Experimental

Chemicals

2,2′-(Ethylene dioxy) bis(ethylamine), acryloyl chloride, triethylamine, dichloromethane (DCM), poly(propylene glycol)bis(2-aminopropyl ether) (Mn~230), acrylamide(AAm), N,N'-dimethylacrylamide(DMAm), 2,2'-methlenebisacrylamide (MBA), tetraethylene glycol diacrylate (tEGDA), poly(ethylene glycol) diacrylate ($PEG_{12}DA$), ammonium persulfate (APS), *N,N,N',N'*-tetramethylethylenediamine (TEMED) and 3-(trimethoxysilyl)propyl methacrylate were obtained from Sigma-Aldrich. Tridecafluoro-1,1,2,2-tetrahydrooctyl-dimethylchlorosilane was from ABCR GmbH Co. KG. Acrylamide was purified by recrystallisation from methanol and dried overnight in a 40ºC vacuum oven. DMAm was distilled at 62ºC in vacuum (11bar) with 4-methoxyphenol as a stabilizer. Other chemicals were used as received.

Analysis facilities

A Microdrop MD-E-401 printer equipped with an AD-K-501 autodrop pipette (Inner nozzle diameter 70μm) and an MD-O-538-85 CCD camera was used with the printing chamber kept at 73±5% relative humidity using a V5100N Vicks ultrasonic humidifier controlled by a humidity controller TH-810H (Advanced Timer Technologies LTD).

Image capture and analyses of hydrogel microarray slides was carried out using a Bioanalyzer 4F/4S fluorescent scanner (Lavision BioTec) or a Nikon Eclipse 50i microscope with the Pathfinder software (IMSTAR S. A., Paris, France).

GPC-SEC analysis (Agilent 1100 series) was carried out on a PLgel 5μm Mixed-C column (300x7.5mm) from Polymer Laboratories. Data was analysed using the ChemStation software (Agilent technologies). Polymer analyses were run using 1-Methyl-2-pyrrolidone (NMP) as the eluent at a flow rate of 0.5ml/min at 55ºC. The polymer was detected by an RID detector. Polystyrenes (Polymer Laboratories) with a peak molecular weight range from 580 to 300,000 g/mol were used as standards.

Synthesis of 2,2'-(ethylenedioxy)bis(ethylamine) mono acylamide (EOA)[1,2]

Acryloyl chloride (10.9ml) was added dropwise to a mixture of 2,2′-(ethylene dioxy) bis(ethylamine) (20ml) and triethylamine (18.8ml) in DCM (100ml) at 0ºC. The mixture was stirred for 1 hour before filtration. The DCM was evaporated *in vacu*. The concentrated product was purified from diethyl ether. The product was analyzed by ^{1}H-NMR in $CDCl_3$ δ ppm: 6.45 (2H, CH_2-*trans*), 5.5 (2H, CH_2-*cis*), 5.8 (1H, CH), 3.8 (8H, CH_2), 3.2 (4H, CH_2) which are similar as reference 1.

Synthesis of poly(propylene glycol)bis(2-aminopropyl ether) monoacrylamide (PPGA)

It was prepared by using similar procedure as above. The product was a light yellow liquid. ^{1}H-NMR δ ppm: 6.5 (2H, CH_2-*trans*), 5.5 (2H, CH_2-*cis*), 5.85 (1H, CH), 3.7 (8H, CH_2), 2.4 (4H, CH) and 1.4 (12H CH_3).

Treatment of glass slides

Normal microscope glass slides (76×26mm) from Menzel GmbH Co. KG were cleaned with water and acetone. Silane treated glass slides were prepared by manually coating 30μL of silane (3-(trimethoxysilyl)propyl methacrylate or tridecafluoro-1,1,2,2-tetrahydrooctyl-dimethylchlorosilane) onto a glass slide[3] and placed in a sealed box for 20 h. The treated glass slides were cleaned with ethanol and acetone before use.

Polymerisation of linear acrylamide copolymers and their GPC analysis[4]

Linear acrylamide copolymers were polymerised in glass vials and on glass slides. The monomer was AAm/DMA(1.7:6.7). The concentration of the monomers was 11.2 % w/w in deionised water. The concentrations of APS were between 0.5 % w/w and 4.76 % w/w. The concentration of TEMED was 2.5vol% in monomer solution. For the polymerization in vials, vacuum degassed monomer solution (100μL) was added into the same volume of the APS solution (100μL), filled with N_2, sealed and shaking for mixing. The solution was left for 1h and froze in liquid nitrogen and dried in vacuum for at least 3 hours. The dried polymer was washed with acetone (twice) before dissolving in ethanol (1mL) and filtered. The filtered polymer solution was dried under a stream of N_2 and then in vacuum for 5h at 40ºC. The dried polymer was dissolved in 1-Methyl-2-pyrrolidinone (3mg/mL) for GPC analysis. For the polymerization by printing, the same solutions were used as above. 20 spots of APS solution were printed on a tridecafluoro-1,1,2,2-tetrahydrooctyl-dimethylchlorosilane treated glass slide with 20 drops/spot under 73±5% RH followed by monomer solution printed with 20 drops/spot on the APS spots. At least 200 spots were printed and the printed slide was left for 1 hour before being in ethanol. The sample was dried and analysed as above.

Preparation of hydrogel microarrays

Method one: 37 mixed monomer solutions were prepared in the ratios giving in Table S1. Monomer solution contained 8.75vol% TEMED except samples 2,3,8,9 and 23-29 (5% v/v TEMED). The concentration of APS was 1% w/w. The chamber of the printer was kept 73±5% relative humidity during printing. 20 spots of APS solution were printed on the acrylate silane treated glass slide with 25 drops/spot, followed by over-printing mixed monomers (Table S1) on the APS spots (12 drops/spot). This was repeated for printing all other monomer solutions. The printed hydrogel glass slides were left for another one hour before being washed with water and acetone (Figure S1).

Method two: 36 hydrogels were prepared by over-printing a series of single monomers (Table S2). 15 spots of APS solution (1% w/w) were printed in line on a the acrylate silane treated glass with 4 drops/spot, $PEG_{12}DA$ solution (0.41 M) was over-printed following DMA (2.5 M) or AAm(2.5 M) or EOA(1.25 M). Finally a solution of TEMED solution (7% w/w) 4 drops/spot was over-printed to initiate polymerization.

Table S1 Method 1: The molar ratios of monomers used for hydrogel printing

Sample	AAm	EOA	PPGA	DMA	MBA	TEGDA	PEG12DA
1				7.8			1
2	1.7			6.1			1
3	3.5			4.3			1
4		1.7		6.1			1
5		3.5		4.3			1
6			1.7	6.1			1
7			3.5	4.3			1
8	1.7			6.1		1	
9	3.5			4.3		1	
10		1.7		6.1		1	
11		3.5		4.3		1	
12			1.7	6.1		1	
13			3.5	4.3		1	
14	1.7			6.1	1		
15	3.5			4.3	1		
16		1.7		6.1	1		
17		3.5		4.3	1		
18			1.7	6.1	1		
19			3.5	4.3	1		
20	1.7			5.2			2
21	3.5			3.5			2
22		1.7		5.2			2
23		3.5		3.5			2
24			1.7	5.2			2
25			3.5	3.5			2
26	1.7			5.2		2	
27	3.5			3.5		2	
28		1.7		5.2		2	
29		3.5		3.5		2	
30			1.7	5.2		2	
31			3.5	3.5		2	
32	1.7			5.2	2		
33	3.5			3.5	2		
34		1.7		5.2	2		
35		3.5		3.5	2		
36			1.7	5.2	2		
37			3.5	3.5	2		

Table S2 Method 2: Drops of initiator (APS), monomers and catalyst (TEMED) over-printed on each spot

Sample	APS	PEG12DA	DMA	AAm	EOA	TEMED
1	4	4	6			4
2	4	4	5	1		4
3	4	4	4	2		4
4	4	4	3	3		4
5	4	4	2	4		4
6	4	4	1	5		4
7	4	4		6		4
8	4	4	5		1	4
9	4	4	4		2	4
10	4	4	3		3	4
11	4	4	2		4	4
12	4	4	1		5	4
13	4	4	2		6	4
14	4	4		5	1	4
15	4	4		4	2	4
16	4	4		3	3	4
17	4	4		2	4	4
18	4	4		1	5	4
19	4	4		2	6	4
20	4	3	5	1		4
21	4	3	4	2		4
22	4	3	3	3		4
23	4	3	2	4		4
24	4	3	1	5		4
25	4	3		6		4
26	4	3	5		1	4
27	4	3	4		2	4
28	4	3	3		3	4
29	4	3	2		4	4
30	4	3	1		5	4
31	4	3	2		6	4
32	4	3		5	1	4
33	4	3		4	2	4
34	4	3		3	3	4
35	4	3		2	4	4
36	4	3		1	5	4

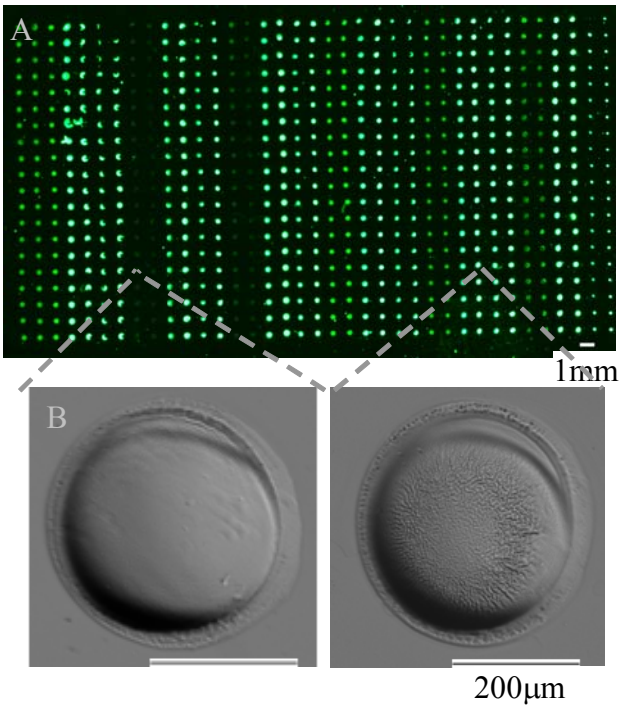

Figure S1 The hydrogel polymer microarray. (A) A fluorescent microscopic image of printed hydrogel microarray on a treated glass slide and (B) two typical hydrogel spots by phase contrast microscope.

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