

Supplementary Information

A High-Throughput Polymer Microarray Approach for Identifying Defined Substrates for Mesenchymal Stem Cells

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1. Polymer microarray fabrication

The polymer microarrays were fabricated as described previously.¹⁻³

Slide preparation. Glass microscope slides were cleaned with oxygen plasma (Europlasma, Belgium) at 20 °C for 5 min, treated with 2% (3-aminopropyl)trimethoxysilane in acetonitrile (w/v) for 1 h, washed with acetonitrile and acetone, and oven dried at 100 °C for 2h. For agarose coating, slides were dipped into a 2% agarose solution (w/v) at 65 °C. The slides were air dried and stored at room temperature.

Contact printing. Polymers were contact printed onto agarose coated slides by using a Qarrayer^{mini} (Genetix, UK) with aQu solid pins (K2785, Genetix) inked from 384-well plates filled with 1% (w/v) polymer solutions in *N*-methylpyrrolidone (NMP). Each spot was stamped 5 times with 200 ms inking time and 100 ms stamping time, resulting in ~ 310 μm spots in diameter. For the initial screen the arrays of the polymers (n = 4) were printed in a 32 × 32 pattern. The slides were dried in a vacuum oven at 40 °C overnight. The ‘hit arrays’ were fabricated in a similar way as described above (polymer n = 6).

2. Synthesis of the lead polymers¹

For the analysis of the polymers see Table S2.

Synthesis of PA338. Methyl methacrylate (5.34 mL, 0.047 mol), glycidyl methacrylate (6.17 mL, 0.047 mol), and AIBN (26 mg, 0.16 mmol) were mixed in anhydrous toluene (58 mL). The solution was purged with N₂ for 15 min, and then heated to 60 °C under a N₂ atm. After 48 h, the reaction mixture was cooled and the polymer precipitated with excess hexane. The epoxy functionalised intermediate was collected by filtering, washed with hexane, and dried *in vacuo* at 40 °C to give a white solid.

The epoxy functionalised intermediate (1g), silica microbeads (50–100 µm, 40 mg), *m*-xylene (16 mL), cyclohexanol (8 mL) and *N*-methylaniline (1.14 mL) were purged with N₂ for 5 min. The reaction mixture was heated to 130 °C for 20 h. After cooling, the solution was decanted to remove the microbeads. The polymer was precipitated with excess hexane, collected by filtering, washed with hexane, and dried *in vacuo* at 40 °C for 24 h. The molecular weight was determined via GPC (Agilent) with 1-methyl-2-pyrrolidinone (NMP) as an eluent.

Synthesis of PU108. Dibutyltin dilaurate (0.06 g, 0.095 mmol) and anhydrous Mw 1000 poly(tetramethylene glycol) (5 g, 5 mmol) in anhydrous toluene (60 mL) were purged with N₂ for 5 min. 1,6-Diisocyanatohexane (1.75 g, 0.01 mol) was added dropwise over 10 min, and the reaction mixture heated to 70 °C for 2 h, after which 3-diethylamino-1,2-propanediol (0.68 g, 4.62 mmol) was added dropwise over 30 min. After 8 h, the reaction mixture was cooled and the polymer was precipitated with excess hexane, collected by filtering, washed with hexane, and dried *in vacuo* at 40 °C for 24 h (GPC analysis in NMP).

Synthesis of PU157. Anhydrous Mw 250 poly(tetramethylene glycol) (5 g, 0.02 mol) and dibutyltin dilaurate (0.14 g, 0.22 mmol) in anhydrous toluene (60 mL) were purged with N₂ for 5 min. 4,4'-Methylenebis(phenylisocyanate) (10.41g, 0.042 mol) was added dropwise over 10 min, and the reaction mixture heated to 70 °C for 2 h. After 2 h, 3-dimethylamino-1,2-propanediol (2.19 g, 0.018 mol) was added dropwise over 30 min. After 8 h, the reaction mixture was cooled and the polymer precipitated with excess hexane, collected by filtering, washed with hexane, and dried *in vacuo* at 40 °C for 24 h (GPC analysis in NMP).

3. Protein extraction from polymer surfaces

Polymer coated coverslips (n = 3) were placed into a 12-well tissue culture plate and sterilised with UV for 10 min. The coverslips were washed twice (10 min) with PBS and 3 mL of culture media (DMEM supplemented with 10% FCS, 4 ng/mL bFGF, 100 units/mL of L-glutamine, and 1% pen/strep) was added to each well. Plates were placed in an incubator at 37 °C with 5% CO₂ for 24 h. The media was removed and the coverslips washed twice with PBS. Each coverslip was placed into 1.5 mL Eppendorf tube and broken up into small pieces using tweezers. For the gelatin and tissue culture plastic controls, surfaces were removed using curved

scalpels. For the culture media control, 40 μL of media was added to a tube. The tubes were centrifuged at 11000 rpm for 5 min. To each tube, 40 μL of sample buffer (0.5 M Tris-HCl (pH 8.8), 4% SDS (w/v), 20% glycerol (v/v), 2% 2-mercaptoethanol (v/v), and 0.0025% bromophenol blue in distilled water) was added and the tubes heated at 100 $^{\circ}\text{C}$ for 10 min. The proteins were separated on a Novex Mini Cell in TGS buffer (Tris 0.025 M, Glycine 0.192 M, 0.1% SDS (w/v)) using a NuPAGE 4–12% Bis-Tris Gel (1mm \times 12 well). Gels were run for 40 min at 200 V, 120 mA. For silver staining, gels were placed in deionised water and stained with Sigma ProteoSilver Silver Stain Kit (PROTSIL1-1KT) following the manufacturer's instructions. For Oriole fluorescent staining, gels were placed directly into 100 mL of premixed fluorescent gel stain (Biorad 161-0496) for 90 min and transferred to deionised water and stored in the dark. Gels were imaged using a BIORAD image analyser and analysed with the Biorad image lab 3.0 software.

4. Contact angle and surface energy calculations

Contact angle

The contact angles of polymer coatings were measured by using a contact angle goniometer JC2000DI (Shanghai Zhongchen digital technic apparatus co.) at room temperature (20 $^{\circ}\text{C}$) and 50% humidity. Distilled water and formamide drops were used for the measurements (n = 3)

Surface energy calculations

Owen-Wendt vequation⁴ was used for surface energy calculation:

$$\gamma_L(\cos\theta+1)=2(\gamma_L^D\gamma_S^D)^{1/2}+2(\gamma_L^P\gamma_S^P)^{1/2}$$

γ_L is the surface tension of liquid, θ is the contact angle between the liquid-air interface and the surface, γ_S is the surface tension of solid.

$$\gamma_L=\gamma_L^D+\gamma_L^P, \text{ and } \gamma_S=\gamma_S^D+\gamma_S^P$$

Where D means dispersion component and P polar component.

For water, $\gamma_L^D = 21.8 \text{ mJ/m}^2$ and $\gamma_L^P = 51.0 \text{ mJ/m}^2$

For formamide, $\gamma_L^D = 39 \text{ mJ/m}^2$ and $\gamma_L^P = 19 \text{ mJ/m}^2$

5. References

1. H. Mizomoto, The synthesis and screening of polymer libraries using a high throughput approach, PhD Thesis, University of Southampton, 2004.
2. S. Pernagallo, J.J. Diaz-Mochon, M. Bradley, *Lab Chip*, 2009, **3**, 397–403.
3. G. Tourniaire, J. Collins, S. Campbell, H. Mizomoto, S. Ogawa, J-F. Thaburet, M. Bradley, *Chem. Comm.*, 2006, 2118–2120.
4. W. Youssef, R.R. Wickett, S.B. Hoath, *Skin Res. Technol.*, 2001, **7**, 10–7.

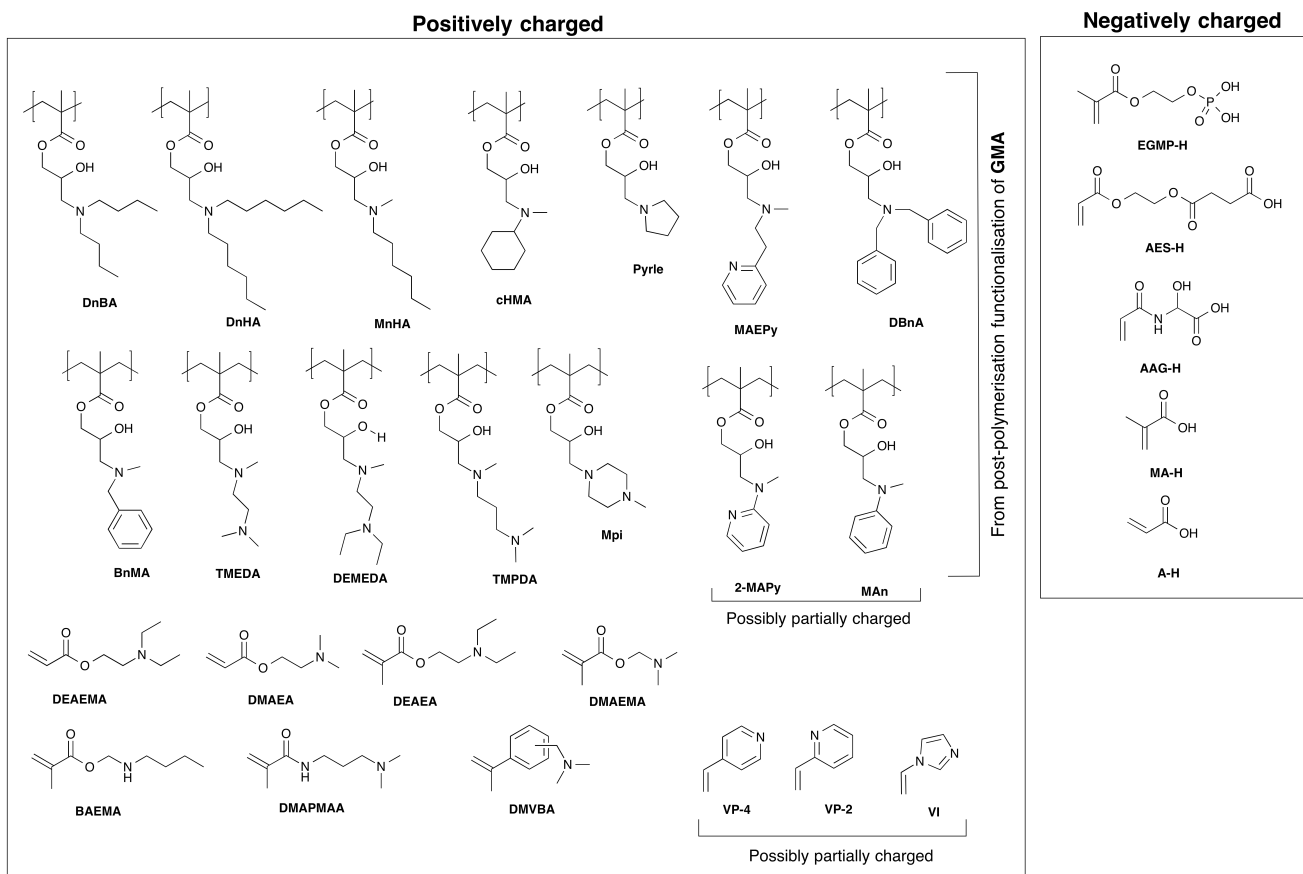


Fig. S1 'PA-library' – Monomers that can be charged at physiological pH.

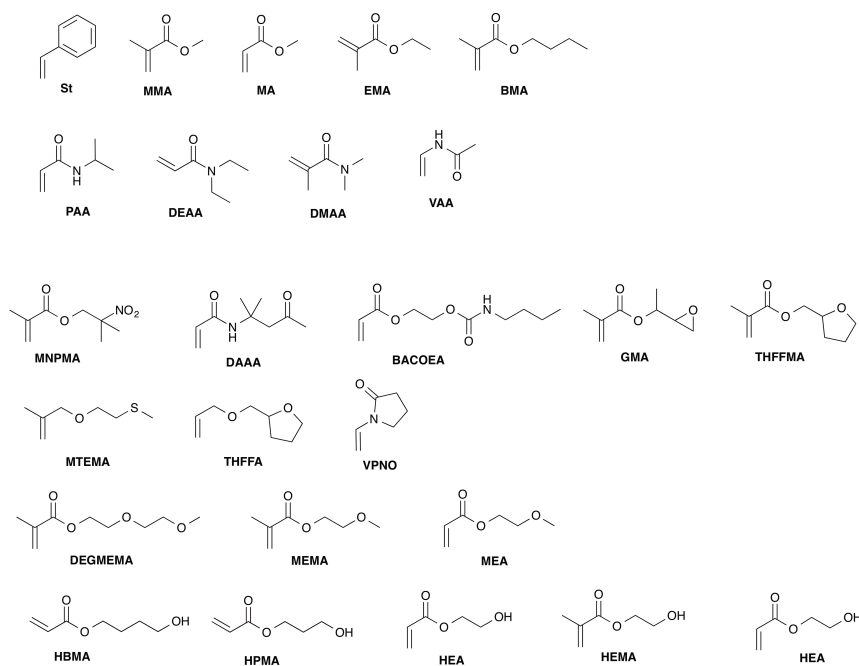


Fig. S2 'PA-library' – Monomers with varying degrees of hydrophilicity/hydrophobicity.

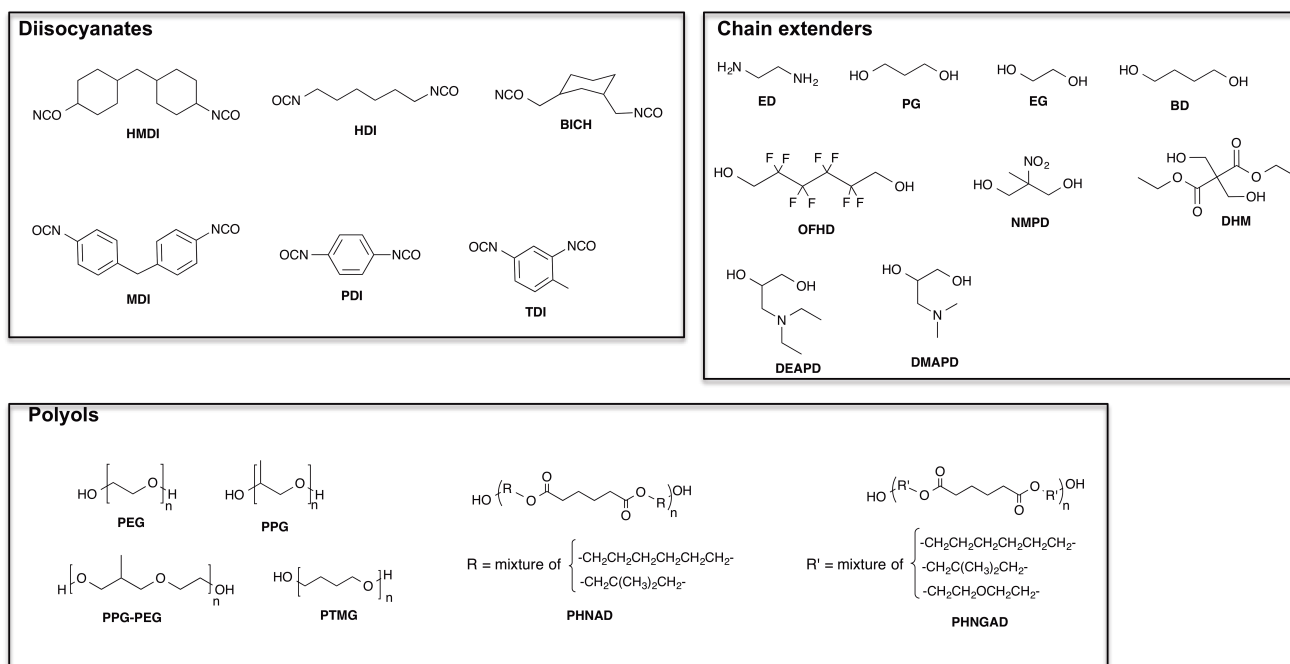


Fig. S3 Monomers used to construct the PU-library.

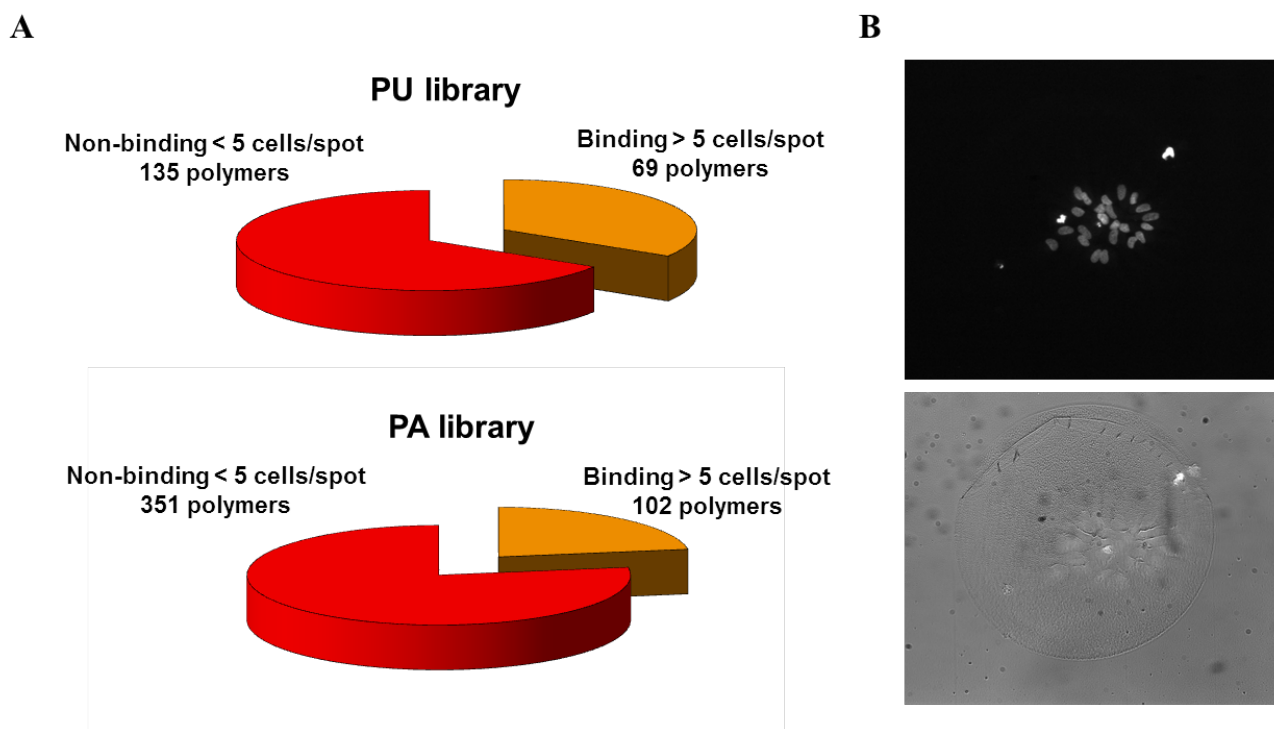


Fig. S4 A) Results of the initial 24 h binding screen (hES-MPs) for the 204 member PU-library and the 453 member PA-library. 69 polymers from the PU-library and 102 polymers from the PA-library were selected for the 'focused' screen. B) An example of a DAPI stained nuclei and bright field images of a polymer binding cells (24 h binding screen).

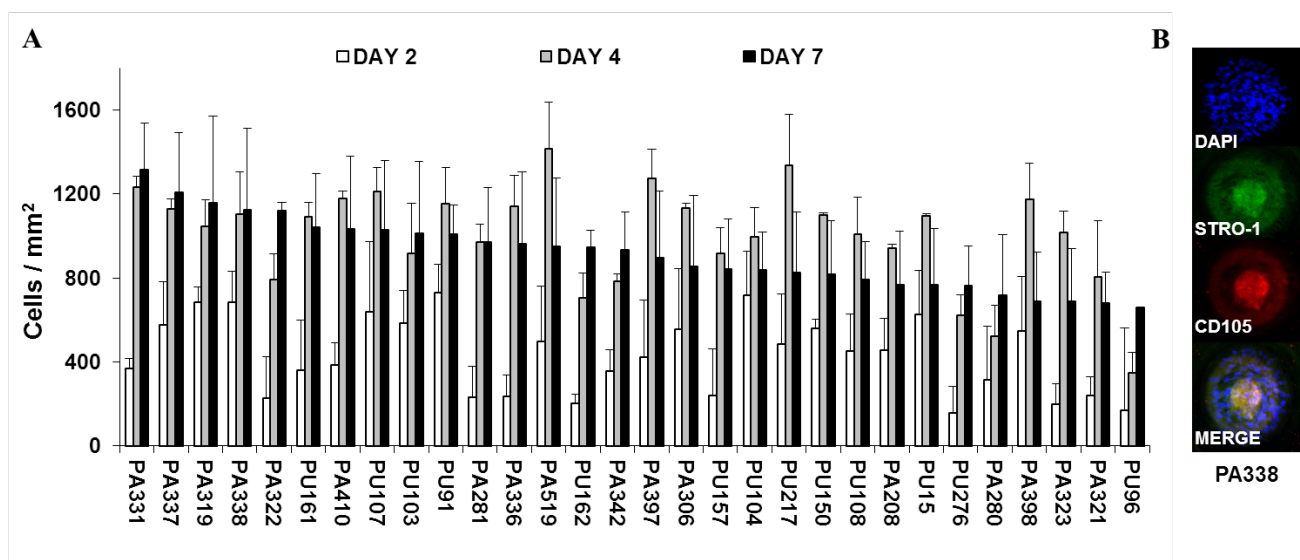


Fig. S5 A) Top 30 polymer candidates for hES-MP binding and growth (cells/mm²) after 2, 4 and 7 days of culture on the ‘focused’ 171-member polymer microarray. B) Staining and imaging of the polymer **PA338** for DAPI (nuclei) and markers STRO-1 and CD105 following cellular incubation on the array.

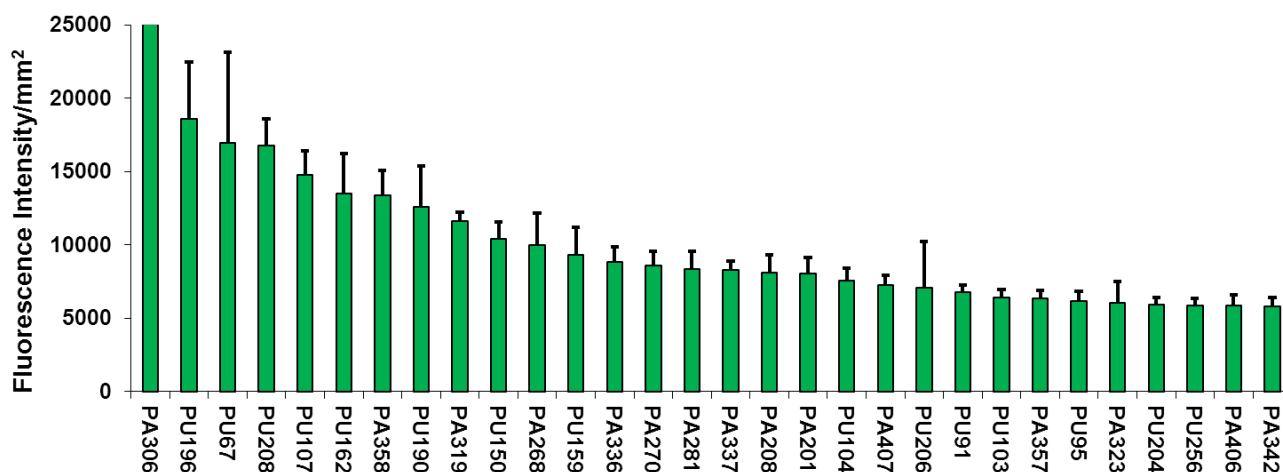


Fig. S6 The top 30 polymers in terms of cell viability measured by fluorescence intensity/mm² ($\lambda_{Ex}/ \lambda_{Em}$ 495/519 nm) from staining of cells on the ‘focused’ polymer microarray with CellTracker Green at day 7. Note that polymer **PA306** is auto-fluorescent.

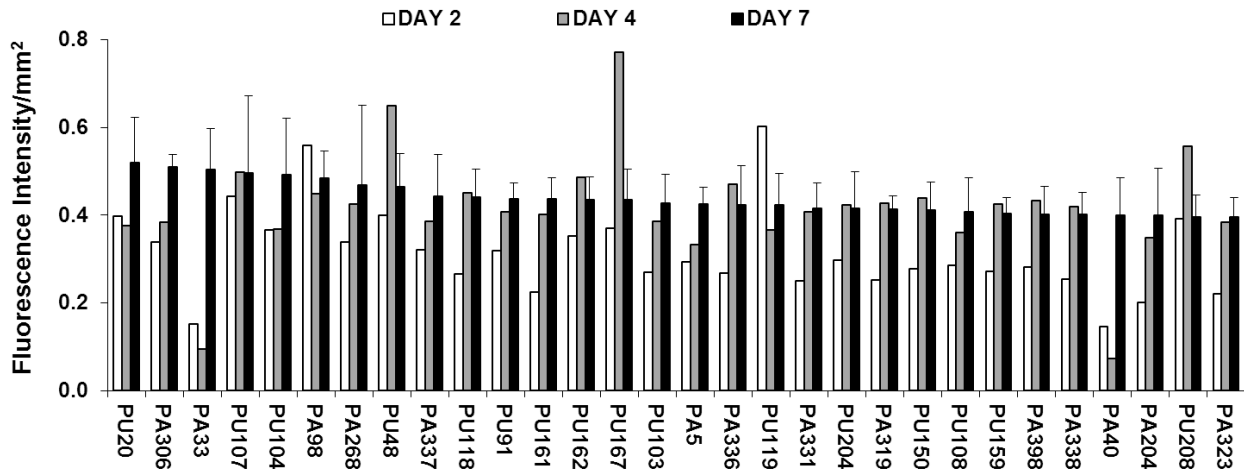


Fig. S7 The top 30 polymers in terms of STRO-1 staining ($\lambda_{Ex}/\lambda_{Em}$ 495/519 nm) from the ‘focused’ polymer microarray measured as fluorescence intensity/mm².

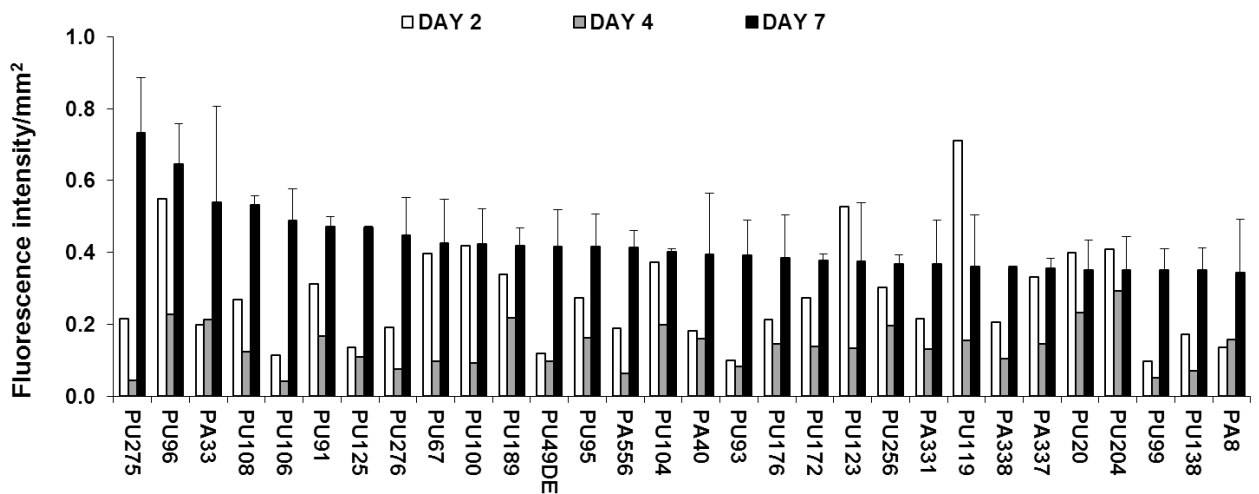


Fig. S8 The top 30 polymers in terms of CD105 staining ($\lambda_{Ex}/\lambda_{Em}$ 555/565 nm) from the ‘focused’ polymer microarray measured as fluorescence intensity/mm².

Table S1. The top 30 performing polymers from the ‘focused’ polymer microarray. The top polymers from the 171 focussed array were ranked for each criterion, *i.e.*, cell count (cells/mm²), STRO-1 intensity, CD105 intensity and cell viability and averaged to give an overall ranking

POLYMER	STRO-1 RANK	CD105 RANK	CELL COUNT RANK	VIABILITY RANK	OVERALL RANKING
PU91	12	7	10	23	1
PA337	10	26	2	17	2
PU104	6	16	19	20	3
PU103	16	35	9	24	4
PU108	24	4	22	36	5
PA338	27	25	4	35	6
PU107	5	73	8	5	7
PA336	18	49	12	14	8
PA331	20	23	1	52	9
PA306	3	75	17	1	10
PU204	21	28	32	28	11
PU161	13	47	6	49	12
PA319	22	82	3	9	13
PU67	38	10	68	3	14
PU162	14	86	14	6	15
PU20	2	27	48	47	16
PU95	40	14	46	26	17
PU190	34	36	49	8	18
PU256	44	22	41	29	19
PU118	11	44	51	41	20
PU96	61	2	31	54	21
PU276	46	9	26	70	22
PU157	35	63	18	45	23
PA204	29	45	65	28	24
PU150	23	115	21	10	25
PA398	26	72	28	57	26
PU208	30	118	34	4	27
PU176	47	19	69	61	28
PA410	63	66	7	62	29
PA357	67	64	43	25	30

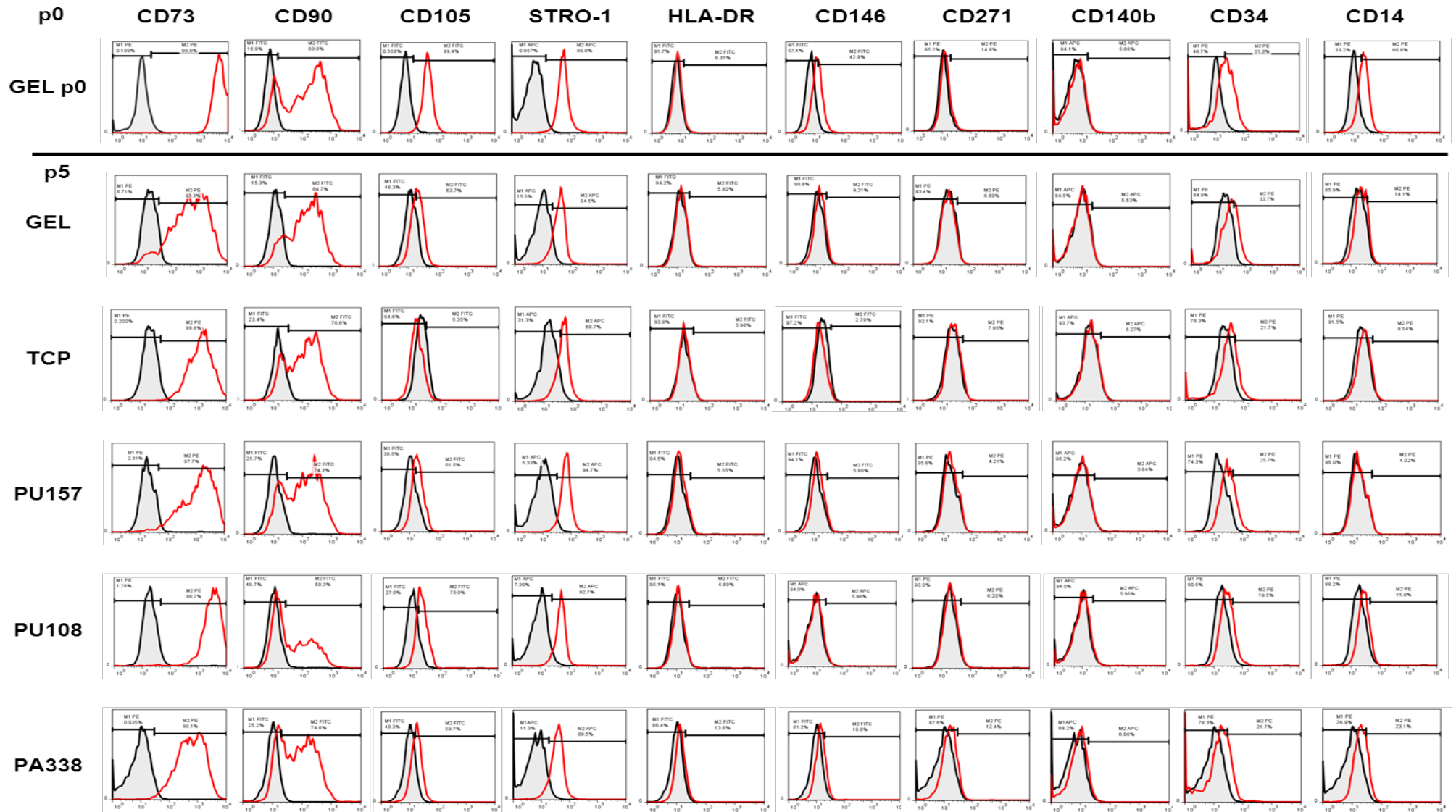


Fig. S9 Representative flow cytometry traces for hES-MPs stained with 10 MSC markers at ‘passage 0’ (p0) prior to long term culture, and after 5 passages (p5) on gelatin (GEL), tissue culture plastic (TCP), **PU157**, **PU108** and **PA338**. The red trace represents the stained cells and the black shaded trace represents the unstained cells for that substrate (n = 3).

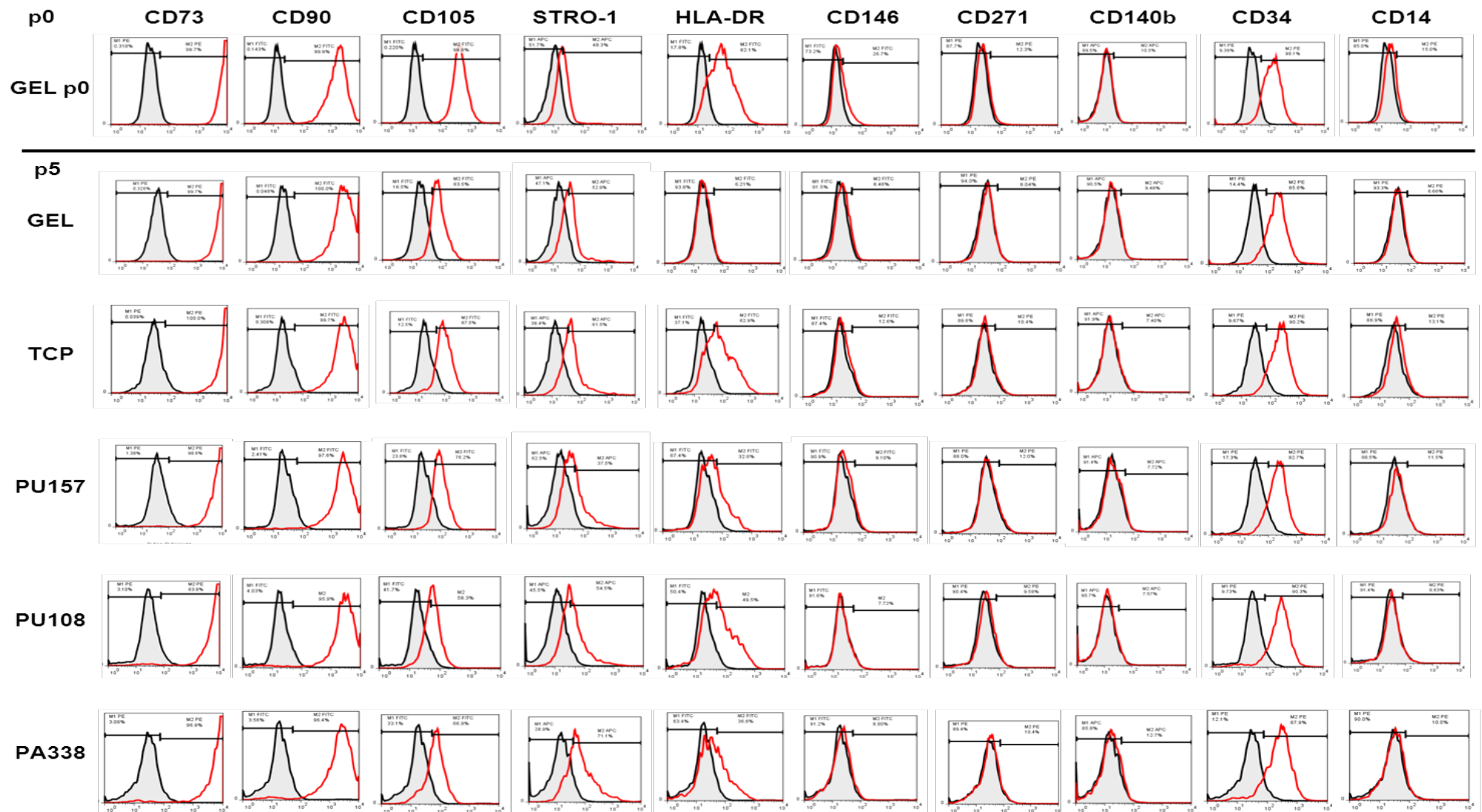


Fig. S10 Representative flow cytometry traces for ADMSCs stained with 10 MSC markers at ‘passage 0’ (p0) prior to long term culture, and after 5 passages (p5) on gelatin (GEL), tissue culture plastic (TCP), **PU157**, **PU108** and **PA338**. The red trace represents the stained cells and the black shaded trace represents the unstained cells for that substrate (n = 3).

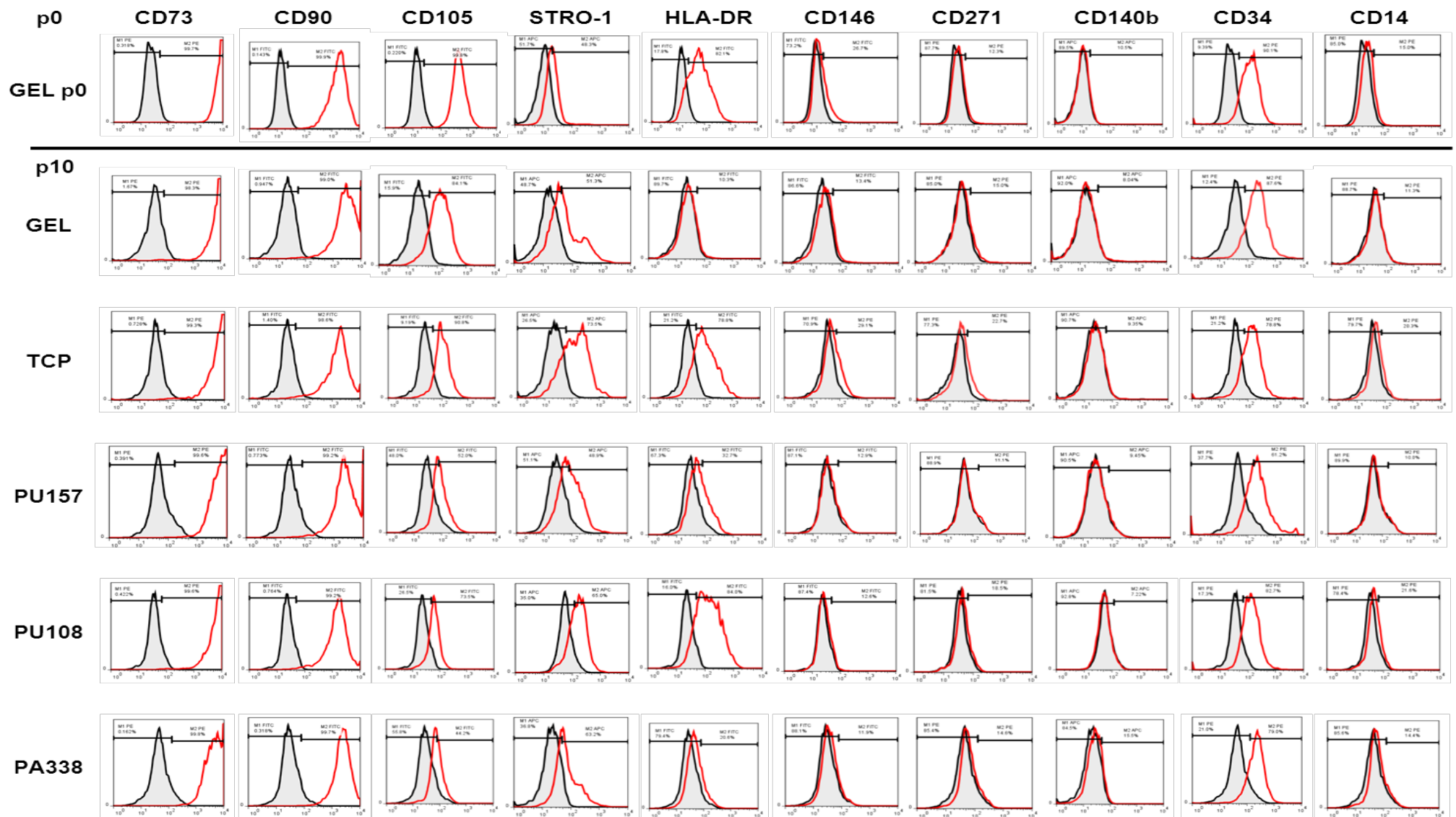


Fig. S11 Representative flow cytometry traces for ADMSCs stained with 10 MSC markers at ‘passage 0’ (p0) prior to long term culture, and after 10 passages (p10) on gelatin (GEL), tissue culture plastic (TCP), **PU157**, **PU108** and **PA338**. The red trace represents the stained cells and the black shaded trace represents the unstained cell for that substrate (n = 3).

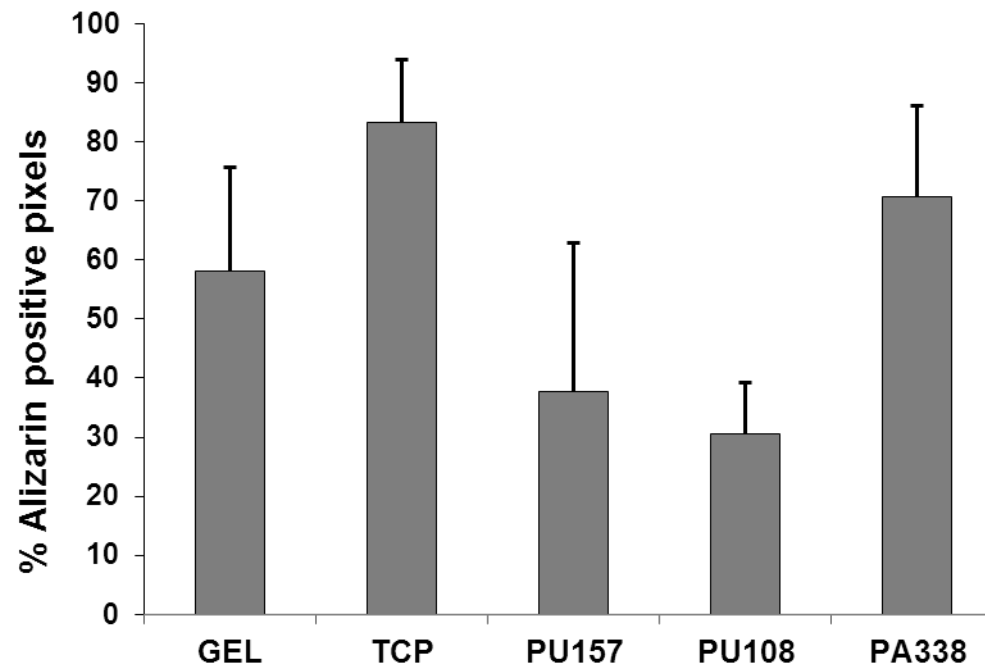


Fig. S12 Comparison of the amount of Alizarin red S positive pixels from osteogenic differentiation images of ADMSCs after 5 passages on gelatin (GEL), tissue culture plastic (TCP), **PU157**, **PU108** and **PA338**. Osteogenic comparison was carried-out using ImageJ by setting the image/adjust/colour threshold option.^a The ‘analyse particles’ option quantified the percentage of pixels above threshold. The values correspond to the percentage of positive pixels on an image that are above a defined minimum threshold.

^a A. Mehlem, C.E. Hagberg, L. Muhl, U. Eriksson, A. Falkevall, *Nat. Protoc.*, 2013, **8**, 1149–1154.

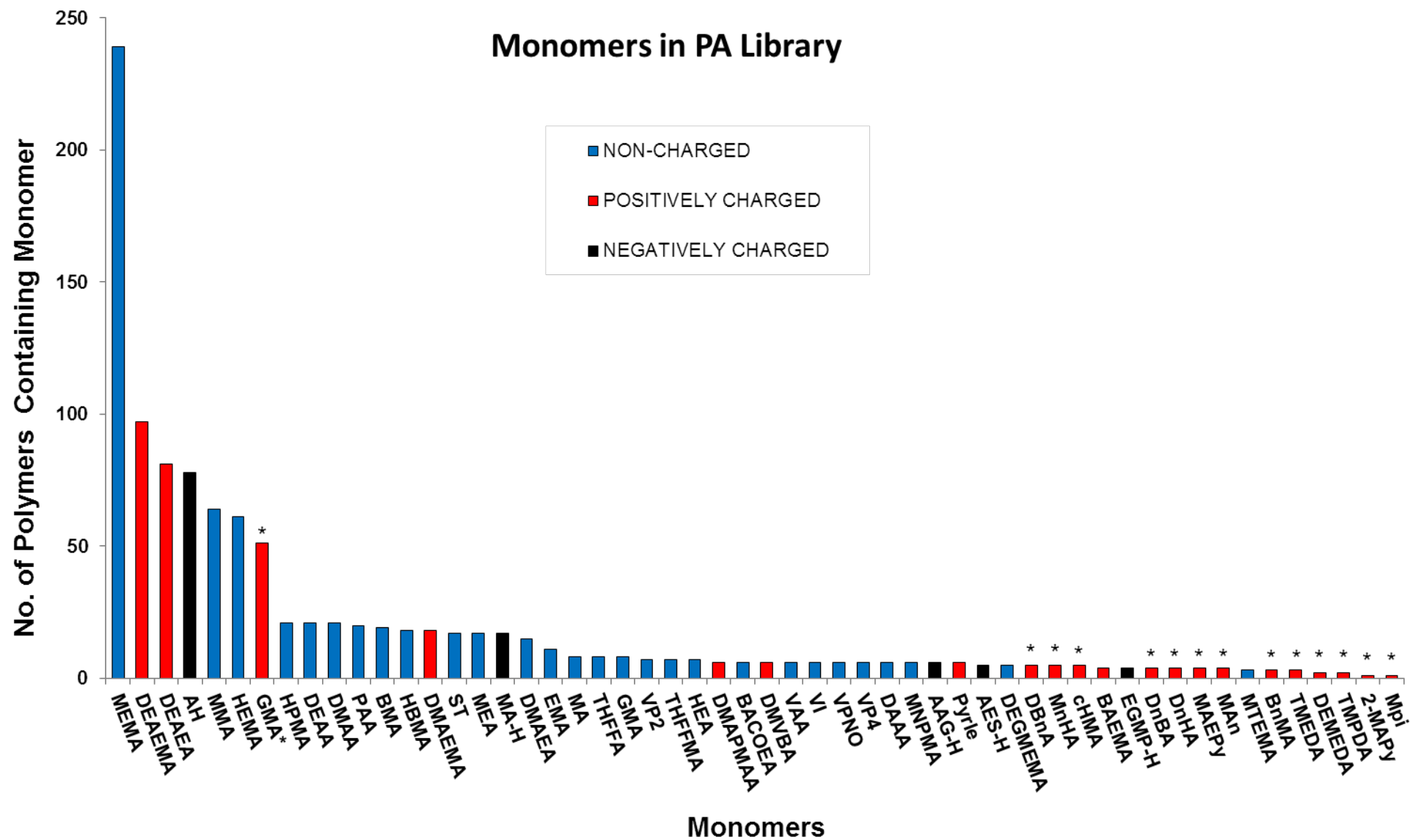


Fig. S13 Monomer distribution in the “PA library”. The GMA* containing polymers were subsequently functionalised with a variety of amines (signified by *). The figure legend shows the likely properties of the monomers at physiological pH (aromatic amines MAn and 2-MAPy mostly uncharged).

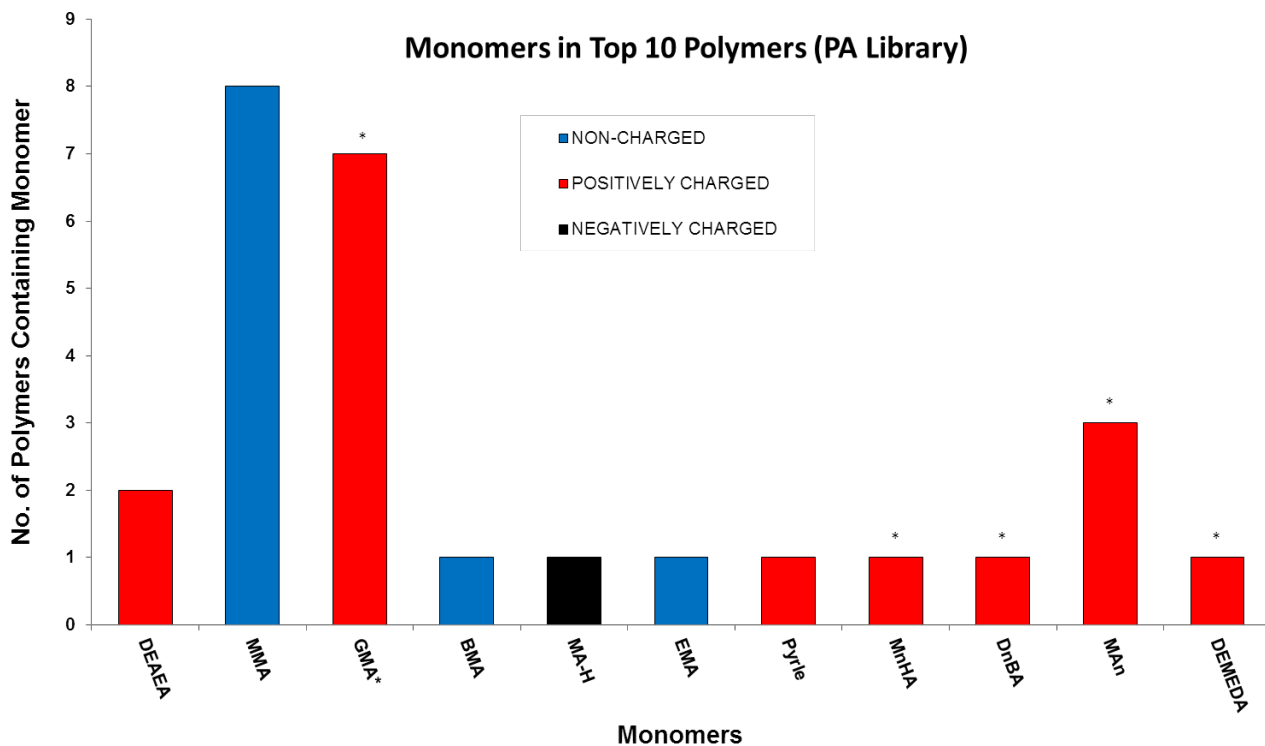


Fig. S14 The monomers found in the top 10 polymers in the PA library in the scale up experiment. The GMA* containing polymers were subsequently functionalised with a variety of amines (signified by *). The figure legend shows the likely properties of the monomers at physiological pH (MAn is an aniline derivative and mostly uncharged).

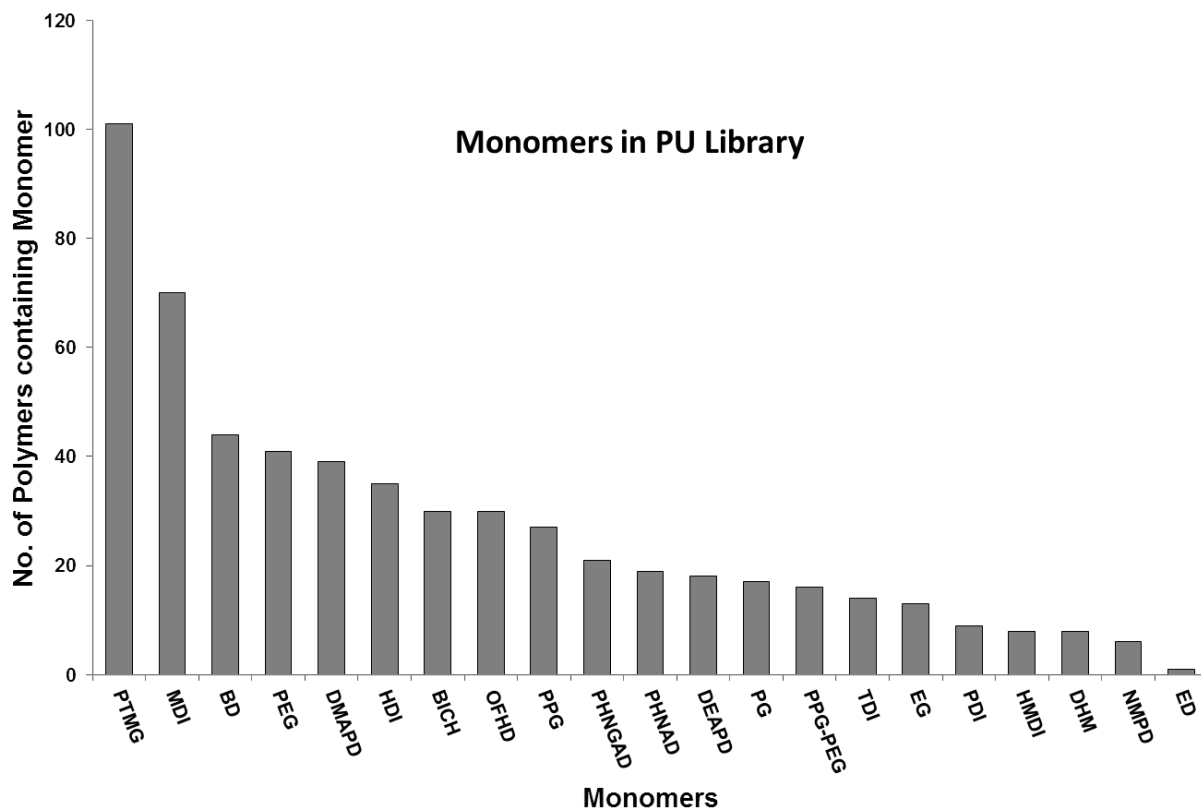


Fig. S15 Monomer distribution in the polyurethane-based PU library.

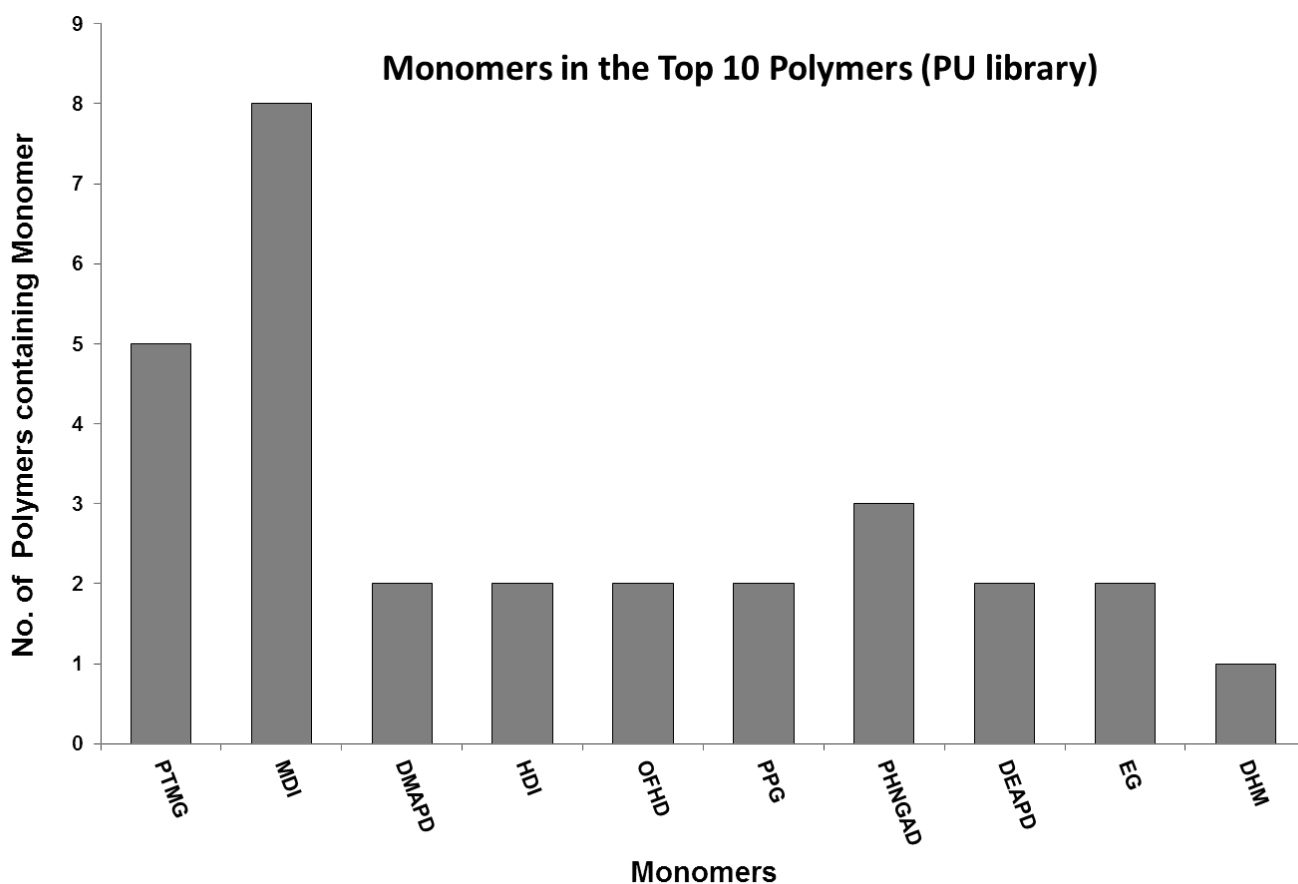


Fig. S16 Monomer distribution in the top 10 PU polymers in the scale up experiment.

Table S2 Molecular weight, polydispersity index (PDI), water and formamide (HCONH₂) contact angles, and surface energy calculations of the 3 lead polymers **PU157**, **PU108** and **PA338**, and representative non-binding polymers **PA554** and **PU6**, and ‘mid-range polymers’ **PA63** and **PU212**.*

Surface	MW	PDI	Contact Angle H ₂ O (°)	Contact Angle HCONH ₂ (°)	Surface Energy (dyne/cm)
PU6	59000	2.0	26.5 ± 1.6	27.1 ± 2.0	66.0 ± 0.9
PU108	233000	2.5	46.1 ± 3.6	27.6 ± 2.2	54.0 ± 1.9
PU212	66000	2.2	61.8 ± 1.2	32.3 ± 0.0	49.8 ± 0.2
PA554	110000	2.2	65.7 ± 0.5	32.3 ± 0.6	51.2 ± 0.7
PA63	52100	4.2	66.7 ± 1.9	46.3 ± 5.1	41.5 ± 3.1
PU157	41000	1.9	69.8 ± 1.2	51.8 ± 1.5	38.0 ± 0.9
PA338	> 2,000,000	N.D	81.1 ± 0.7	62.1 ± 0.6	31.8 ± 0.6

* **PA63** is a copolymer of HBMA and PAA, and **PA554** of AH and HEMA. **PU212** was synthesised from PHNAD and HDI with PG as a chain extender, **PU6** from PEG and BICH. For the monomer structures see Fig. S1–S3.

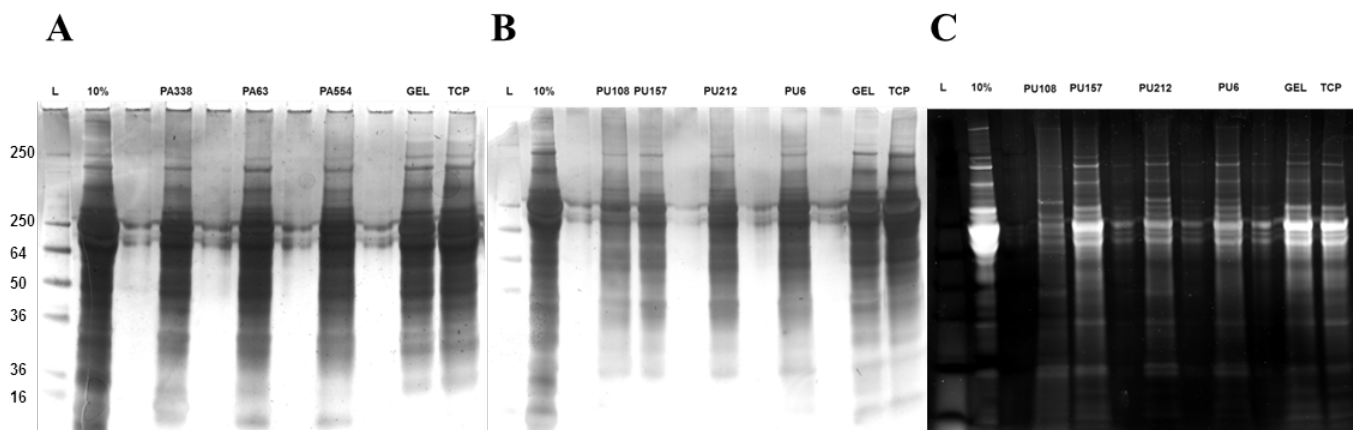


Fig. S17 Protein analysis on polyacrylamide gels (silver or fluorescent staining) after protein extraction from polymer-coated coverslips, gelatin and tissue culture plastic (TCP), incubated for 24 h with culture media (DMEM containing 10% FCS, 4 ng/mL bFGF, 200u/mL of L-glut, 1% pen/strep). The first lane on the left (L) contains a protein ladder (unit kDa) and the next lane contains culture media.