

Supporting information

A dendronised polymer architecture breaks the conventional inverse relationship between porosity and mechanical properties of hydrogels

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1 Supplemental figures

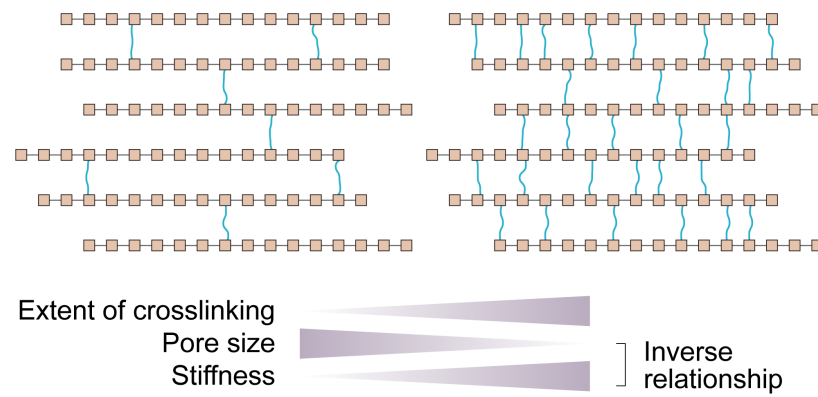


Figure S1. In a traditional hydrogel structure, the extent of crosslinking varies directly with viscoelastic properties and inversely with pore size.

2 Experimental

2.1 Materials

All chemicals were purchased from Sigma-Aldrich Australia unless otherwise stated.

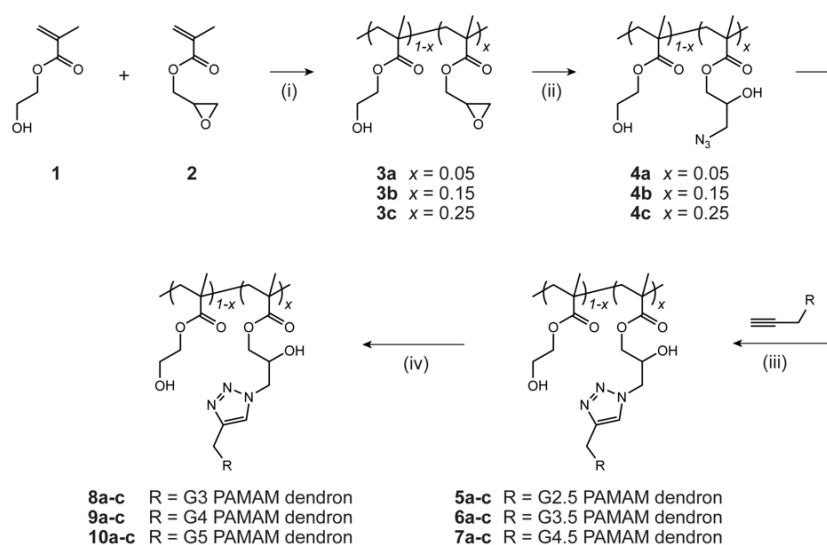
2.2 Synthesis

Dendronised polymers were synthesised as previously reported (Scheme S1).¹ Briefly, statistical copolymer backbones were prepared by ATRP of HEMA **1** and GMA **2** to give **3a-c**.

Table S1. Monomer feed ratios for ATRP reactions to synthesis linear backbone of various monomers ratio.

	Feed ratio	
	1 HEMA/MeOH	2 GMA/MeOH
3a	4.61 mL (38.0 mmol)	0.27 mL (2.0 mmol)
3b	4.12 mL (34.0 mmol)	0.82 mL (6.0 mmol)
3c	3.64 mL (30.0 mmol)	1.36 mL (10.0 mmol)

Azide functionalisation of **3** was achieved by treatment with NaN₃ (45 eq relative to epoxide) and NH₄Cl (41 eq relative to epoxide) in DMF at 60 °C for 72 h to give **4**. The solution was cooled and centrifuged to remove insoluble byproducts. The supernatant was purified by repetitive precipitation in ether and dried under vacuum. Propargyl-functionalised PAMAM dendrons of generations G2.5, G3.5 or G4.5 were prepared based on the methods of Lee *et al.*² and Lin *et al.*³ and attached to azido-functionalised poly(HEMA-*ran*-GMA) **4** via copper-catalysed alkyne–azide click reaction (adapted from Zhao *et al.*⁴). The dendron generations were completed to form G3, G4 and G5 by reaction with excess ethylenediamine before being purified by dialysis against deionised water and lyophilised.



Scheme S1. Synthesis of dendronised polymer. Statistical copolymer backbones were prepared by ATRP of HEMA and GMA before attachment of propargyl-functionalised PAMAM dendrons *via* copper-catalysed alkyne–azide click reaction. Reaction conditions: (i) CuBr, bpy, ME-Br, MeOH, 80 °C, 2 h. (ii) NaN₃, NH₄Cl, DMF, 60 °C, 72 h. (iii) CuBr, PMDETA, DMF, r.t., 72 h. (iv) ethylenediamine, MeOH, 0 °C–r.t., 7 d.

2.3 Propargyl poly(amido amine) dendron structures

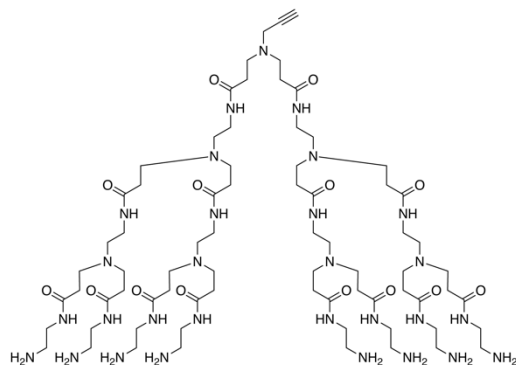


Figure S2. Structure of G3 propargyl PAMAM dendron.

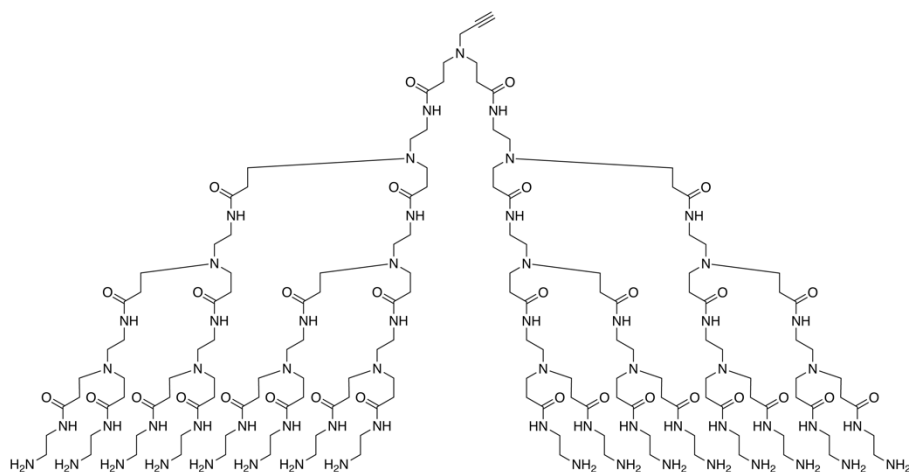


Figure S3. Structure of G4 propargyl PAMAM dendron.

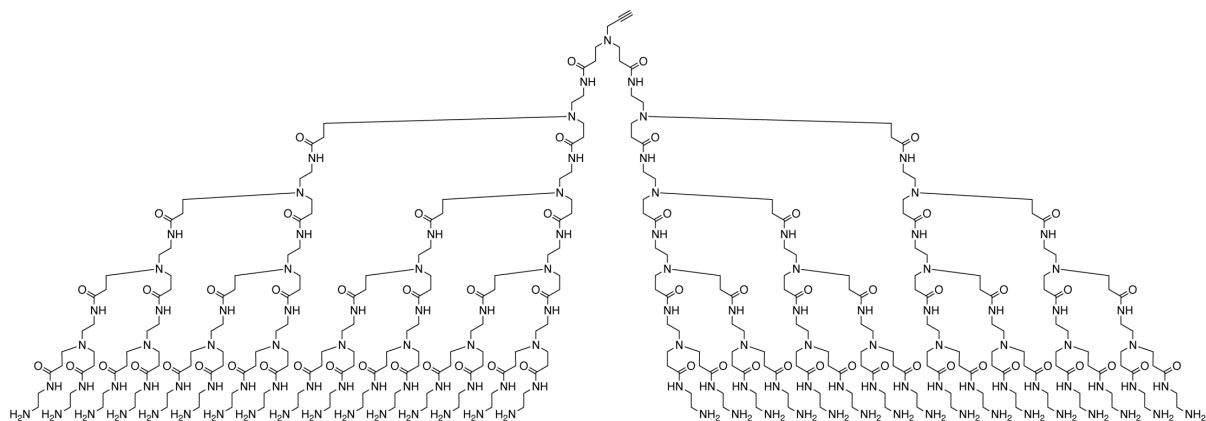
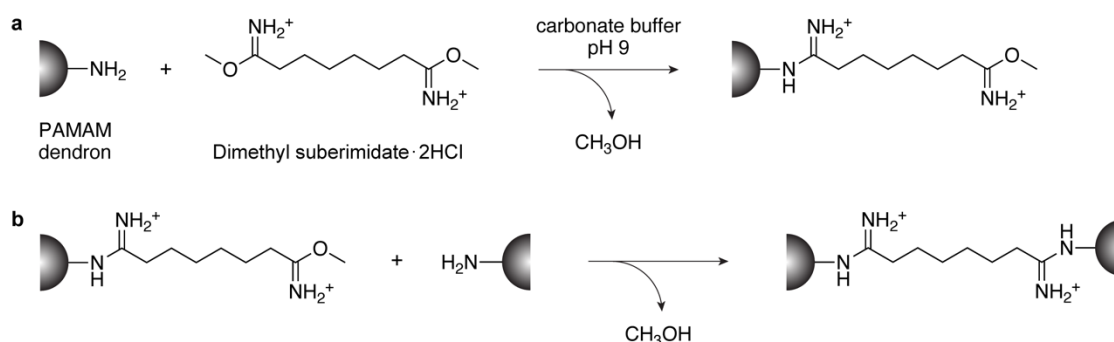


Figure S4. Structure of G5 propargyl PAMAM dendron.

2.4 Hydrogel formation

Polymers (**8a-c**, **9a-c**, **10a-c**) were reconstituted to 20% w/v in sodium carbonate buffer (pH 9). Dimethyl suberimidate (DMS) and dimethyl adipimidate (DMA) imidoester crosslinkers were dissolved at a concentration of 12% w/v in the same buffer. Immediately after the crosslinker solution was prepared, it was added to the polymer in a 1:1 ratio by volume. The resulting mixture was dropcast onto paraffin wax strips and clean glass coverslips were placed over the droplets. The coverslips were left in a humidified chamber for 30 min at 25 °C to allow the crosslinking reaction to proceed to completion. Gels were lifted off the wax strips and kept in phosphate buffered saline (PBS, pH 7.4) before being used in further experiments. The reaction of an imidoester crosslinker with PAMAM dendrons is exemplified in Scheme S3.



Scheme S2. Crosslinking of dendronised polymers to form gels. (a) In the first step, the imidoester dimethyl suberimidate (DMS) reacts with a peripheral primary amine of a polymer-anchored PAMAM dendron. (b) In the second step, further reaction with dendron primary amines leads to the formation of crosslinks between dendronised polymers.

2.5 Scanning electron microscopy

Hydrogel samples were prepared for SEM by the critical point drying method. Samples were washed with PBS pH 7.4 and immersed successively in increasing concentrations of ethanol (50%, 70%, 90%, and twice in 100%); after each step, samples were dehydrated in a specialised microwave (PELCO, BioWave 34700 Laboratory Microwave System). Critical point drying using CO₂ completed the dehydration process. Coverslips were mounted (ProSciTech, cat. No. G040) with carbon tabs and sputter-coated with 3 nm Pt. Images were recorded using a Zeiss 1555 VP-FESEM with in-lens detector at 4–5.6 mm working distance, 30 μm aperture, and accelerating voltage 5 kV. Pore sizes in the images were analysed with FIJI v. 2.0.0-rc-69/1.52p (ImageJ)⁵ by thresholding using identical settings for all samples.

2.6 AFM measurement of Young's modulus

Surface indentation was used to measure the compressive stiffness of hydrogels using an MFP-3D atomic force microscope (Asylum Research). Pyramid-shaped 200 μm chromium/gold-coated, pyrex-nitride cantilevers with triangular-shaped tips were used (PNP-TR, NanoWorld). Measurements were taken on samples immersed in PBS, probed with indentations with a 2 μm s⁻¹ approach velocity, 10 μm s⁻¹ retract velocity, and 2 nN trigger force. *Post-hoc* analysis using Wavemetrics Igor Pro v. 6.2 was carried out using the linear portion of the force curves.⁶ Six randomly chosen points across the hydrogel were indented in

triplicate and averaged.⁷ Samples used for stiffness measurements were fabricated regularly during the experimental timeline to ensure robustness and reproducibility of fabrication methodology.

2.7 AFM pore size

AFM images were acquired using a Keysight 5500 atomic force microscope. A sharp silicon tip (MikroMasch) with a spring constant of 40 N m^{-1} was cleaned by UV/ozone treatment for at least 10 min before use. Gels were made up to $10 \mu\text{L}$ per coverslip and transferred to AFM immediately after incubation at $25 \text{ }^\circ\text{C}$ for 30 min. Gels were submersed in PBS and imaging was carried out using tapping mode. The scan line and the sampling point for each scan line were both 256; the scan rate was 2 Hz. Scan sizes of $5 \times 5 \mu\text{m}$ and $10 \times 10 \mu\text{m}$ were used in this work. Analysis of the pore sizes were carried out using watershed thresholding using Gwyddion v2.55 as described previously.⁸ An example of the watershed thresholding is presented in Figure S1. Each sample was processed using identical settings to minimise bias.

2.8 Rheology

Measurements were performed on an Anton Paar MCR 302 rheometer using a 25 mm stainless steel parallel plate geometry configuration and analysed using RheoPlus v3.61 software. In a typical measurement, $550 \mu\text{L}$ hydrogel was cast onto one of the stainless steel plates (gap distance 1 mm), lowering the other plate to the measurement position, and allowing 30 min of incubation at $25 \text{ }^\circ\text{C}$ to ensure complete gelation. A Peltier temperature control hood and solvent trap was used to reduce evaporation and maintain a temperature of $25 \text{ }^\circ\text{C}$ for frequency and amplitude sweeps. Frequency sweeps were performed with a log ramp frequency f of 0.01–10 Hz and constant strain γ equal to 0.2%. Amplitude sweeps were performed at constant $f = 1 \text{ Hz}$ and log ramp strain $\gamma = 0.1$ –100%. Plots displayed are an average of at least three replicates and error bars denote two standard deviations from the log-averaged mean.

2.9 Statistical analyses

All comparisons were analysed by one-way ANOVA (Tukey multiple comparisons correction) using GraphPad Prism v. 8.0.2. Pore sizes for both SEM and AFM used a minimum sample size of $n = 1000$ replicates.

3 Supplemental data

3.1 Sample numbering key

Table S2. Summary of dendronised polymer samples prepared in this work.

ID	PAMAM dendron generation	Backbone GMA mol%	Name in manuscript
8a	3	5	5 mol% G3
8b	3	15	15 mol% G3
8c	3	25	25 mol% G3
9a	4	5	5 mol% G4
9b	4	15	15 mol% G4
9c	4	25	25 mol% G4
10a	5	5	5 mol% G5
10b	5	15	15 mol% G5
10c	5	25	25 mol% G5

3.2 Copolymer characterisation

Copolymers were characterised by GPC using PMMA standards. The mol% GMA incorporated in the polymers was determined by integrating one of the epoxide CH₂ protons in the ¹H NMR spectrum (CD₃OD) at *ca.* 2.9 ppm (H_f, 1H) relative to the methylene bridge at 2.0 ppm (H_h, 2H).

Table S3. Molecular weight and PDI measurements of copolymers were determined by GPC.

Polymer	M _w / kDa	Đ	mol% GMA by ¹ H NMR integration
3a	15.7	1.21	5.04%
3b	21.5	1.30	15.7%
3c	17.5	1.30	25.6%

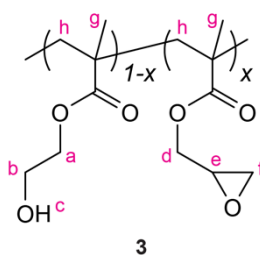


Figure S5. Poly(HEMA-*ran*-GMA) ¹H NMR (400 MHz, CD₃OD, δ_H/ppm): 4.38 (br s, H_d), 4.05 (br s, H_a), 3.78 (br s, H_{b,d}), 3.30 (br s, H_e), 2.88 and 2.71 (br s, H_f), 2.00 (br d, H_h), 1.11 and 0.95 (br s, H_g).

3.3 Dendronised polymer characterisation

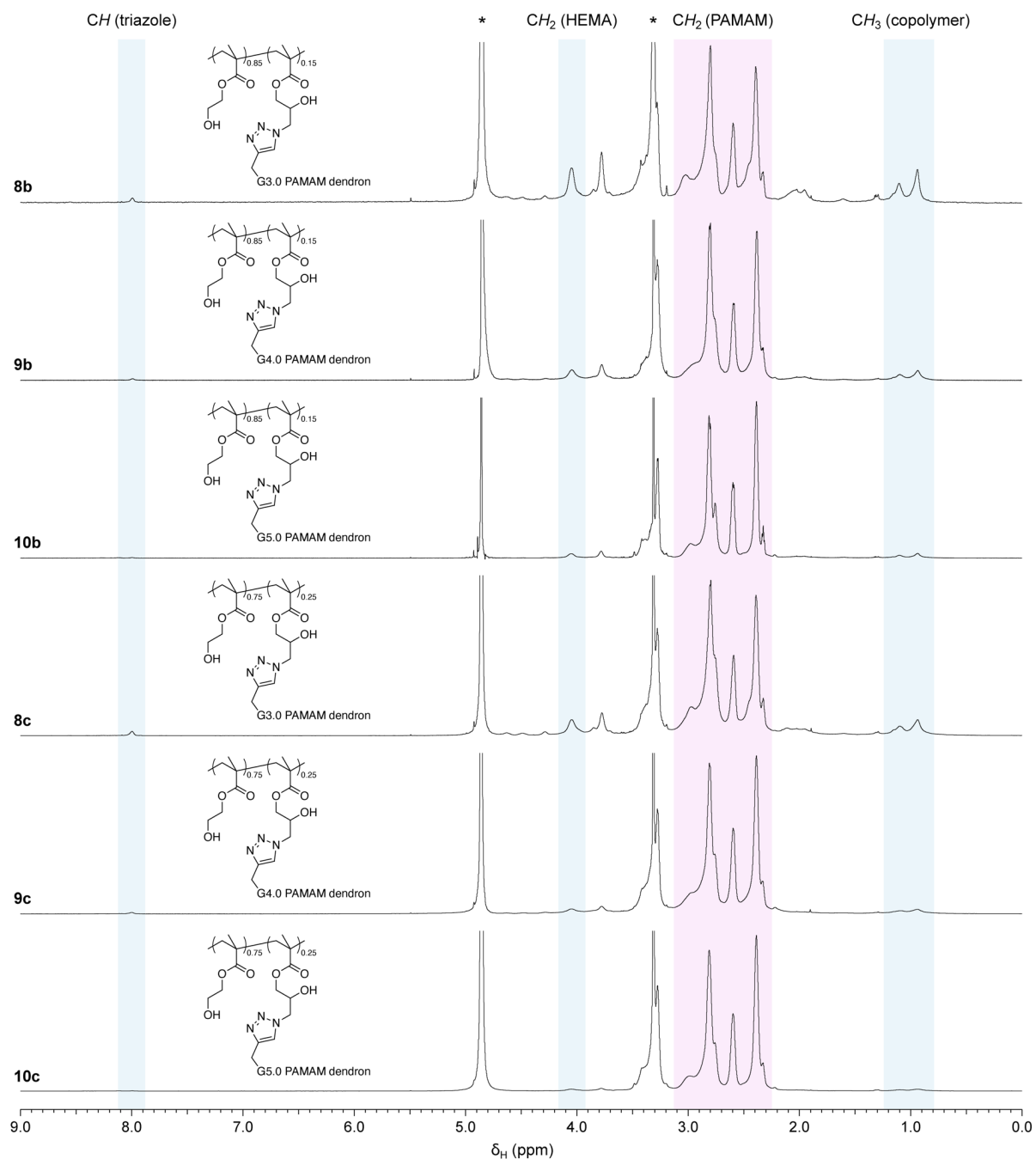


Figure S6. ^1H NMR spectra (600 MHz, CD_3OD) of 15 mol% and 25 mol% G3, G4, G5 dendronised polymers **8b**, **9b**, **10b**, **8c**, **9c**, **10c**. Integration of dendrons (pink) was performed by normalising to the copolymer backbone (blue). Asterisks indicate water (4.87 ppm) and methanol (3.31 ppm) peaks respectively.

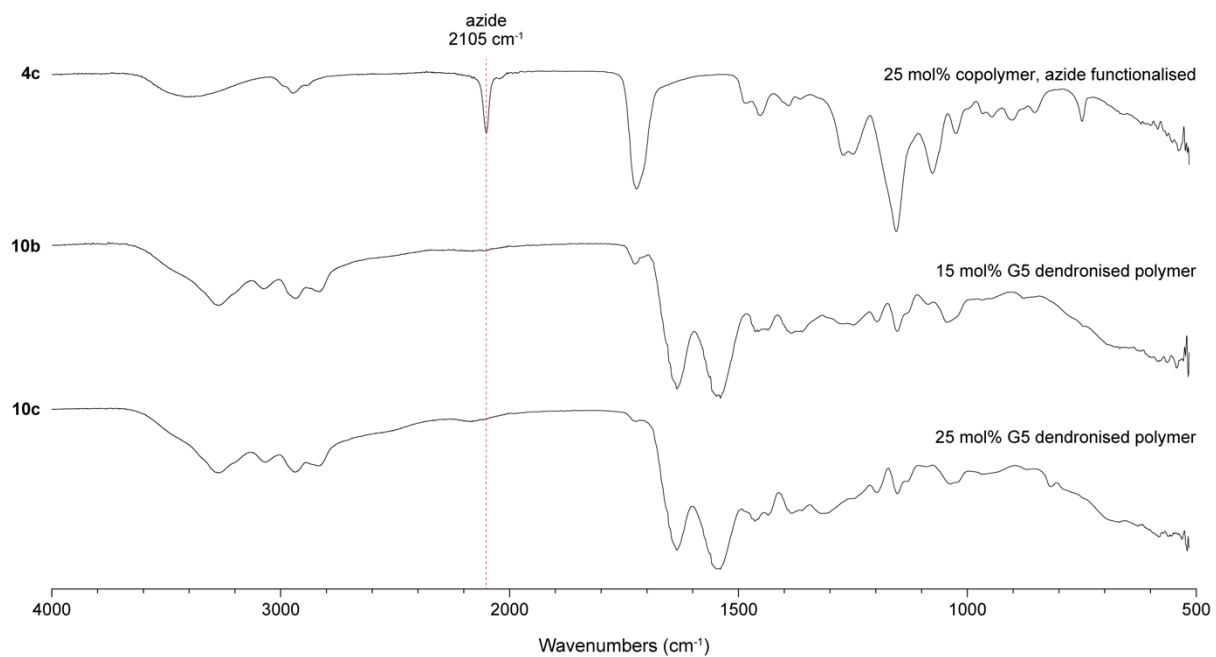


Figure S7. FT-IR spectra of 25 mol% azide functionalised copolymer **4c** and G4 and G5 dendronised polymers **10b**, **10c**. The disappearance of the azide peak at 2105 cm⁻¹ confirms that the click reaction of dendrons to the copolymer backbone has proceeded to completion.

3.4 AFM images

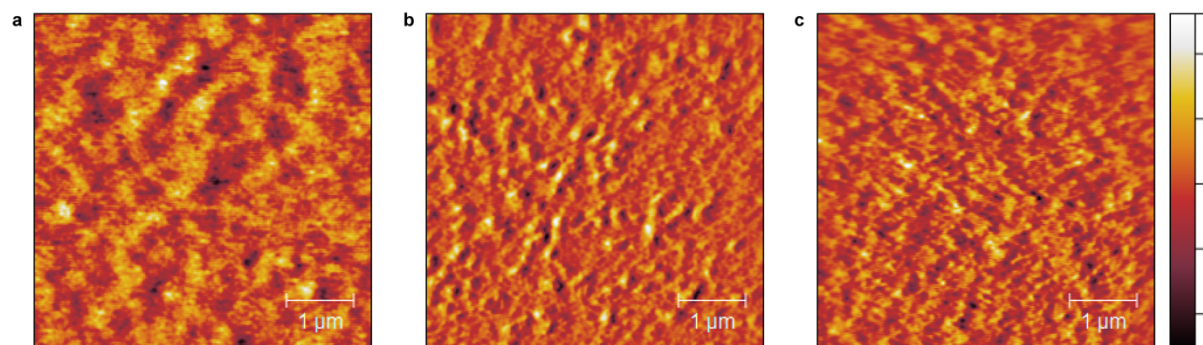


Figure S8. Representative AFM image used for surface topology determination of DMS-crosslinked gels prepared from 15 mol% polymers (8b, 9b, 10b). 2D images of (a) 15 mol% G3, (b) 15 mol% G4, (c) 15 mol% G5 hydrogels.

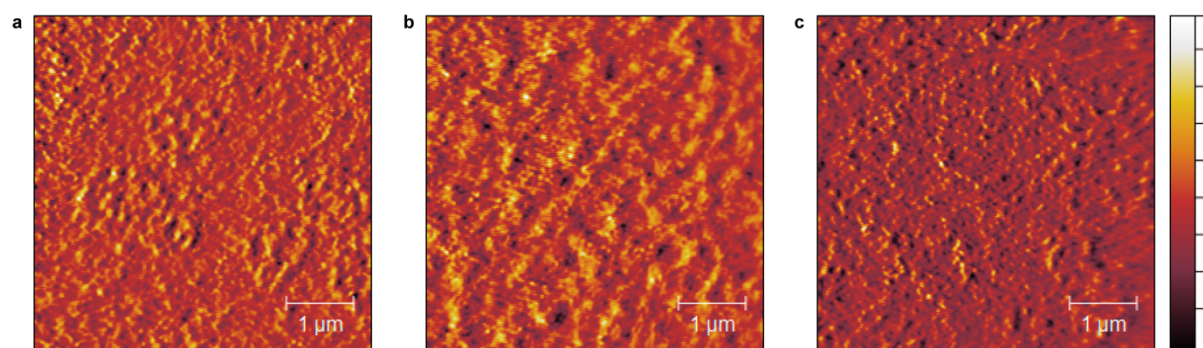


Figure S9. Representative AFM image used for surface topology determination of DMS-crosslinked gels prepared from 25 mol% polymers (8c, 9c, 10c). 2D images of (a) 25 mol% G3, (b) 25 mol% G4, (c) 25 mol% G5 hydrogels.

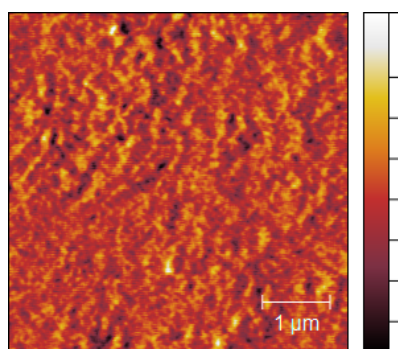


Figure S10. Representative AFM image used for surface topology determination of DMA-crosslinked gel prepared from 25 mol% G5 polymer (10c).

4 References

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