Electronic Supplementary Information (ESI)

Optimisation of grafting of low fouling polymers from three dimensional scaffolds via surface-initiated Cu(0) mediated polymerisation.

Lina Duque-Sánchez [a,b], Narelle Brack [a], Almar Postma [b], Paul J. Pigram [a],

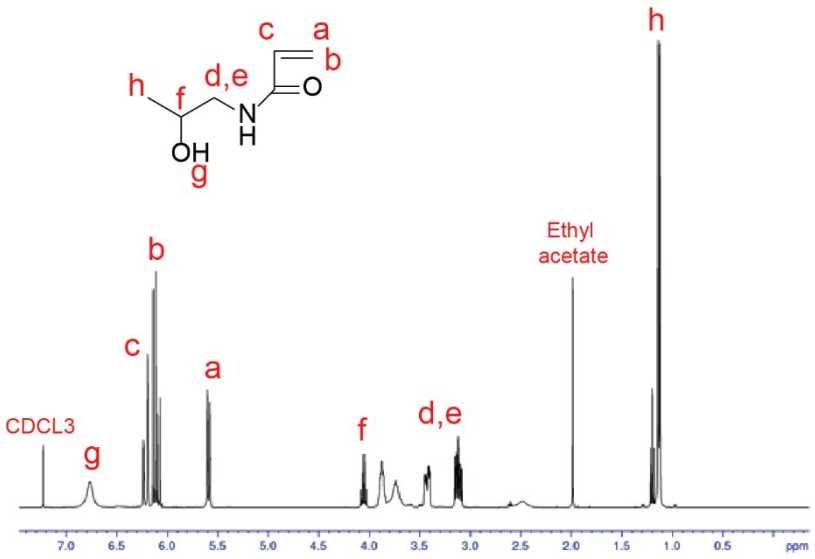
Laurence Meagher [c*]

[a] Centre for Materials and Surface Science and Department of Chemistry and Physics, La Trobe University, Melbourne, Victoria 3086, Australia.

[b]CSIRO Manufacturing, Bayview Avenue, Clayton, Vic 3168, Australia.

[c]Monash Institute of Medical Engineering and Department of Materials Science and Engineering, Monash University, Clayton, Vic 3800, Australia.

1. *N*-(2-hydroxypropyl)acrylamide (HPA)



^{1}H NMR spectrum of N-(2-hydroxypropyl) acrylamide (HPA) monomer

2. Cell culture experiments

For cell culture experiments, the L929 fibroblast cell line was employed. DMEM media was supplemented with 1 % Pen-Strep and 10 % FBS to obtain complete media. The confluence of the cell cultures was assessed under the microscope and passaged was completed before full confluency ~80 % was achieved. A biological safety cabinet was used, and all procedures were undertaken under sterile conditions. For cell passaging, medium from the flask was aspirated, then cells were gently rinsed with DPBS. DPBS was carefully and completely removed before adding the TrypLe agent. Once TrypLE was added, the flask was gently moved and placed into the incubator for 1-2 minutes in order to promote cell detachment. Once cell detachment had occurred additional media was added to the flask and the cell suspension was collected and transferred to a falcon tube. The cell suspension and complete media were then transferred to a new flask in an adequate ratio. Cells were incubated at 37 ^{o}C, 5 % CO_2.

2.1 Cell seeding onto electrospun fibres

Electrospun ultrafine fibres were sterilised prior to cell seeding as follows: samples of 9 mm diameter were cut using a wad punch and placed into a 48 well plate. Sterilisation was completed by adding 200 µL of 2x antibiotic-antimycotic solution to each well and leaving it overnight. After sterilisation, samples were rinsed once with DPBS. Complete media was then added to the wells and the samples were incubated overnight. After overnight incubation, the medium was replaced by seeding 100.000 cells/well in 0.4 mL of complete media onto the samples. Cell counts were achieved by loading 10 µL aliquot of the cell suspension onto a hemocytometer; the hemocytometer was placed under the microscope and cells were counted.

2.2 Cell fixation and staining

Once the desired incubation times were completed, cell culture medium was removed, and samples were gently rinsed with DPBS. A pre-determined amount of 4 % PFA, sufficient to cover the sample, was added to each well and allowed to react for 15 minutes at room temperature. Samples were immediately transferred to a fume hood where the PFA was removed and the samples were rinsed twice with DPBS. Permeabilisation of the samples was achieved by adding 0.1 % Triton-X-100 in DPBS solution to the samples and leaving for 15 min at room temperature. Once the permeabilisation time was completed, samples were incubated in 3 % BSA for 30

minutes at room temperature. For staining, ActinRed (2 drops for each mL of 3 % BSA) and trihydrochloride, trihydrate (Hoechst 33342) (1 µL for each mL of 3 % BSA) reagents were added to each well. The well plate was covered in aluminum foil and left in the dark for 1 hour. Samples were then rinsed five times with DPBS and stored in the dark 4 °C until imaging with confocal microscopy was attempted.

Table S1 Optimisation of the Cu(0) mediated solution-based polymerisation of all monomers in H_2O:MeCN (95:5) solvent solution at 0 °C for 15 min.

Sample	[M]:[I]:[Cu(I)Br]:[Me_6TREN]	Conv.(%)	$M_n^{(theo)}$	$M_n^{(GPC)}$	$Đ$
	N,N-dimethylacrylamide (DMA)				
1	[200]:[1]:[0.4]:[0.4]	46	9300	158000	3.15
2	[200]:[1]:[0.8]:[0.4]	94	18800	22600	1.12
3	[200]:[1]:[0.8]:[0.6]	89	17800	30800	1.55
	N-acryloylmorpholine (NAM)				
1	[200]:[1]:[0.8]:[0.4]	96	27300	25000	1.23
2	[200]:[1]:[0.8]:[0.6]	99	28100	22500	1.30
3	[200]:[1]:[1.6]:[1]	95	27000	24700	1.11
	N-(2-hydroxypropyl) acrylamide (HPA)				
1	[200]:[1]:[0.6]:[0.6]	15	4100	188000	1.73
2	[200]:[1]:[0.6]:[0.8]	80	21000	31800	1.28
3	[200]:[1]:[0.6]:[1]	45	11900	112000	2.03
4	[200]:[1]:[0.8]:[0.6]	82	21500	41200	1.31
5	[200]:[1]:[0.8]:[0.8]	64	16800	84100	1.92
6	[200]:[1]:[1]:[0.6]	87	22800	38500	1.31
7	[200]:[1]:[1.2]:[0.6]	47	12400	24700	1.27
8	[200]:[1]:[1.2]:[0.8]	74	19400	28700	1.52
9	[200]:[1]:[1.6]:[1]	95	24900	54900	1.26

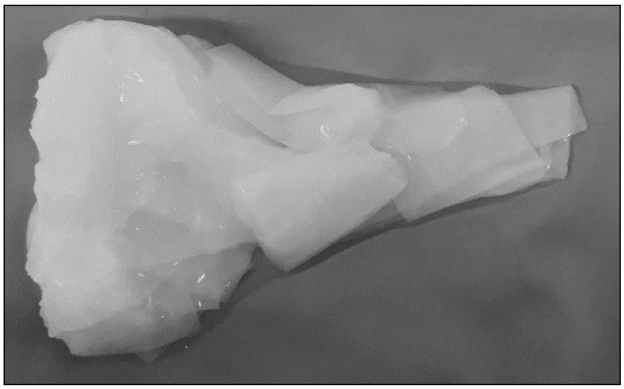


Figure S1. Electrospun ultrafine fibres after surface initiated Cu(0) mediated polymerisation without sacrificial initiator. Thickening or gelation throughout the solution and around the fibres indicates less than ideal controlled of polymerisation and hindered further characterisation.

Table S2 Analysis of the solution from the surface-initiated Cu(0) mediated polymerisation without sacrificial initiator. Conditions [DMA]:[Cu(I)Br]:[Me_6TREN]:[I]; [200]:[0.8]:[0.4]:[1] in H_2O:MeCN (95:5) solvent solution at 0 °C.

Sample	Conv.(%)	$M_n^{(theo)}$	$M_n^{(GPC)}$	$Đ$
Without sacrificial initiator	22	4600	109900	1.62

Initiator density

PLA density (g/m3)	1250000
Volume of a cubic packing (m3)	1.00E-23
Area (nm2)	1.00E+04
MW PLGA (g/mol)	75000
MW PLA-Br (g/mol)	10000
Avogrado's number	6.02E+23

Assuming a cubic packing of 1 cm each side. We calculated the mass of the cubic based on the PLA density

Mass (g) 1.25E-17

Then, taking into account the 1:1 (50%:50%) ratio of the blend we obtained the number of moles of each polymer based on the molecular weight of each polymer

Polymer	Mass(g)	# of moles	# of molecules
PLGA	6.25E-18	8.33E-23	5.02E+01
PLA-Br	6.25E-18	6.25E-22	3.76E+02

Finally, the number of molecules of PLA-Br were divided by the area of the cubic paking to obtain the molecules nm-2

Initiator density 3.76E-02 molecules.nm-2

We approximated this value to 0.04 molecules nm-2

Grafting density

Surface area of electrospun fibres (m2/g)	12.52
Amount of degrafted PDMA (g)	0.0035
Molecular weight of degrafted PDMA (g/mol)	66000
Sample size for BET (g)	0.1
Avogrado's number	6.02E+23

Based on surface area and samples size of BET, the area was obtained

Area(nm2) 1.252E+18

Then, based on the molecular weight of the degrafted PDMA and the amount of PDMA retrieved, the number of moles of degrafted PDMA were obatined

# of moles 5.30303E-08

Using the avogrado's number, the number of molecules were calculated

# of molecules 3.19E+16

Finally, the grafting density was obtained

Grafting density 2.55E-02 molecules.nm-2

We approximated this values to 0.03 molecules.nm-2

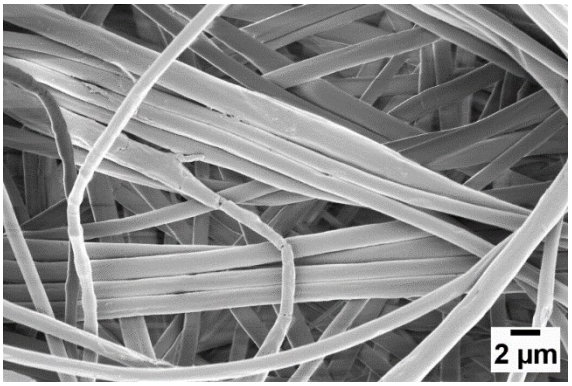


Figure S2 SEM images of the PHPA-grafted electrospun fibres when the monomer concentration was set at 0.7 M.

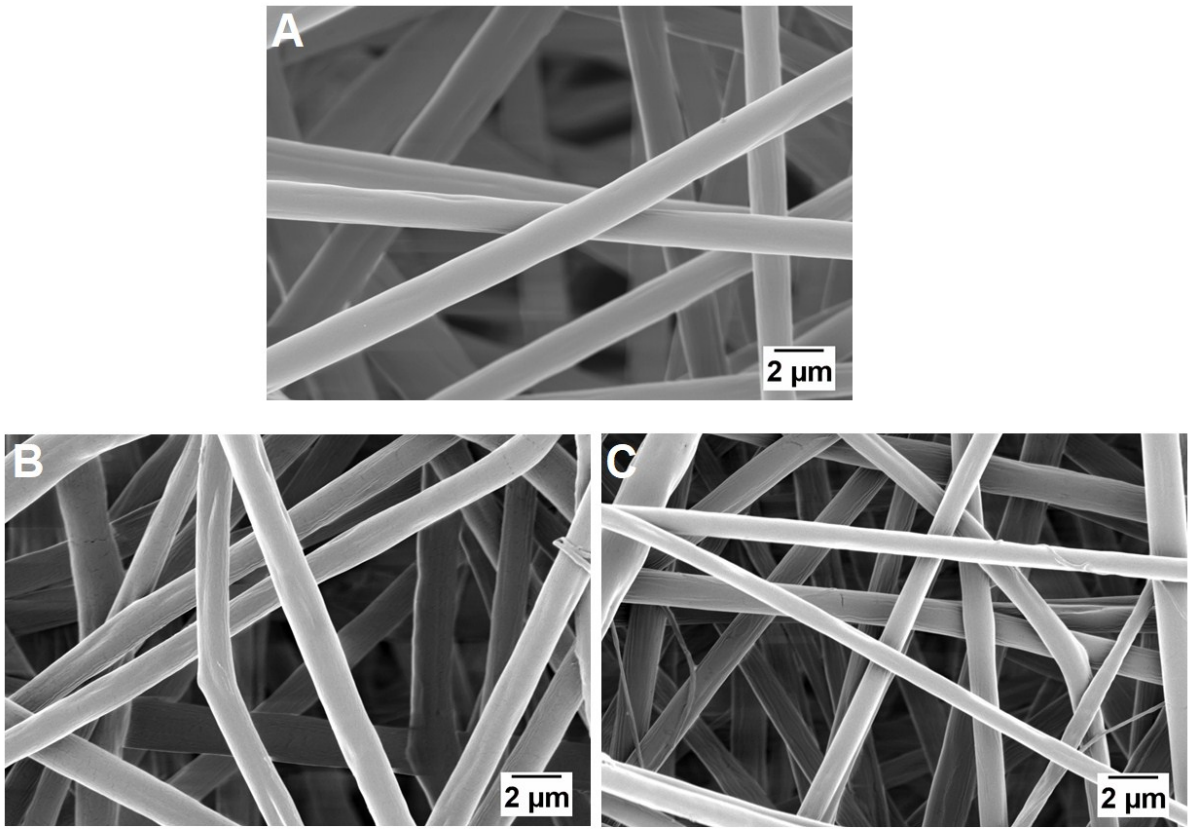


Figure S3 SEM images of the electrospun ultrafine fibres. A) Before surface modification. B) PHPA-grafted electrospun fibres when the monomer concentration was set at 0.5 M. C) PNAM-grafted electrospun fibres when the monomer concentration was set at 0.7 M.

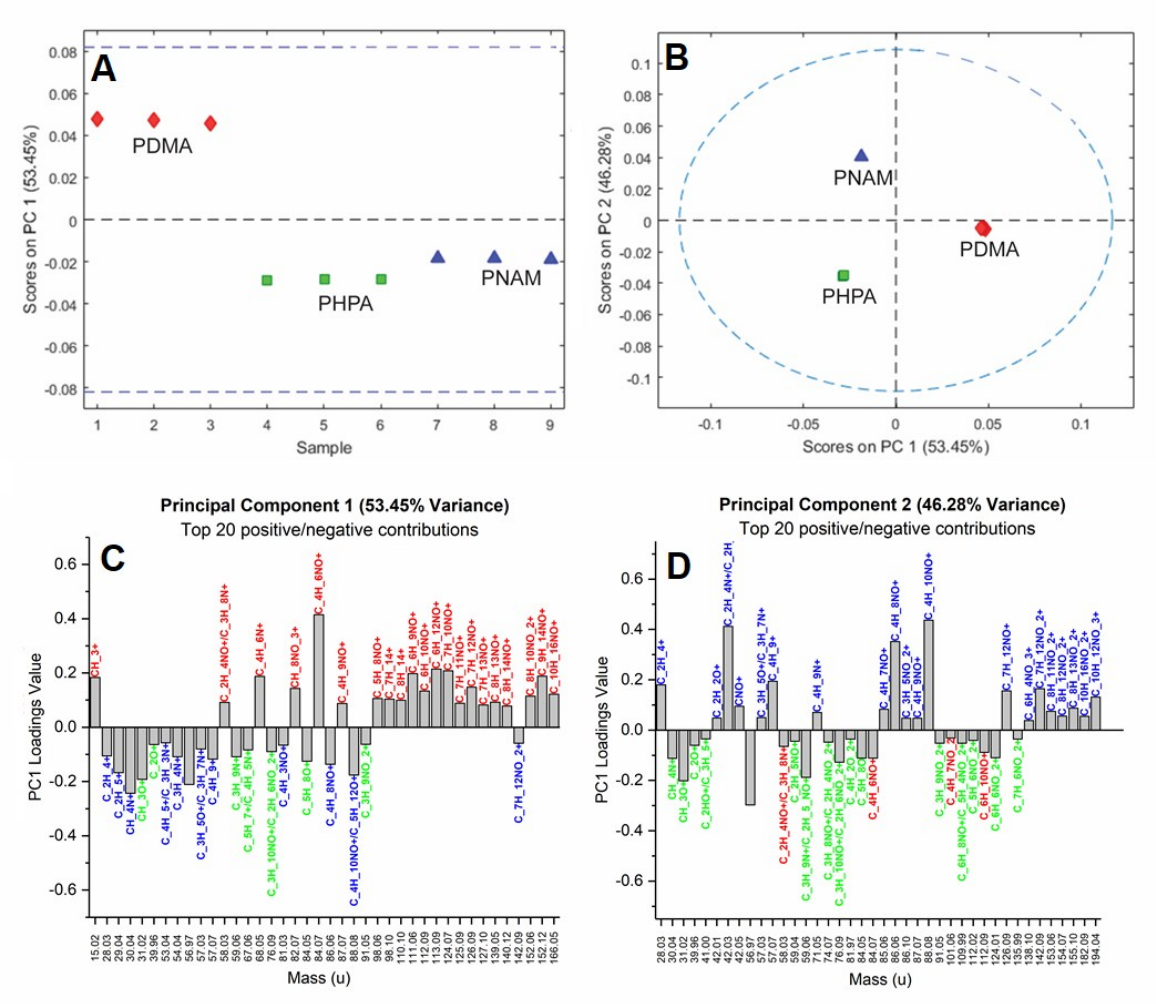


Figure S4 PCA analysis of PDMA/PHPA/PNAM-grafted (DP 200) electrospun samples. A) The scores plot of PC1, encompassing 53.45 % of the variation. B) Top 20 positive and negative loadings plot for PC1. C) The scores plot of PC1 *vs* PC2, where PC2 encompassing 46.28 % of the variation. D) Top 20 positive and negative loadings plot for PC2.

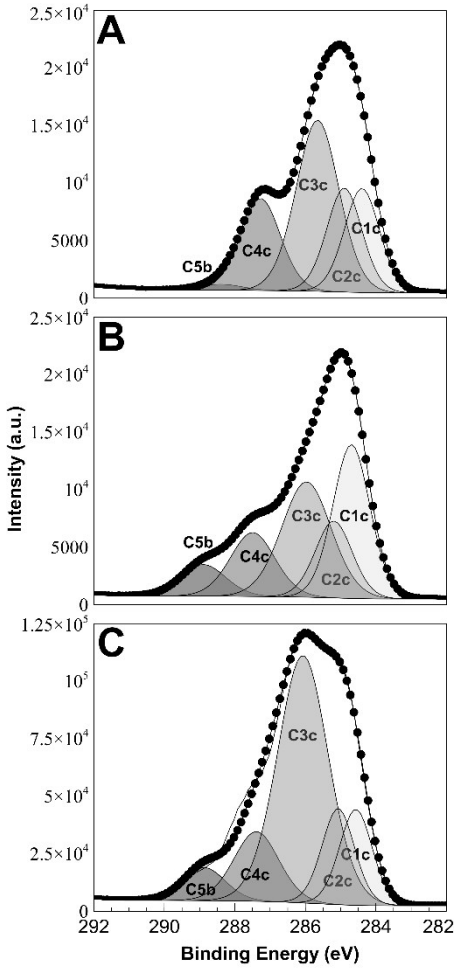


Figure S5 High resolution C 1s spectra resulting from XPS analysis of the surface of the fibres grafted using the following conditions. A) PDMA at DP 400. B) PHPA at DP 400. C) PNAM at DP 400.

Table S3 C 1s XPS components (Cx/C ratios) of samples from SI-Cu(0) mediated polymerisation using PDMA, PHPA and PNAM at DP 400. C_{1c} (C–C/C–H), C_2 assigned to secondary shifted (C–C–X), C_{3c} (C–O)/(C–N), C_{4c} (N–C=O) and C_{5b} (O=C–O–C).

Polymer	(DP 400)				
	C_{1c}/C	C_{2c}/C	C_{3c}/C	C_{4c}/C	C_{5b}/C
PDMA	0.26	0.26	0.53	0.26	0.02
PHPA	0.44	0.22	0.44	0.22	0.11
PNAM	0.22	0.23	0.67	0.19	0.08