Supporting Information for:

Supramolecular surfaces functionalization via catechols for the improvement of cell-material interactions

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1.1 General methods

Instrumentation

^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 MHz NMR (Varian Mercury Vx or Varian 400MR) operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Proton chemical shifts are reported in ppm downfield from tetramethylsilane (TMS) and carbon chemical shifts in ppm downfield from TMS using the resonance of the deuterated solvent as internal standard. Abbreviations used are s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet. IR spectra were acquired on a Perkin-Elmer spectrum Two equipped with a UATR Two sample stage. Matrix assisted laser desorption/ionisation mass spectra were obtained on a Bruker autoflex speed spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) and 2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) as matrices. DSC spectra were obtained on a TA-instruments DSC Q2000 with heating and cooling rates of 10 °C/min at the first heating run. Gel permeation chromatography (GPC) was carried out on a Shimadzu Prominence-i LC-2030C 3D system equipped with refractive index and UV/vis detectors (Shimadzu RID-10A and Shimadzu SPD-M10A photodiode array detector respectively) using Polymer Laboratories PL Gel 5 µm MIXED-C and MIXED-D columns. Liquid chromatography mass spectrometry (LC-MS) data was obtained with a Thermoscientific LCQ fleet spectrometer. Fluorescent images were taken on the Zeiss Axiovert 200M fluorescent microscope, the Zeiss LSM510 META NLO confocal laser scanning microscope or Leica TCS SP5X confocal laser scanning microscope.

Materials

Bifunctional UPy-modified polycaprolactone (compound **1**, M_n = 2.7 kg/mol) was provided by SymoChem BV. Unless stated otherwise, all reagents and chemicals were obtained from commercial sources at the highest purity available and used without further purification. All solvents and chemicals were purchased from Sigma-Aldrich or Biosolve. Priplast 3196 was obtained from Croda (Gouda, the Netherlands). 2(6-Isocyanatohexylaminocarbony-lamino)-6-methyl-4[1H]pyrimidinone (UPy-C6-NCO) was synthesized as described.[1] Phosphate buffered saline (PBS) tablets were purchased from Sigma-Aldrich (pH 7.20-7.60). Gelatin from porcine skin Type A was purchased from Sigma (gel strength = 300 g

Bloom). Trypsin-EDTA solution was purchased from Sigma (0.5 g/L porcine trypsin and 0.2 g/L EDTA in Hank's Balanced Salt Solution with phenol red). Fibronectin from Bovine plasma was purchased from Biomedical Tecnhologies Inc.

List of antibodies used for immunofluorescent staining

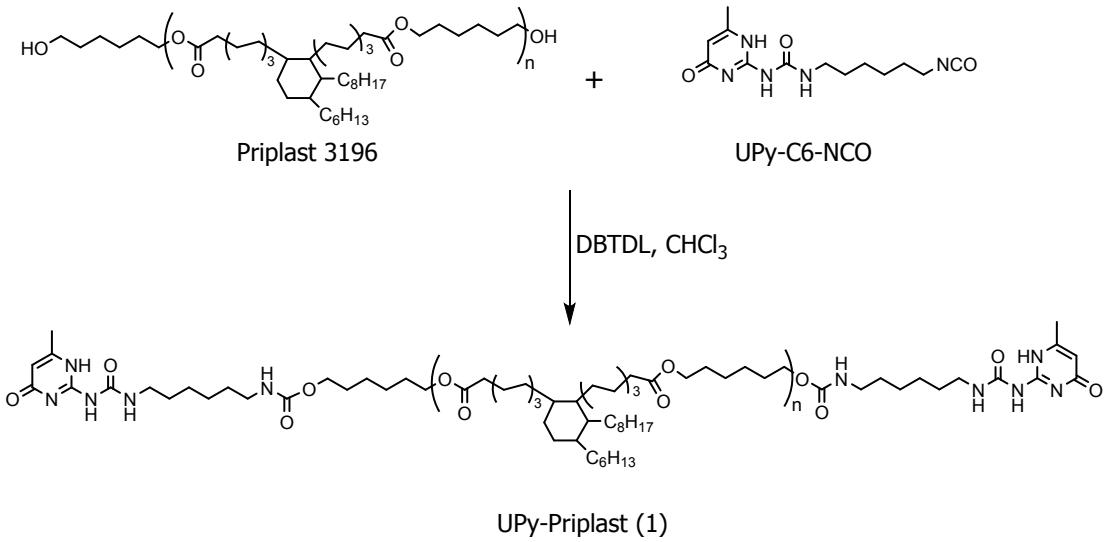
Antigen	Source	Cat. no	Isotype	Label	Species	Dilution
Integrin-β1	SC	sc-53711	IgG1	-	Mouse	1:50
Collagen I	USBiological	C7510-17K	IgG	-	Goat	1:50
Collagen IV	Abcam	ab86042	IgG1	-	Mouse	1:100
Collagen III	Abcam	ab7778	IgG	-	Rabbit	1:100
Fibronectin	Sigma	F-3648	IgG	-	Rabbit	1:200
Laminin γ1	Abcam	ab17792	IgG1	-	Rat	1:50
Rabbit IgG	Invitrogen	A31572	IgG (H+L)	Alexa 555	Donkey	1:300
Mouse IgG1	Molecular Probes	A21121	IgG1 (H)	Alexa 488	Goat	1:300
Mouse IgG1	Molecular Probes	A21127	IgG1 (H)	Alexa 555	Goat	1:200
Goat IgG	Invitrogen	A21432	IgG (H+L)	Alexa 555	Donkey	1:50
Rat IgG	Molecular Probes	A21434	IgG (H+L)	Alexa 555	Goat	1:200

List of primer sequences using for qPCR

Gene of interest		Sequence	Annealing temperature
GATA-4	fw	TCCAGCAACTCCAGCAAC	60
	rev	AGACATCGCACTGACTGAG	60
Nkx2.5	fw	CCCCTGGATTTTGCATTCAC	60
	rev	CGTGCGCAAGAACAAACG	60
Connexin43	fw	TTTCTTCAAGGGCGTTAAGGATC	60
	rev	AGGAGGAGACATAGGCGAGAG	60
Versican	fw	GGCACCTGTTATCCTACTGAAA	60
	rev	ACACAAGTGGCTCCATTACG	60
Link protein (HAPLN3)	fw	TGGTTCACCCGCATCCTAAC	60
	rev	GTAAACACCGTACAAGCGGC	60
Nidogen	fw	CCGAGAATGTGTTCTCGCTC	60
	rev	TGACGCCTTCTGCTACAACA	60
Laminin γ1 (LAMC1)	fw	CGCACATAGGTGATGTCAAAA	60
	rev	ACCGACTACAACAACCAGGC	60
Perlecan α1 (HSPG2)	fw	GGTGTATCGCAACTTCCCAC	60
	rev	CCAGCTCTCTTTTGGCAACT	60
Collagen IV	fw	ACTCTTTTGTGATGCACACCA	60
	rev	AAGCTGTAAGCGTTTGCGTA	60
Fibronectin	fw	AAGACCAGCAGAGGCATAAGG	60
	rev	CACTCATCTCCAACGGCATAATG	60
Collagen I	fw	AATCACCTGCGTACAGAACGG	60
	rev	TCGTCACAGATCACGTCATCG	60
Decorin	fw	TGCAGGTCTAGCAGAGTTGTGT	60
	rev	AATGCCATCTTCGAGTGGTC	60
Collagen III	fw	ATCTTGGTCAGTCCTATGC	60
	rev	TGGAATTTCTGGGTTGGG	60

1.2 Synthesis of UPy building blocks

Synthesis of UPy-Priplast:



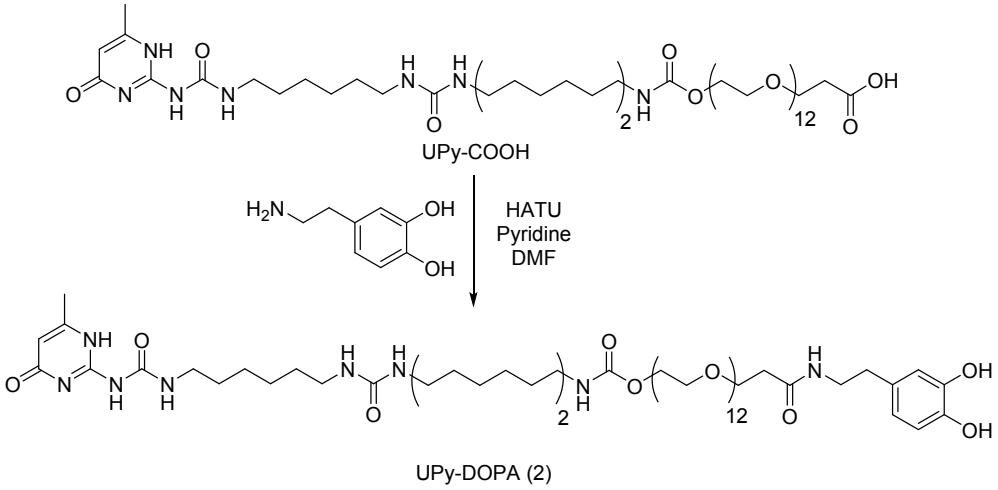
Supplementary Scheme S1: Synthesis of the UPy-Priplast (1), n~4, M_n = 4990 g/mol.

Priplast 3196 (25 g, 9.24 mmol) was dissolved in 150 mL chloroform, UPy-C6-NCO (5.69 g, 19.42 mmol) and dibutyltin dilaurate (0.29 g, 0.46 mmol) were added. The reaction mixture was stirred at reflux under argon for 5 h, then overnight at 70 °C. The reaction mixture was diluted with 1 L chloroform and filtered over a glass filter (pore size = 1), then over a glass filter (pore size = 1) with hyflo, and again over a glass filter (pore size = 3) with hyflo until a clear solution was obtained. The solvent was evaporated and product was further dried in vacuo at 35 °C affording 22 g (72 %) of UPy-Priplast (1).

^{1}H NMR (399 MHz, $CDCl_3$) δ 13.14 (s, 1H), 11.86 (s, 2H), 10.15 (s, 2H), 7.05 – 6.59 (m, 2H), 5.85 (s, 2H), 4.89 (s, 2H), 4.31 – 3.84 (m, 22H), 3.20 (d, J = 39.2 Hz, 8H), 2.53 (s, 4H), 2.31 – 2.17 (m, 24H), 1.93 – 0.73 (m, 387H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.90, 173.11, 156.72, 156.54, 154.68, 148.23, 106.66, 64.57, 64.12, 40.69, 39.63, 37.40, 37.12, 34.67, 34.35, 33.67, 33.22, 32.76, 32.36, 31.91, 30.15, 30.02, 29.77, 29.70, 29.64, 29.50, 29.34, 29.29, 29.18, 28.96, 28.55, 27.10, 26.70, 26.29, 26.12, 25.61, 25.54, 25.00, 23.15, 22.68, 19.71, 18.92, 14.11. Maldi-TOF m/z calcd ($C_{200}H_{364}N_{10}O_{24}$) 3293; found 3674 [M-UPy-C6, n=5,]$^+$, 3001 [M-UPy-C6, n=4]$^+$, 2364 [M-UPy-C6, n=3]$^+$, 1720 [M-UPy-C6, n=2]$^+$,1071 [M-UPy-C6,n=1]$^+$ FT-IR (ATR): υ (cm^{-1}) =2923, 2853, 1736, 1701, 1664, 1587, 1524, 1461, 1376, 1249, 1169, 845, 755, 725, 602, 564, 523. DSC (10 °C/min, first heating run): T_g = -51.9 °C; T_c = 37.1 °C, ΔH = 3.99 J/g; T_m = 77.3 °C, ΔH = 5.61 J/g. M_n (GPC, RI, THF): (**1**) 4.99 kDa; D: 2.33; Priplast 3196 4.95 kDa; D: 2.30.

Synthesis of UPy-DOPA:

The synthesis of the UPy-COOH synthon has been described previously.[2]



Supplementary Scheme S2: Synthetic route of monofunctionalized UPy-DOPA (2)

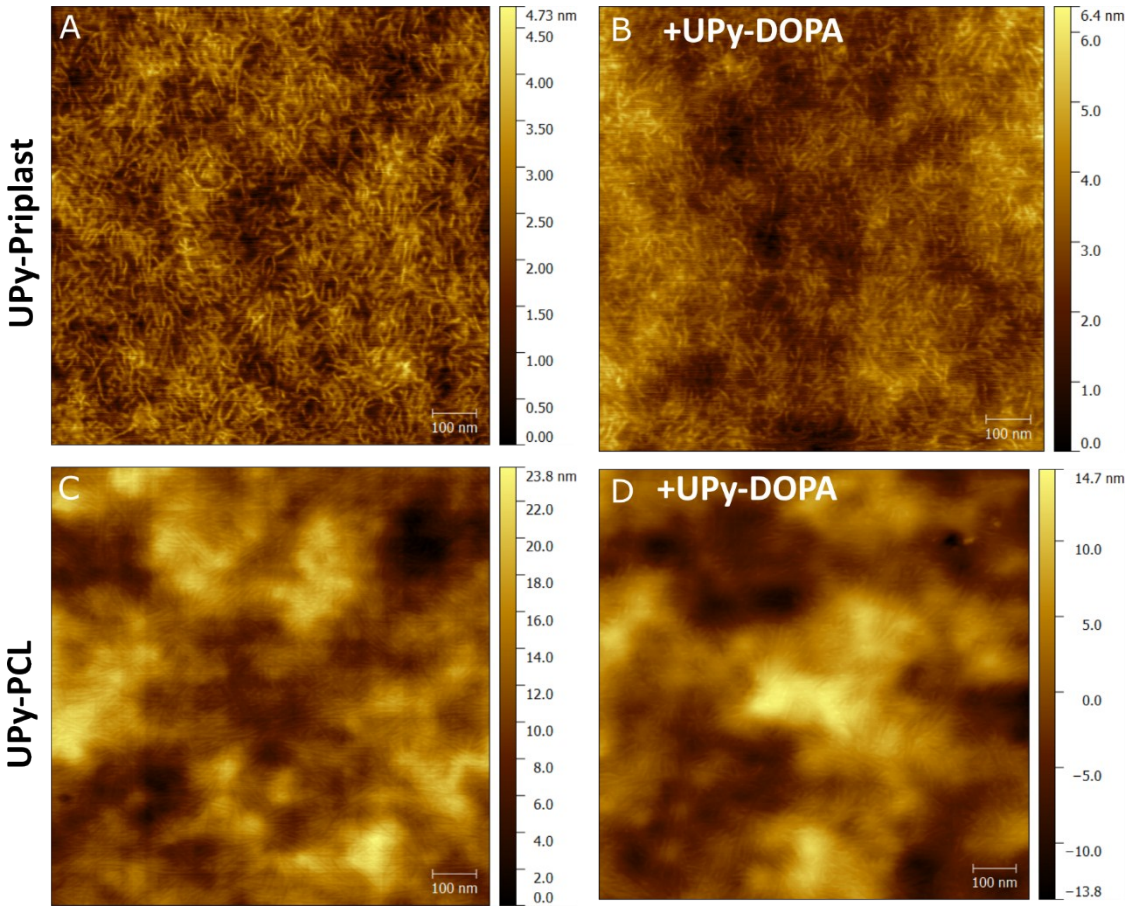
UPy-COOH (170 mg; 0.15 mmol) was dissolved in 5 mL DMF under argon. Pyridine (70 µL, 0.77 mmol) and HATU (70 mg, 0.17 mmol) were added to the reaction mixture and stirred for 30 min. After pre-activation, 3,4-dihydroxyphenethylamine hydrochloride (31 mg, 0.17 mmol) was added and the reaction was stirred overnight under argon. The reaction mixture was precipitated in a 1% (v/v) solution of formic acid in water. The precipitate was collected and freeze-dried yielding 166 mg (87%) of the product as a yellowish white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 13.18 (s, NH), 11.75 (s, NH), 9.79 (s, NH), 8.63 (s, 1H),), 8.27 (s, 1H), 6.75 (m, 1H), 6.69 (m, 1H), 6.55 (dd, 1H), 5.86 (s, 1H), 5.01 (s, NH), 4.18 (t, 2H), 3.63 (m, 48H), 3.23 (t, 2H), 3.20 (m, 6H), 2.64 (t, 2H), 2.44(t, 2H), 2.23 (s, 3H), 1.28 (m, 6H), 1.25 (m, 28H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.31, 172.22, 144.90, 143.59, 131.43, 120.89, 116.40, 115.99, 106.88, 71.08, 71.03, 71.00, 70.97, 70.94, 70.92, 70.90, 70.88, 70.80, 70.77, 70.61, 70.52, 70.12, 67.69, 67.16, 64.24, 41.36, 40.94, 40.86, 40.82, 39.91, 37.29, 35.45, 35.14, 30.72, 30.67, 30.35, 30.23, 30.14, 29.97, 29.93, 29.78, 29.67, 27.36, 27.16, 26.62. LCMS: calc. MW = 1273.6 g/mol, found m/z: 1273.7 $[M+H]^+$, 637.1 $[M+2H]^{2+}$.

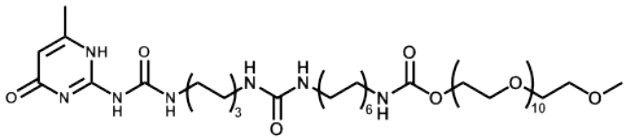
1.3 Silanized glass coverslips

Glass coverslips (diameter = 13 mm, water contact angle = 64.9 ± 3.4°) were made hydrophobic (water contact angle = 97.4 ± 2.1°) by first etching with Piranha solution (3:1 of sulphuric acid and hydrogen peroxide), followed by reacting it with dimethyldichlorosilane (in heptane) and subsequent drying with N_2 air.

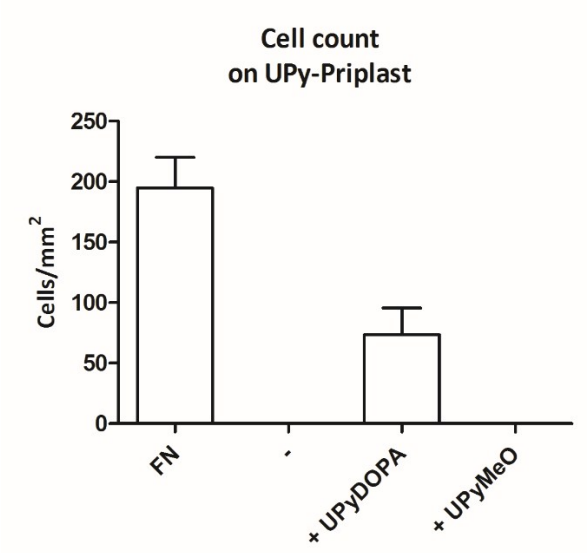
1.4 Additional results



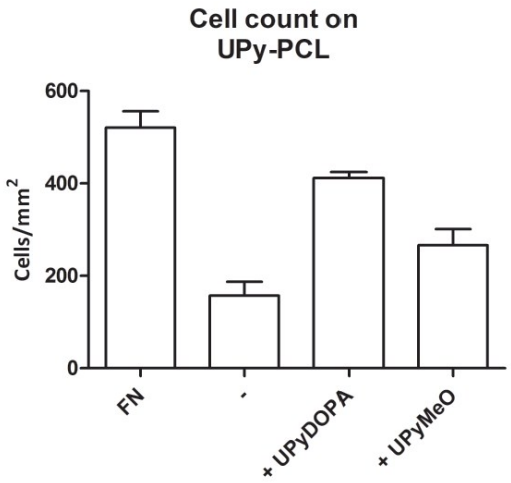
Supplementary Figure S1: Corresponding atomic force microscopy height images of dropcast films consisting of A) UPy-Priplast, B) UPy-Priplast + 10 mol% UPy-DOPA, C) UPy-PCL and D) UPy-PCL + 10 mol% UPy-DOPA



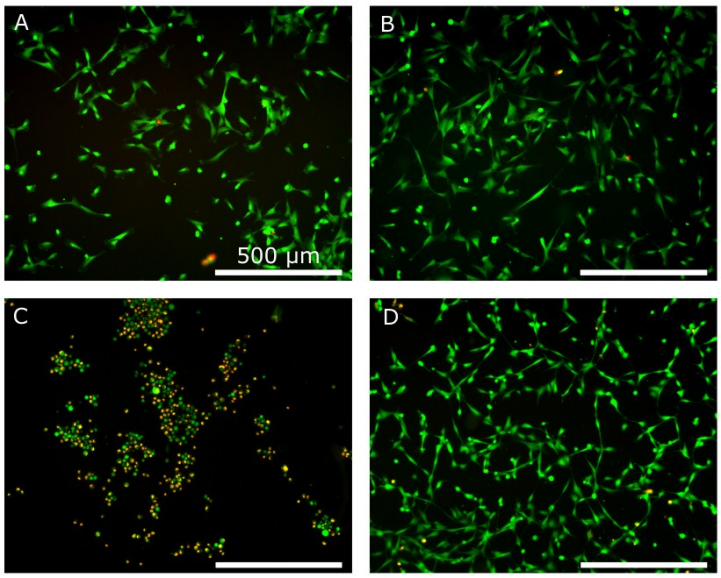
Supplementary Figure S2: Chemical structure of UPy-MeO. The synthesis of the UPy-MeO synthon has been described previously.[3]



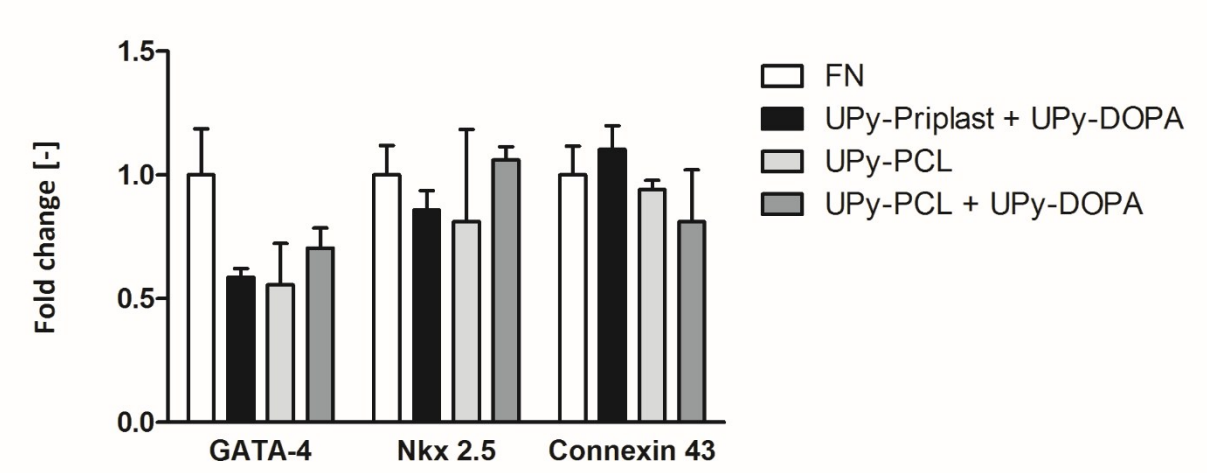
Supplementary Figure S3: Quantification of CMPC adhesion (cells/mm2) after culturing for 24h on fibronectin-coated glass (FN), UPy-Priplast (-), UPy-Priplast + 10 mol% UPy-DOPA and UPy-Priplast with 10 mol% UPy-MeO.



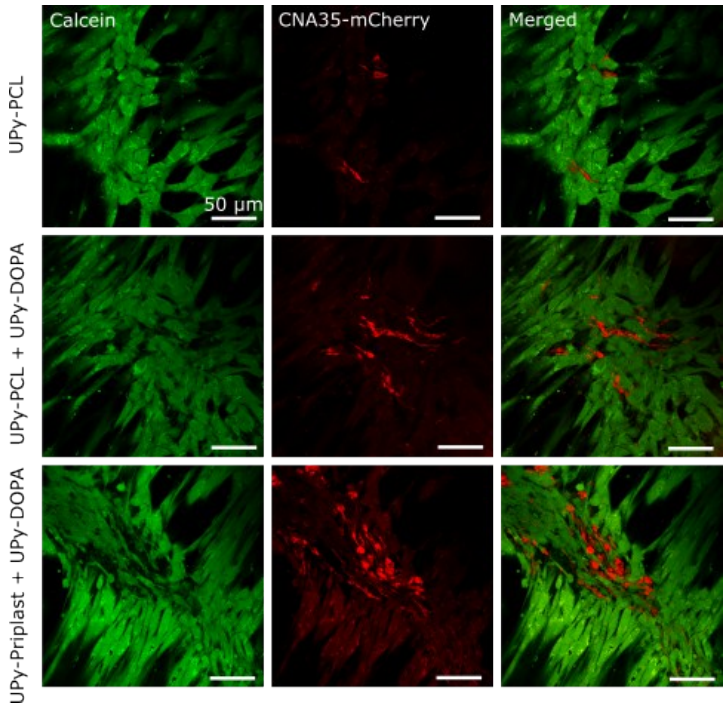
Supplementary Figure S4: Quantification of CMPC adhesion (cells/mm2) after culturing for 24h on fibronectin-coated glass (FN), UPy-PCL (-), UPy-PCL + 10 mol% UPy-DOPA and UPy-PCL with 10 mol% UPy-MeO.



Supplementary Figure S5: Live dead assay of CMPCs after 1 day of culturing on A) UPy-PCL, B) UPy-PCL + UPy-DOPA, C) UPy-Priplast and D) UPy-Priplast + UPy-DOPA



Supplementary Figure S6: Gene expression of cardiac markers by CMPC after 7 days on fibronectin-coated glass (FN), UPy-Priplast + UPy-DOPA, UPy-PCL and UPy-PCL + UPy-DOPA.



Supplementary Figure S7: : Fluorescence micrographs showing extracellular collagen production by CMPC after culturing for 7 days on dropcast films. From top to bottom; UPy-PCL, UPy-PCL + UPy-DOPA, and UPy-Priplast + UPy-DOPA. From left to right; Calcein, CNA35-mCherry and merged image of both Calcein and CNA35-mCherry. Scale bar = 50 µm.

1.5 Additional references

(1) Folmer, B. J. B.; Sijbesma, R. P.; Versteegen, R. M.; Van Der Rijt, J. a J.; Meijer, E. W. *Adv. Mater.* **2000**, *12*, 874–878.

(2) De Feijter, I.; Goor, O. J. G. M.; Hendrikse, S. I. S.; Comellas-Aragonès, M.; Söntjens, S. H. M.; Zaccaria, S.; Fransen, P. P. K. H.; Peeters, J. W.; Milroy, L. G.; Dankers, P. Y. W. *Synlett* **2015**, *26* (19), 2707–2713.

(3) Kieltyka, R. E.; Pape, A. C. H.; Albertazzi, L.; Nakano, Y.; Bastings, M. M. C.; Voets, I. K.; Dankers, P. Y. W.; Meijer, E. W. *J. Am. Chem. Soc.* **2013**, *135* (30), 11159–11164.