mono-Boc-protected by dissolution in 50 mL MeOH, addition of BOC_2O (4.27 g, 19.5 mmol) and Et_3N (3 mL, 21.3 mmol) under constant stirring at r.t. under argon overnight. Solvent was evaporated and the product was obtained as clear oil (yield = 6.64 g, 97%). RPLC-MS: In the chromatogram the product was primarily observed in one peak with m/z = 382.17 $[M+H]^+$, 404.33 $[M+Na]^+$. Also m/z = 282.33 (M-Boc); Partial deprotection happened during RPLC-MS analysis because of the presence of formic acid in the eluent. Calculated MW = 381.47 g/mol. ^{1}H-NMR ($CDCl_3$): δ = 5.12 (s, br, 1H), 3.72-3.60 (m, 22H), 3.54 (t, 2H), 3.31 (q, 2H), 2.64 (s, br, 1H), 1.44 (s, 9H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 156.0, 79.1, 77.3, 77.0, 76.7, 72.5, 70.6, 70.5, 70.3, 70.2, 61.7, 40.3, 31.2, 28.4 ppm.

*Synthesis of compound **13***

Compound **12** (6.5 g, 17 mmol) was dissolved in 70 mL THF. NaOH (1.36 g, 34 mmol) dissolved in 15 mL H_2O was added. The mixture was cooled to 0 °C and TsCl (3.9 g, 20 mmol) dissolved in 30 mL THF was added dropwise. The reaction mixture was allowed to warm to r.t. and was stirred overnight. THF was evaporated, 100 mL H_2O was added, followed by two extractions with 50 mL $CHCl_3$ and washing with 50 mL brine. The product was dried over $MgSO_4$, concentrated by solvent evaporation and purified using SiO_2 column chromatography with 5% isopropanol in $CHCl_3$ as eluent. The product was obtained as a clear oil (yield = 8.6 g, 94%). Calculated MW = 535.7 g/mol. ^{1}H-NMR ($CDCl_3$): δ =7.80 (d, 2H), 7.34 (d, 2H), 5.01 (s, br, 1H), 4.16 (t, 2H), 3.70 (t, 2H), 3.65-3.58 (m, 16H), 3.55 (t, 2H), 3.31 (q, 2H), 2.45 (s, 3H), 1.44 (s, 1H) ppm. ^{13}C-NMR ($CDCl_3$): δ = 156.0, 144.6, 133.0, 129.8, 128.0, 79.1, 77.3, 77.2, 77.0, 76.7, 70.7, 70.6, 70.5, 70.2, 69.2, 68.7, 40.3, 28.4, 21.6 ppm.

*Synthesis of compound **14***

Compound **13** (8.4 g, 15.7 mmol) and NaN_3 (1.52 g, 23.5 mmol) dissolved in 50 mL DMF was stirred overnight at 50 °C. The reaction mixture was cooled to r.t. Then, 1L H_2O was added, followed by 8 extractions with 100 mL DCM. The product was dried over $MgSO_4$ and solvent was evaporated under reduced pressure. The product was obtained as a clear oil (yield = 6.1 g, 95%). Calculated MW = 406.5 g/mol. ^{1}H-NMR ($CDCl_3$): δ = 5.03 (s, br, 1H), 3.69-3.61 (m, 18H), 3.54 (t, 2H), 3.39 (t, 2H), 3.31 (q, 2H), 1.44 (s, 9H) ppm. ^{13}C-NMR ($CDCl_3$): δ =77.3, 77.0, 76.7, 70.7, 70.6, 70.5, 70.2, 70.0, 50.7, 40.3, 36.4, 31.4, 28.4 ppm.

*Synthesis of compound **15***

Compound **14** (5.9 g, 14.5 mmol) was dissolved in 80 mL MeOH and 20 mL 25% ammonia solution and reacted with H_2 in presence of Pd/C (10%, 0.56 g) in a Parr reactor for 3 h. Complete reduction was confirmed with FTIR. The reaction mixture was filtered over hyflo and solvent was evaporated. The product was then dissolved in THF/EtOAc, dried over $MgSO_4$, filtered, and the solvent was evaporated. The product was obtained as clear oil (yield = 4.95 g, 90%). Calculated MW = 391.5 g/mol. ^{1}H NMR ($CDCl_3$): δ = 5.18 (s, 1H), 3.66-3.50 (m, 20H), 3.31 (q, 2H), 2.86 (t, 2H), 1.44 (s, 1H) ppm. ^{13}C-NMR ($CDCl_3$): δ =109.9, 79.1, 77.3, 77.0, 76.7, 73.4, 70.6, 70.5, 70.3, 41.8, 40.3, 28.4 ppm.

*Synthesis of compound **16***

CBz-protected aminehexanoic (604 mg, 2.38 mmol), EDC (548 mg, 2.86 mmol) and few crystals of DMAP were stirred in 20 mL DCM for 30 min. Compound **15** (1.0 g, 2.6 mmol) dissolved in 5 mL DCM was added dropwise and the reaction mixture was stirred at r.t. under argon overnight. An isocyanate-functionalized resin (Sigma Aldrich) was added, and the mixture was stirred for 2 h. The mixture was filtered, washed with 10% citric acid and washed with brine. Then the organic phase was dried over $MgSO_4$ and the solvent was evaporated. The product was purified by SiO_2 column chromatography with 10% isopropanol in $CHCl_3$ as eluent. The product was obtained as a clear oil (yield = 950 mg, 64%). ^{1}H-NMR ($CDCl_3$): δ = 7.35-7.27 (m, 5H), 6.29 (s, br, 1H), 5.09 (s, br, 2H), 4.94 (s, br, 1H), 3.65-3.62 (t, 18H), 3.53 (q, 2H), 3.43 (q, 2H), 3.30 (q, 2H), 3.18 (q, 2H), 2.18 (t, 2H), 1.65 (m, 2H), 1.52

(m, 2H), 1.44 (s, 9H) 1.34 (m, 2H) ppm. ^{13}C-NMR ($CDCl_3$): δ =172.9, 136.7, 128.5, 128.0, 79.2, 77.3, 77.0, 76.7, 70.5, 70.2, 70.1, 69.9, 40.8, 40.3, 39.1, 36.3, 29.6, 28.4, 26.3, 25.3, 25.2 ppm.

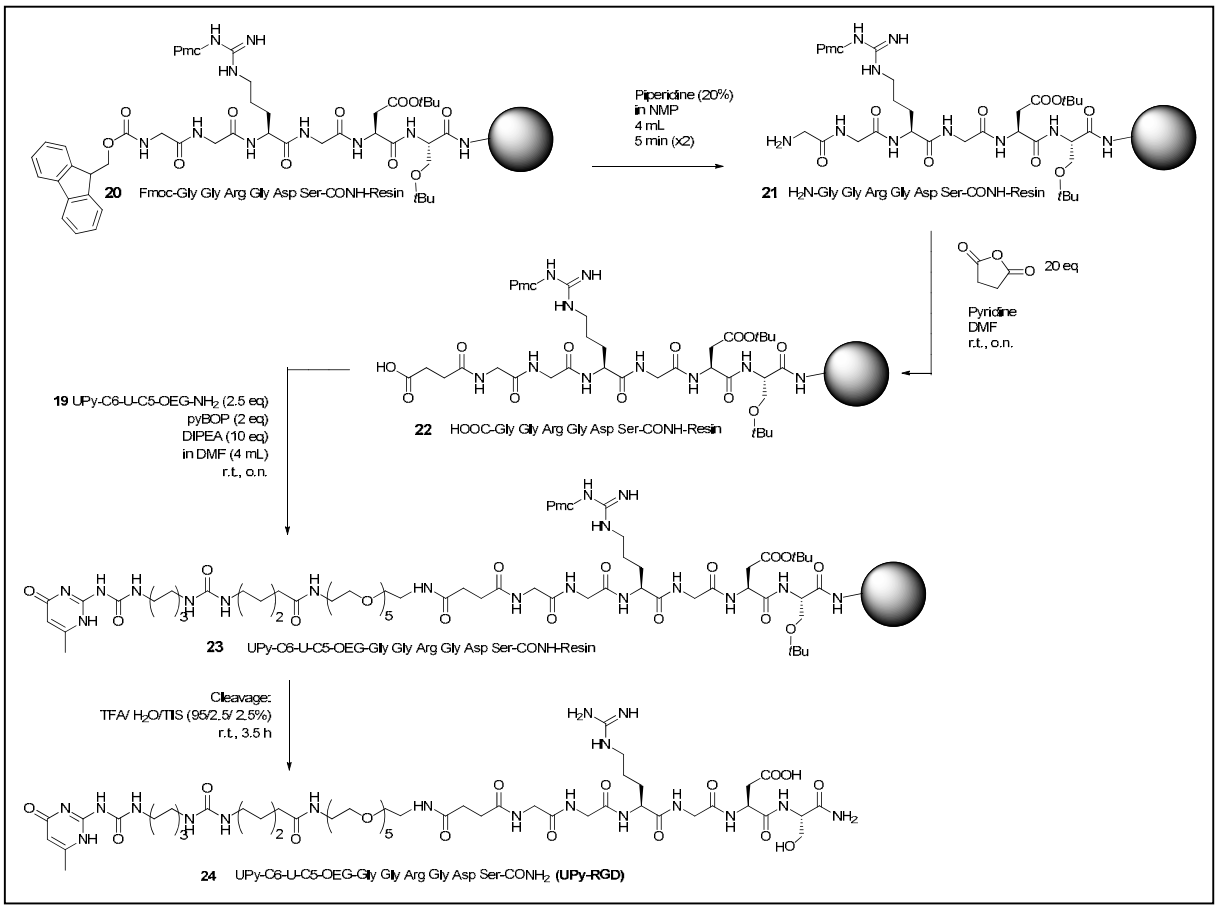
*Synthesis of compound **18***

Compound **16** (850 mg, 1.35 mmol) was dissolved in 5 mL MeOH and perfused with argon. Then Pd/C (10%, 60 mg) was added. To the stirred mixture under argon, Et_3SiH (680 uL, 4.25 mmol) was added dropwise. The mixture was stirred at r.t. for 2 h and then filtered over hyflo, and the solvent was evaporated. The residue (**17**) was added to the UPy-synthon **1** (475 mg, 1.62 mmol) dissolved in 100 mL $CHCl_3$. The mixture was stirred for 1.5 h. An amine-functionalized resin (Sigma Aldrich) was added, and the mixture was stirred over weekend. The mixture was filtered and solvent was evaporated. The residue was dissolved in approximately 10 mL $CHCl_3$ and 5 mL MeOH. Ether was added dropwise until a gel-like state was reached. The product was precipitated in hexane (yield = 800 mg, 80%). MW = 786.97 g/mol. ^{1}H-NMR ($CDCl_3$): δ = 13.14 (s, br, 1H), 11.82 (s, br, 1H), 10.06 (s, br, 1H), 6.43 (s, br, 1H), 5.82 (s, 1H), 5.15 (s, br, 1H), 4.90 (d, 2H), 3.66-3.52 (m, 20H) 3.43 (q, 2H), 3.31 (q, 2H), 3.23 (q, 2H), 3.14 (q, 4H), 2.19 (t, 2H), 2.08 (s, 3H), 1.49-1.26 (m, 14H), 1.44 (s, 9H) ppm. ^{13}C-NMR ($CDCl_3$): δ =173.2, 148.5, 106.6, 79.2, 77.3, 77.2, 77.0, 76.7, 70.5, 70.2, 70.1, 69.9, 40.4, 40.1, 40.0, 39.6, 39.1, 36.2, 31.9, 29.9, 29.8, 29.30, 28.4, 26.3, 25.1, 22.7, 18.9 ppm. IR (ATR): ν= 3332, 2931, 2858, 2160, 1701 (UPy), 1666 (UPy), 1642, 1620 (urea), 1578 (UPy), 1524 (UPy), 1461, 1364, 1254, 1172, 1101, 1039, 943, 870, 807, 783, 767, 740 cm-1.

*Synthesis of compound **19***

Compound **18** (780 mg) in 30 mL DCM was heated until completely dissolved. Then, 15 mL TFA was added and stirred at r.t. for 5 h. Solvent was evaporated and the residue was dissolved in DCM and precipitated in ether. After decantation of the ether, the residue was again dissolved in DCM (5 mL) and precipitated in 40 mL ether. The product was obtained as a white powder (yield = 700 mg). This TFA salt was stored at -20 °C until further use. ^{1}H-NMR (d7-DMF): δ= 8.27 (s, br, 3H), 7.84 (s, br, 1H), 5.95 (s, br, 1H), 5.80 (s, 1H) 3.80 (t, 2H), 3.66-3.49 (m, 20H), 3.32 (q, 4H), 3.23(q, 2H), 3.09 (q, 4H), 2.16 (m, 5H), 1.57-1.33 (m, 14H) ppm. IR (ATR): ν= 3332, 2932, 2858, 1700, 1665 (UPy), 1644 (UPy), 1620 (urea), 1578 (UPy), 1526 (UPy), 1480, 1462, 1447, 1414, 1379, 1350, 1303, 1277, 1256, 1200, 1176, 1119, 1039, 943, 870, 830, 798, 784, 766, 739, 720 cm-1.

1Eii. Synthesis of RGD-peptide and coupling with the UPy-OEG-linker



Scheme SI-4: *Synthesis of RGD-peptide and coupling with the UPy-OEG-moiety.*

Synthesis of compound ***20***

Peptide synthesis was performed according to standard solid phase peptide synthesis by hand in a syringe. The Fmoc-protected Gly-Gly-Arg-Gly-Asp-Ser-$CONH_2$ peptide was synthesized on a Rink amide MBHA resin (Nova Biochem) on 200 µmol scale, and was stored on the resin at -30 °C.

Synthesis of compound ***22***

The peptide on the resin **20** (154 mg, 0.2 mmol) was set to swell in NMP (4 mL) for 30 min, followed by deprotection with 20% piperidine in NMP (4 mL, 8.3 mmol) while shaken 2 times for 5 min, yielding compound **21** on the resin. The resin was washed 5 times with 3 mL NMP and 5 times 3 mL DMF. Succinic anhydride (400 mg, 4 mmol) and pyridine (2 mL) were dissolved in 3 mL DMF, the resultant solution was added to the peptide resin and shaken overnight at r.t. The product **22** was washed 6 times with 3 mL DMF, and 6 times with 3 mL DCM. Then the peptide on the resin was allowed to dry *in vacuo*, and stored at -30 °C.

*Synthesis of compound **24** (**UPy-RGD**)*

Compound **22** (0.1 mmol) on the resin was set to swell in DMF (5 mL) for 2 h. A stock solution of compound **19** was prepared by dissolution (200 mg, 0.25 mmol) in 4 mL DMF with DIPEA (174 µL, 1 mmol) and PyBOP (104 mg, 0.2 mmol). This mixture was added to the peptide on the resin and shaken at r.t. overnight. The product **23** was washed 6 times with DMF, and 6 times with 3 mL DCM. Then the peptide on the resin was allowed to dry *in vacuo*, and stored at -30 °C. Cleavage of the UPy-functionalized peptide and deprotection of the amino acid protecting groups was performed using a cleavage cocktail consisting of TFA (95%, 4.75 mL), H_2O (2.5%, 0.125 mL) and TIS (2.5%, 0.125 mL). This mixture was added to the syringe and shaken at r.t. for 3.5 h, followed by precipitation of the product in ice-cold diethylether (20 mL). The precipitate was pelleted by centrifugation at 2000 rpm for 10 min, re-dissolved in 10% ACN in H_2O, and lyophilized. The product **24** was purified with preparative RPLC-MS on a C18 column using a gradient of 18-22% ACN in H_2O, containing 0.1% TFA (yield = 18 mg, 13.7%). RPLC-MS: In the chromatogram one peak with m/z = 1315.5 $[M+H]^+$, 658.4 $[M+2H]^{2+}$, 439.3 $[M+3H]^{3+}$ was measured). Calculated MW = 1315.6 g/mol.

2. Additional Figures

2A. UPy-based supramolecular polymer biomaterial – a modular approach

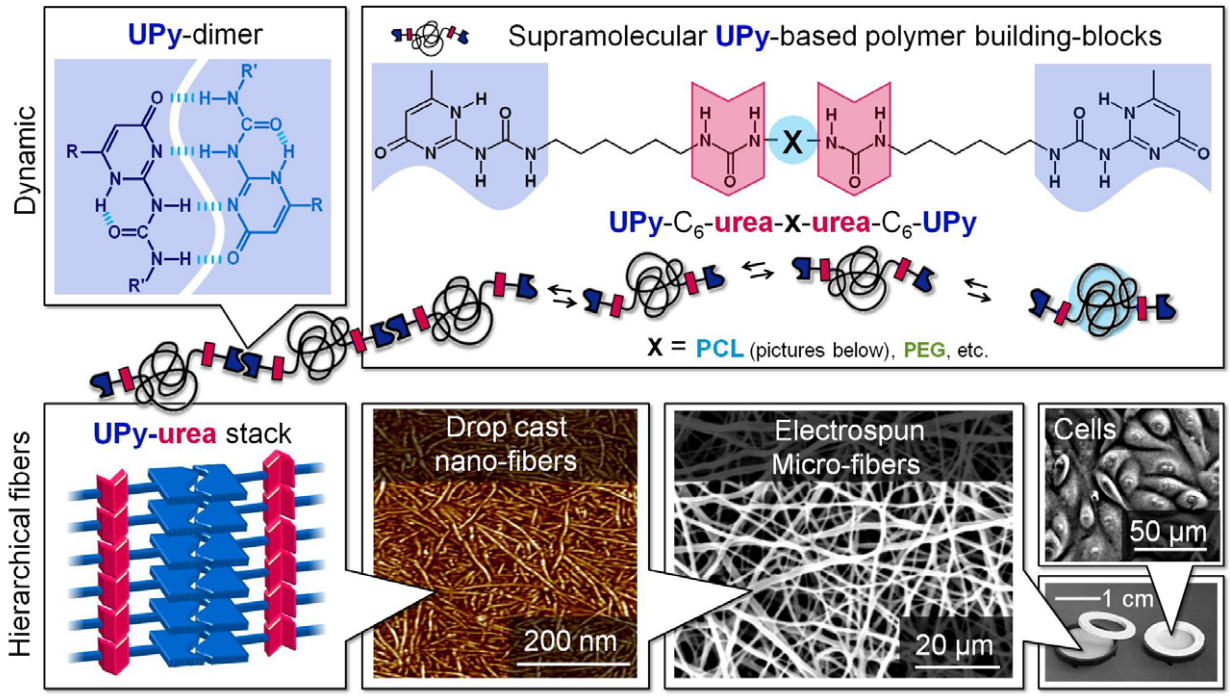


Figure SI-1: *Supramolecular material assembly of UPy-based polymers; UPy-dimerization leads to supramolecular chain extension of telechelic UPy-functionalized covalent polymer building blocks. UPy-dimer stack formation results from via pi-pi interactions and lateral hydrogen-bridges between adjacent urea linkers. These stacks are visualized as 'hard' nano-fibers in the AFM phase image of dropcast UPy-PCL. UPy-PCL can be processed into microfibers by electrospinning. The dimensions of the randomly oriented microfibers and resulting pores allow cells to grow in a monolayer fashion.*

2B. Processing of UPy-PEG with electrospinning setup results in electrospraying

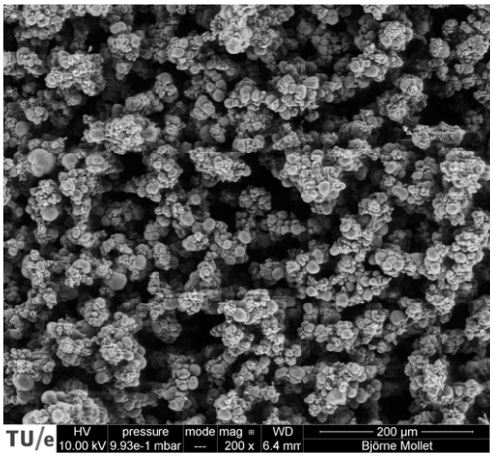


Figure SI-2: *Scanning electron micrograph of UPy-PEG, processed via electrospinning. The settings as applied for the electrospinning of UPy-PCL into fibrous scaffolds here result in electrosprayed material deposition without a clear fibrous structure.*

2C. Cell adhesion and cell morphology on bilayer membranes

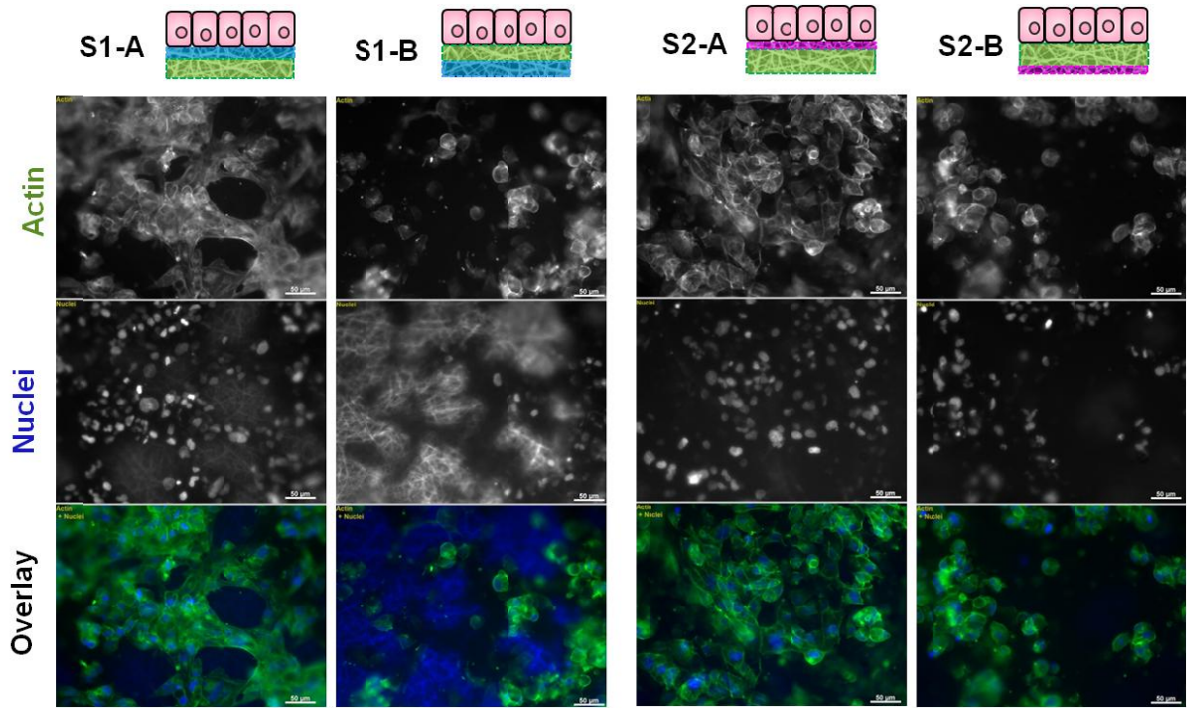


Figure SI-3: *Fluorescence microscope images of HK-2 cells, 14 h after seeding on the different bilayered scaffold sides. The actin cytoskeleton and nuclei of the cells were stained and visualized. Scale bars represent 50 µm. In some samples visualization of the nucleic staining is interfered by background staining/auto-fluorescence of the polymer scaffold.*

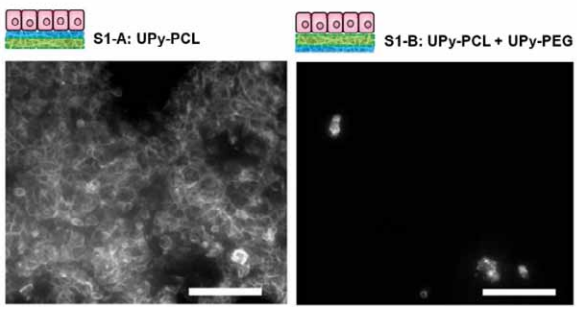


Figure SI-4: *Fluorescence microscope images of HK-2 cells on bilayered scaffold 1. The actin cytoskeleton of the cells was stained and visualized 3 days after seeding 180 x 10^3 cells per sample. Compared to SI-3, the HK-2 cells appear to proliferate on the pure UPy-PCL scaffold side to form larger confluent patches. On the contrary, for the UPy-PEG containing scaffold side S1-B, the amount of cells present on that scaffold side decreased over time. Scale bars represent 200 µm.*

2D. Electrospun meshes on glass.

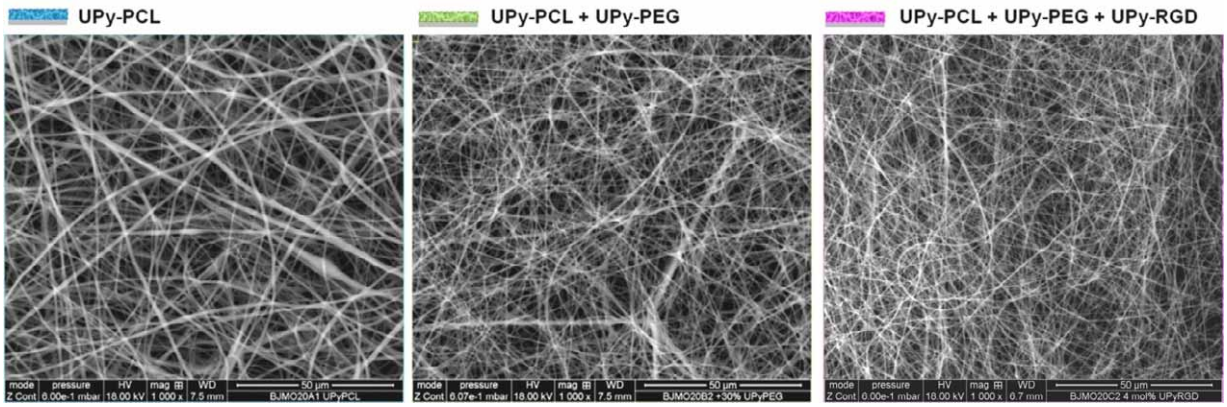


Figure SI-5: *Scanning electron microscopy images of electrospun meshes, prepared as thin layers of randomly oriented microfibers on glass coverslips, give an impression of the overall mesh morphology (scale bars represent 50 µm). From higher magnification micrographs (not shown here), average fiber diameters were determined: UPy-PCL: 827 ± 364 nm, UPy-PCL+UPy-PEG: 534 ± 146 nm, UPy-PCL+UPy-PEG+UPy-RGD: 437 ± 90 nm.*

2E. *HK-2 cells on dropcast films and electrospun meshes on glass*

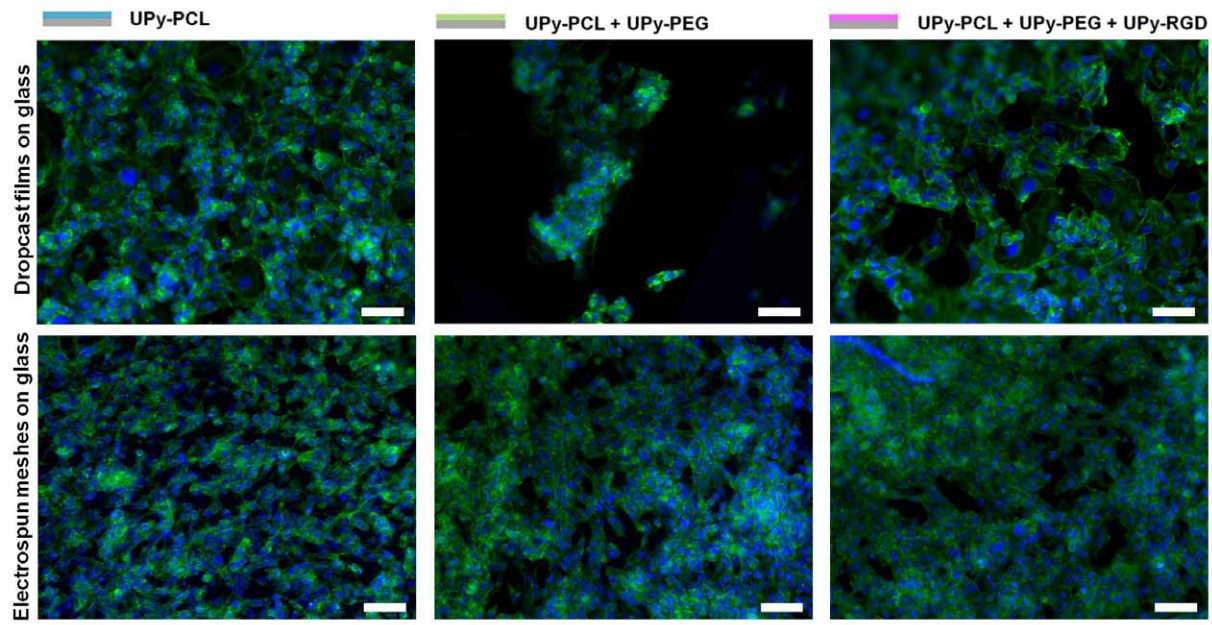


Figure SI-6: *Fluorescence micrographs of HK-2 cells, 14h after seeding of a high cell density (180 x 10^3/sample) on different UPy-biomaterial dropcast films (top row) and electrospun meshes (bottom row). Staining of cell nuclei (blue) and actin (green) shows the cell density and spreading of the cells. A similar result is observed compared to a low cell seeding density (36 x 10^3 cells/sample, shown in main article Fig. 5b); the anti-cell adhesive effect of UPy-PEG and cell adhesion inducing effect of UPy-RGD is clearly seen for the dropcast films, whereas no/less clear differences are observed for the electrospun meshes. Scale bars represent 100 µm.*

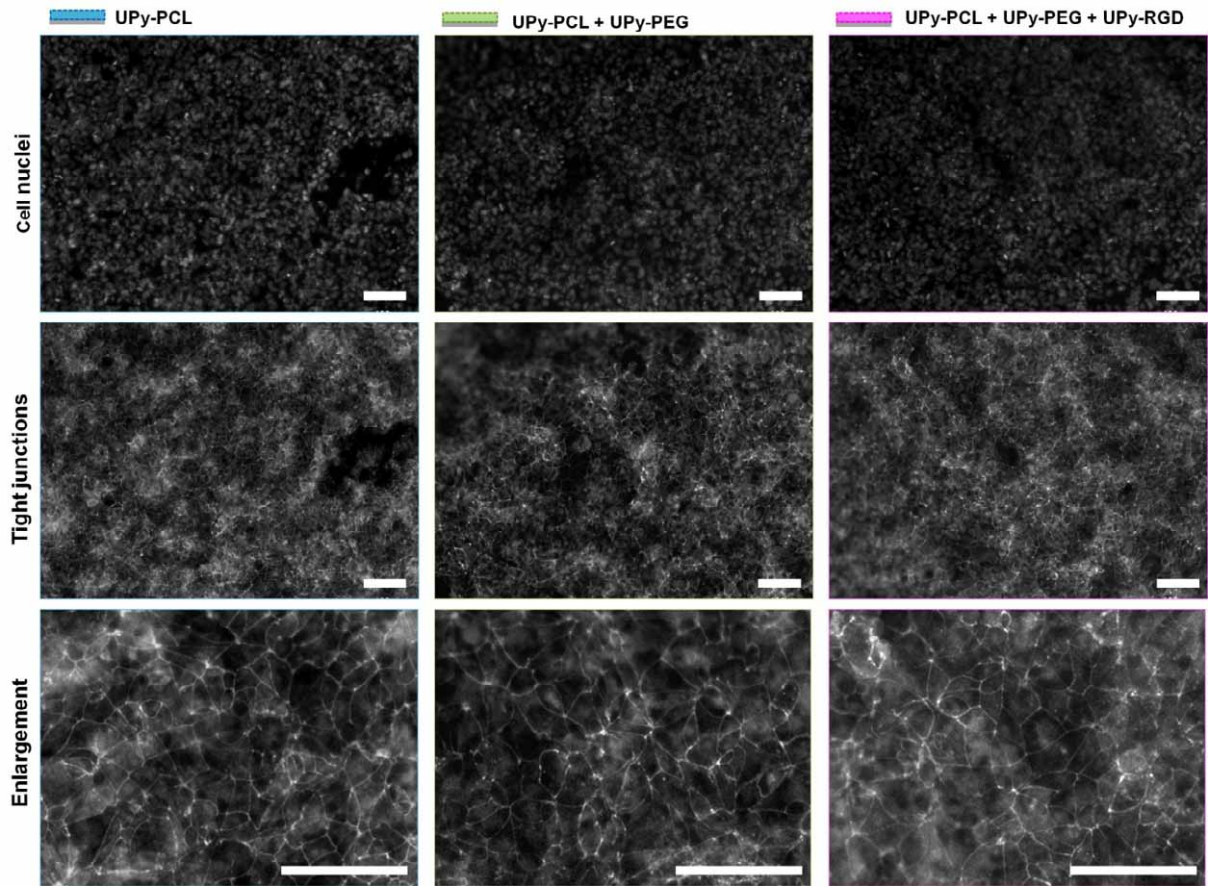


Figure SI-7: *Fluorescence micrographs of HK-2 cells, 3 days after seeding of a low cell density (36 x 10^3/sample) on different electrospun UPy-biomaterial meshes. Large patches of confluent cells are observed for all three mesh compositions. Staining of cell nuclei (top row) indicates a high cell density, and staining of zona occludens-1 (ZO-1) protein shows the presence of tight junctions throughout the confluent cell layer. The magnified micrographs of ZO-1 staining show the characteristic fine-lined pattern for confluent epithelial cells (scale bars represent 100 µm).*

The images shown in Figure SI-7, compared to images taken at 14h after seeding (shown in main article, Fig 5b), indicate that the cells that adhered to the meshes after seeding in a sub-confluent cell density, are able to proliferate to form a confluent cell layer. This is supported by an increase in signal in the resazurin mitochondrial activity assay performed on these samples (Figure SI-8).

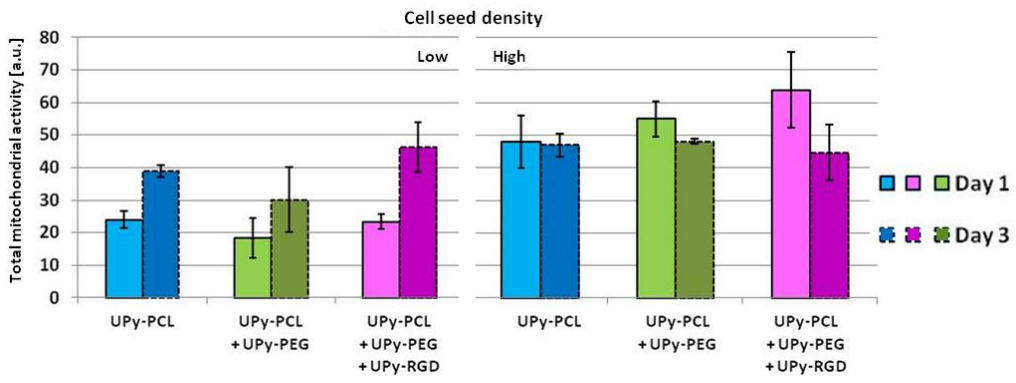


Figure SI-8: *Resazurin mitochondrial activity assay of HK-2 cells cultured on electrospun meshes of different UPy-biomaterial composition. The assay was performed at day 1 and day 3 on the same samples (n=3), after seeding HK-2 cells in high (180 x 10^3/sample) or low (36 x 10^3/sample) cell density. Independent of chemical composition, the cells adhere in higher density when seeded in a higher density. Assuming that an increase of total mitochondrial activity relates to an increase of viable cells, these results indicate that HK-2 that attached to the meshes remain viable and are able to proliferate when seeded in a sub-confluent density.*

3. *Resazurin mitochondrial activity assay - method*

To assess cell viability a mitochondrial activity assay was used based on the mitochondrial conversion of non-fluorescent resazurin to fluorescent resorufin. Resazurin salt (Sigma Aldrich) dissolved in PBS in a 88 10^{-5} M stock solution, was diluted 20 x in complete media to yield a 4.4×10^{-5} M concentration. The HK-2 cells cultured on meshes on glass were incubated for 3 h at 37 °C and 5% CO_2 in a humidified atmosphere with 800 µL of the resazurin solution. Per mesh, three samples of 200 µL resazurin solution were transferred to a black 96-well plate (Thermo Scientific) and fluorescence was measured (λ_{ex} = 550, λ_{em} = 584) with a Fluoroscan plate reader (Thermo Fisher Scientific). The non- invasive assay was performed at day 1 and day 3 after cell seeding. The average total mitochondrial activity of n=3 for each UPy-biomaterial mix was calculated at both time points and expressed ± standard deviation.

References

1. J. S. Moore and S. I. Stupp, *Macromolecules*, 1990, **23**, 65–70.
2. B. J. B. Folmer, R. P. Sijbesma, R. M. Versteegen, J. a. J. van der Rijt, and E. W. Meijer, *Adv. Mater.*, 2000, **12**, 874–878.