Electronic Supplementary Information

Transiently malleable multi-healable hydrogel nanocomposites based on responsive boronic acid copolymers

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Methods

^{1}H nuclear magnetic resonance (NMR) spectra were acquired on a 400 MHz Bruker spectrometer and analysed using MestReNova software. The molecular weight of the polymer was determined using size-exclusion chromatography (SEC) equipped with a refractive index detector (Viscotek). The system was equipped with an on-line degasser and the chromatograms were recorded at 30 ºC. The eluent (THF) was used at a flow rate of 1 mL min^{-1}. Cloud point temperatures and fluorescence intensities were measured using a SpectraMax M2e spectrophotometer (Molecular Devices). Fourier transform-infrared - attenuated total reflectance (FTIR-ATR) spectra were recorded using a Spectrum 100 IR spectrometer (PerkinElmer) at room temperature over the range 4000–500 cm^{-1} with a resolution of 4 cm^{-1} and accumulation of 16 scans. The microstructure of the hydrogels was examined using scanning electron microscopy (SEM). Prior to examination, the samples were freeze-dried, cross-sectioned and sputter-coated with gold. Analysis was conducted on a FEI Quanta 200 instrument. Transmission electron microscopy (TEM) was used to analyse the size distribution of the gold nanoparticles (AuNPs). AuNPs were deposited on a TEM copper grid by direct droplet casting from the stock solution. The samples were left to dry at room temperature before examination with a Philips CM 120 BioTwin microscope. Dynamic light scattering (DLS) and zeta potential measurements were recorded on a Zetasizer NanoZS (Malvern Instruments) at room temperature, and at a backward scattering angle of 173º. The zeta potential, average hydrodynamic particle diameter (z-average) and polydispersity index (PDI) of the particles were determined with the Zetasizer software. UV/Vis absorption spectra of the AuNPs (in aqueous solution) and hydrogel nanocomposites (freshly prepared in a quartz cuvette) were acquired using a Cary 100 spectrophotometer (Agilent Technologies). Rheological characterisation was performed using a Bohlin Gemini HR nano rheometer (Malvern Instruments) fitted with a parallel-plate geometry. Thermogravimetric analysis (TGA) was performed using a Discovery TGA analyser (TA Instruments). The samples were placed in aluminium (or platinum) pans and sealed with a pin-holed lid. After stabilisation, the samples were heated from 30 to 500/700 ºC at a ramp rate of 10 ºC min^{-1} under a flow of nitrogen (40 mL min^{-1}). The data was analysed using the TA Universal Analysis software. An EVOS® FL Cell Imaging System (Life Technologies) was used for microscopy studies.

Cloud point measurements

The lower critical solution temperature (LCST) turbidity assay was performed by measuring the absorbance of the polymer solutions in respect to temperature. P1 solutions (5 mg mL^{-1}) were prepared in PBS (pH 7.4, 0.01 M) and DMEM (pH 7.4). The LCST was considered to be the initial onset of a sharp increase in absorbance at 500 nm.

Alizarin fluorescence assay

A stock solution of alizarin red S was prepared in glycine buffer (0.1 mM, pH 9) and *ca.* 5 mg of P1 was dissolved in 1 mL (immediate change in colour from pink to orange was observed upon addition of the polymer). The fluorescence intensity was measured at 580 nm in a 96-well plate (the excitation wavelength was at 465 nm), and recorded at temperatures below and above the LCST (at least 5 minutes were allowed for stability before recording each spectrum). Kinetics of the ARS dissociation from the APBA was recorded at 45 ºC (mean ± logSD).

Glucose-triggered degradation of the gels

Freshly prepared gels with initial weight W_0 were placed in a vial and immersed in a 2.5% (w/v) glucose solution. The samples were incubated at 25 ºC (statically), removed at specific time intervals, gently wiped and finally weighted (W_f), to determine the weight loss according to the formula: $$\%w = \frac{W_f}{W_0} \times 100$$.

Light irradiation study

AuNP-gel composites response test to light was carried out using a LED illuminator (DiCon, 1050 mW) at 521 nm. The samples were placed in close proximity to the light source (*ca.* 1 cm) and left until visual phase transition was observed. The temperature changes of the gels were monitored using a digital thermometer (Digitron TM-22).

Calculation of mesh size

An approximate value of the initial mesh size of the hydrogels was calculated, as described in the literature,[1, 2] using the G' value to calculate the molecular weight between crosslinks (we considered PVA as the most influent constituent of the gels to carry out the calculations).
The gels were incubated in water, dried and weighted to calculate the swelling ratio (q_F) and volume fraction polymer (v_2):

(1) $$q_F = \frac{mass\ of\ gel\ after\ preparation}{mass\ of\ gel\ after\ freeze-drying},$$

(2) $$v_2 = \left(1 + (q_F - 1) \times \frac{\rho_P}{\rho_W}\right)^{-1},$$

where ρ_P is the density of PVA (1.26 g cm^{-3}) and ρ_w is the density of water (1.0 g cm^{-3}).
The crosslink density (η) was calculated from the Flory-Rehner equation:

(3) $$\eta = \frac{-\left(\ln(1 - v_2) + v_2 + \chi_1 v_2^2\right)}{V_1\left(v_2^{1/3} - 0.5 v_2\right)},$$

where χ_1 is the Flory-Huggins interaction parameter (≈0.5), V_1 is the molar volume of the solvent (water, 18 cm^3 mol^{-1}) and η represents the number of active network chain segments per unit of volume (mol cm^{-3}).
The molecular weight between crosslinks was calculated from the measured G' values:

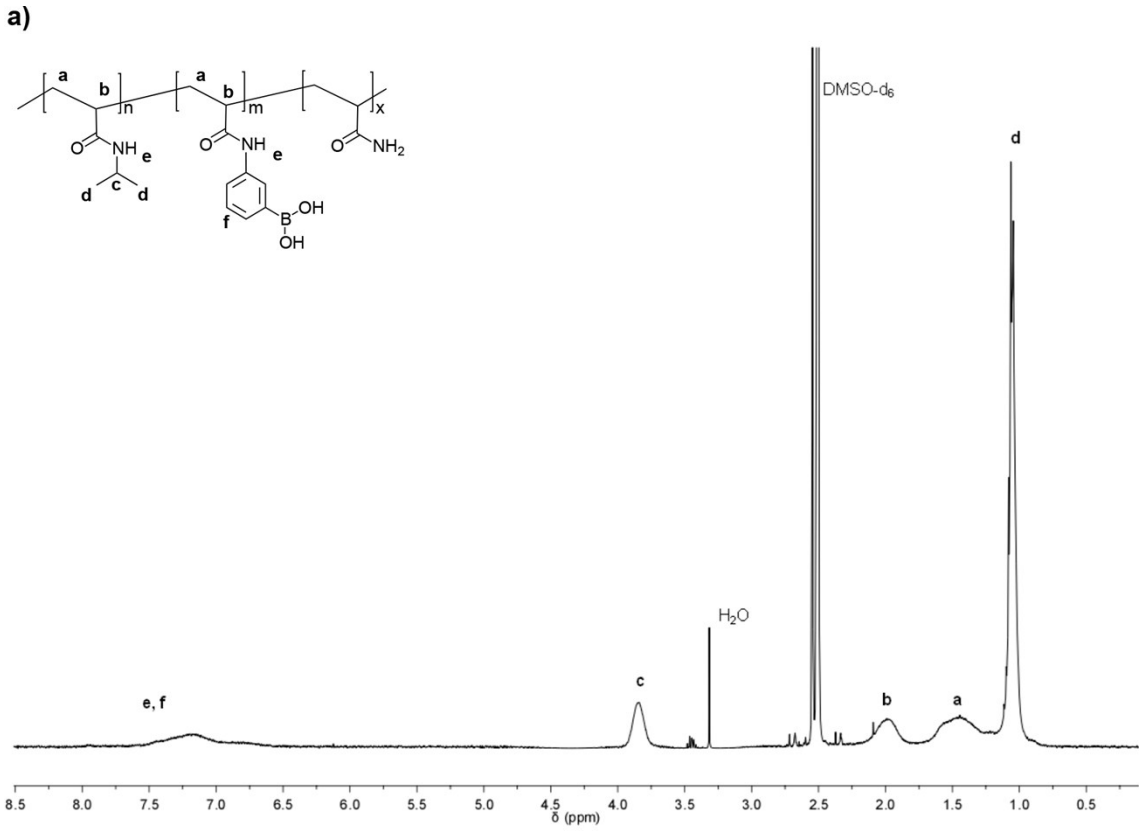
(4) $$M_c = \frac{c_P RT}{G'},$$

where c_p is the concentration of PVA in solution (5 or 7.5 wt%), R is the gas constant (8.314 m^3 Pa mol^{-1} K^{-1}) and T is the temperature (K).
Finally, the mesh size (ξ) was calculated from Equation (5) as follows:

(5) $$\xi = v_2^{-1/3} \times l \times \left(\frac{2M_c}{M_r}\right)^{1/2} \times C_n^{1/2},$$

where l is the carbon–carbon bond length of monomer unit (1.54 Å), M_r is the molecular weight of the repeating unit (44 g mol^{-1}) and C_n is the characteristic ratio (8.9 for PVA).

Supplementary Figures



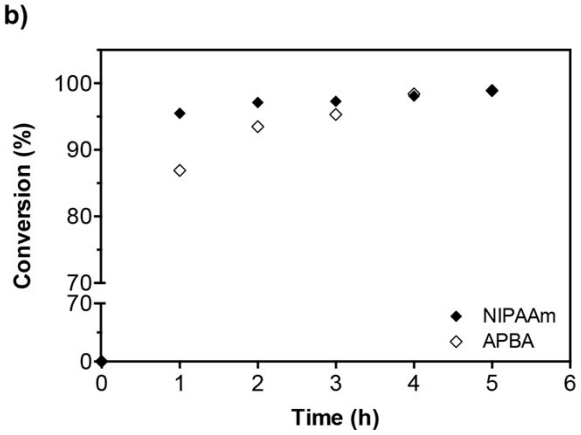


Fig. S1 (a) ^{1}H NMR spectrum (DMSO-d_6) of P1 and assignment of the related peaks, and (b) monomers' conversion with time of the model copolymer poly(NIPAAm-APBA).

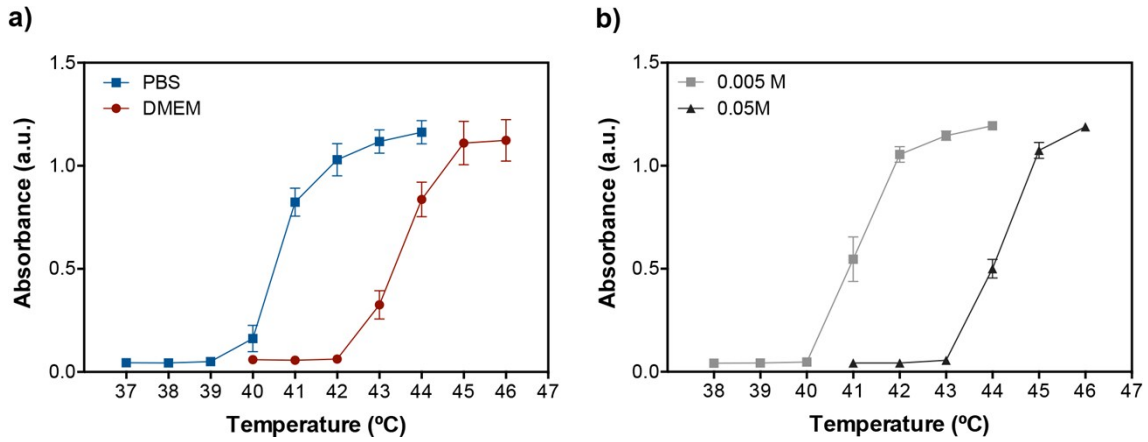


Fig. S2 (a) LCST profile of copolymer P1 in PBS (pH 7.4) and DMEM. (b) The effect of glucose addition on the LCST of P1 in PBS (mean ± SD from triplicates).

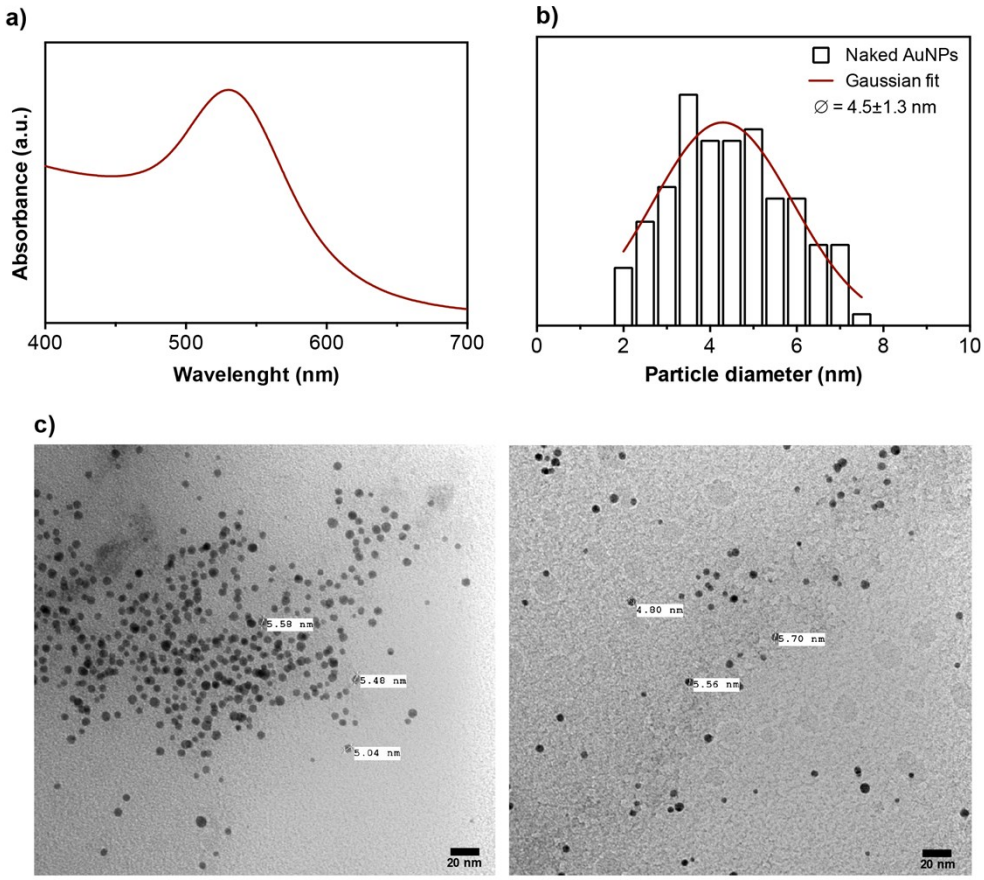


Fig. S3 (a) UV/Vis spectrum of the as prepared AuNPs in aqueous solution. (b) Size distribution histogram derived by measuring the diameter of 100 nanoparticles. (c) TEM microphotographs of PVP-coated AuNPs (scale bars = 20 nm).

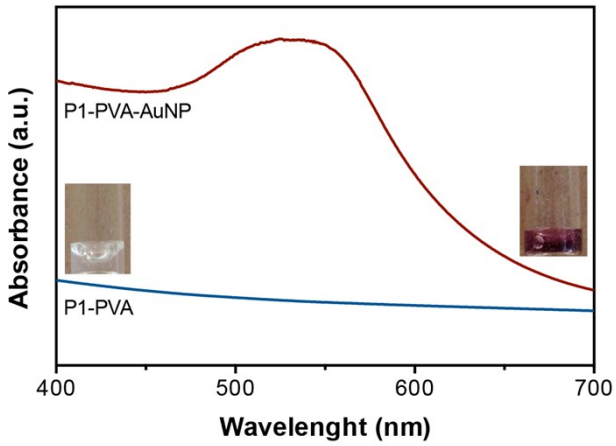


Fig. S4 UV/Vis spectra of P1-PVA and P1-PVA-AuNP hydrogels. The latter exhibits a characteristic absorbance peak around 525 nm owing to the incorporation of AuNPs within the matrix.

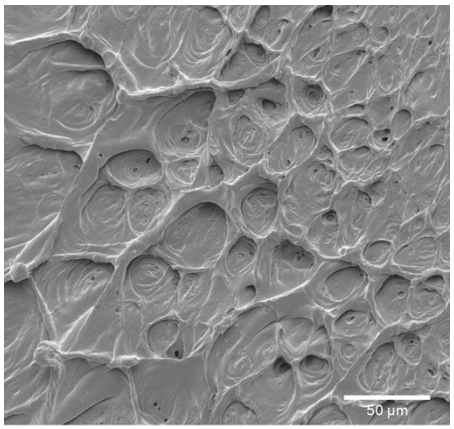
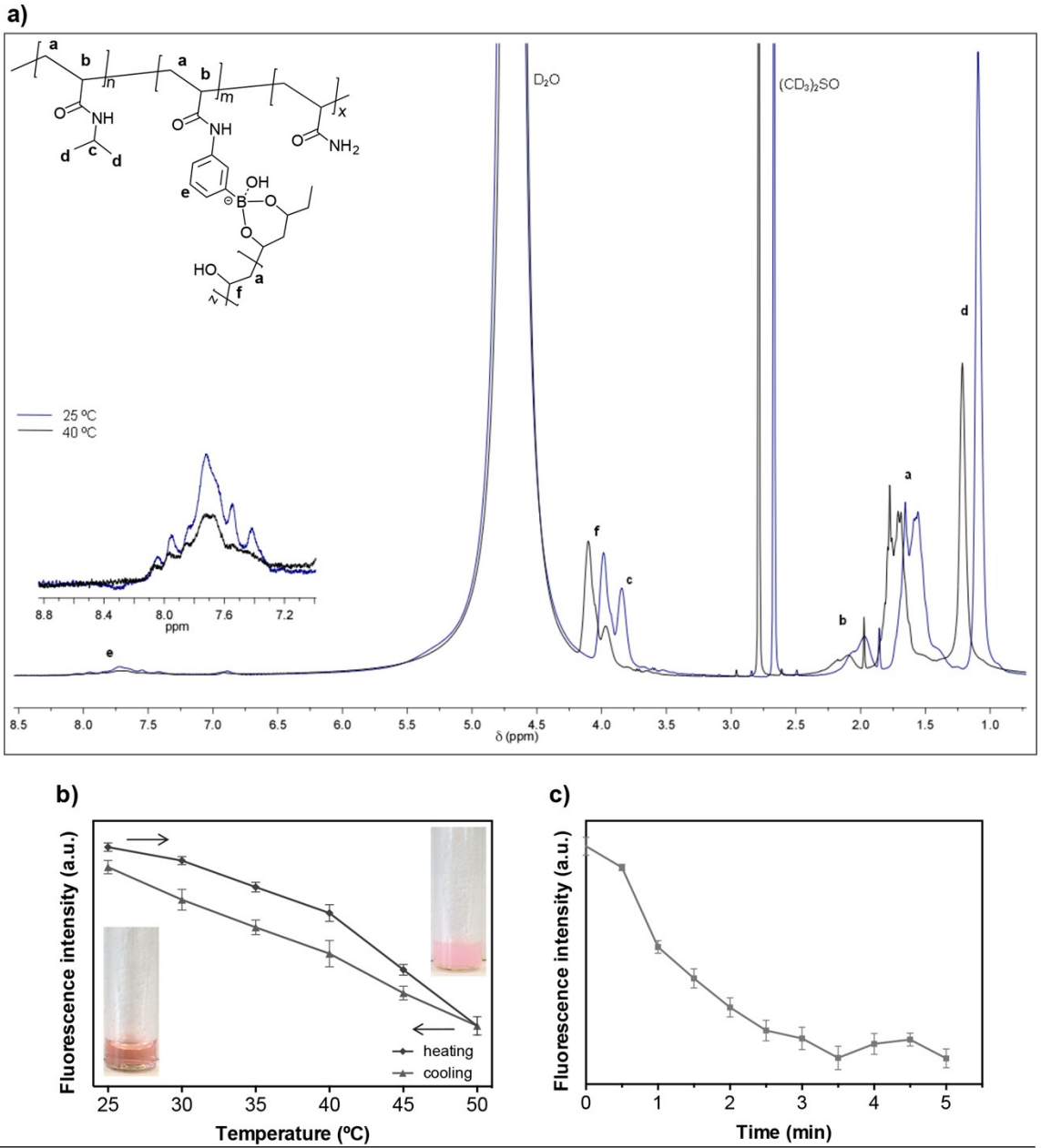


Fig. S5 Typical SEM image of freeze-dried P1-PVA gel.

Table S1 Fabrication of hydrogels (experimental conditions).

PVA (wt%)	P1 (wt%)		
	2.5	3.7	5
1.3	-	-	+/-
2.5	+/-	+	+



3.7	+/-	+	+
5	+	+	+

Fig. S6 (a) ^{1}H NMR spectra of P1-PVA at 25 and 40 ºC in D_2O (with a small trace of DMSO-d_6 to facilitate better solubility, 400 MHz and 400 scans). P1-ARS interaction in glycine buffer (pH 9) showing the variation of fluorescence intensity with (b) temperature (heating-cooling cycle; inlets are digital photographs of P1-ARS solutions at 25 and 45 ºC), and (c) time at 45 ºC (Ex. 465 nm, mean ± SD from triplicates).

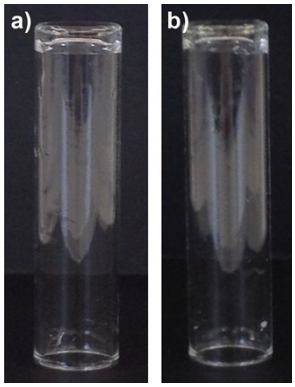


Fig. S7 Digital photographs of control P1-PVA gel (a) before and (b) after irradiation with light at 521 nm.

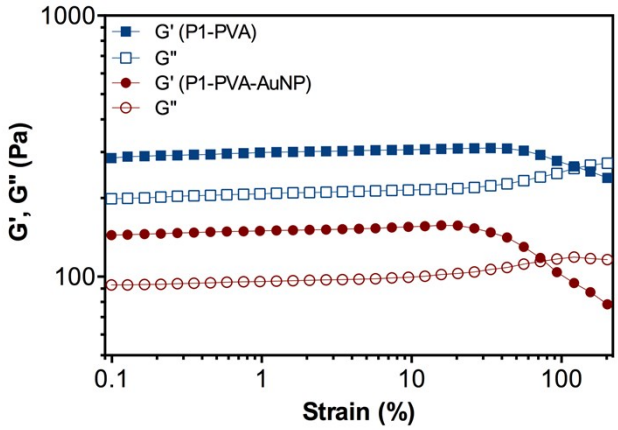


Fig. S8 (a) Strain sweep measurements of P1-PVA and P1-PVA-AuNP at fixed frequency (1 Hz, 25 ºC).

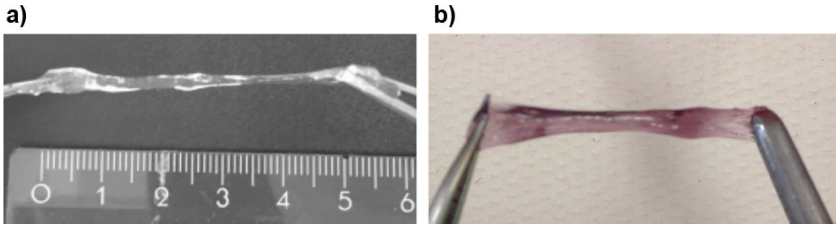


Fig. S9 Gel stretching of (a) P1-PVA and (b) P1-PVA-AuNP into string-like dimensions at 25 ºC.

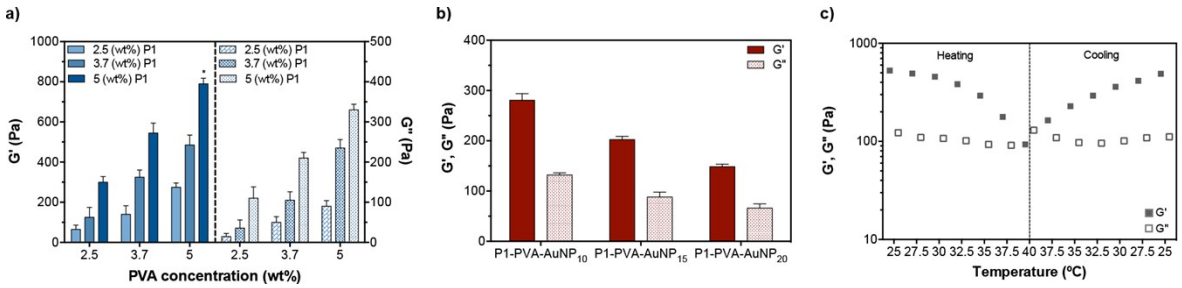


Fig. S10 G' and G'' moduli of the hydrogels in relation to the concentration of (a) P1/PVA and (b) AuNPs (time sweep at 25 ºC, mean ± SD from triplicates, $^{*}p<0.05$). (c) Thermoreversibility of P1-PVA upon heating and cooling.

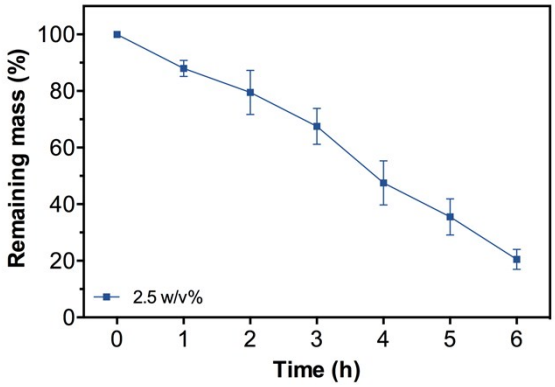


Fig. S11 Degradation behaviour of P1-PVA hydrogel in a 2.5% (w/v) glucose concentrated solution at 25 ºC (pH 7.4, mean ± SD).

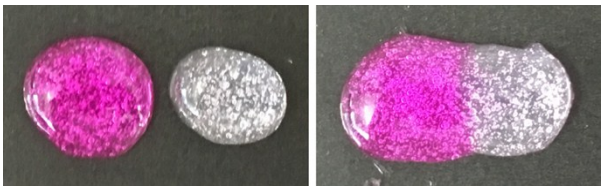


Fig. S12 Photographs of the self-healing behaviour of P1-PVA gel after cut in two pieces (with and without pink dye for better contrast) and mechanically rejoined at 25 ºC.

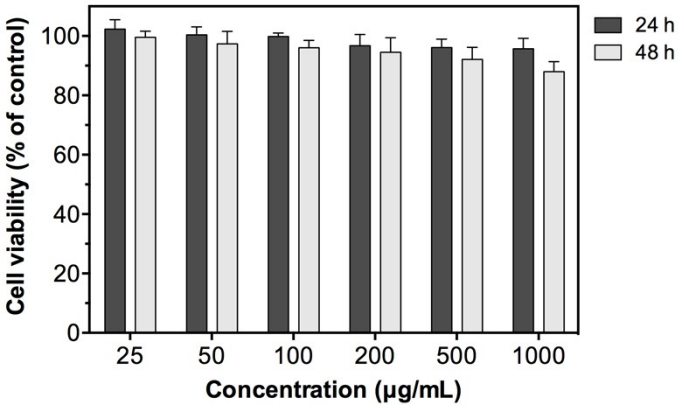


Fig. S13 Effect of P1 concentration on the cell viability (resazurin assay). The data are shown as a percentage of the cell viability with respect to the control corresponding to untreated cells (mean ± SD obtained from triplicates).

Movie S1 Thermally-induced gel rejoining of two hydrogel samples.
Movie S2 Self-healing of a cut hydrogel sample driven by a thermal stimulus.
Movie S3 Optically-induced gel-sol-gel transition of a sample with green light (speed 2x).
Movie S4 Mechanical rejoining of an initially cut bulk hydrogel from two different pieces at room temperature (speed 2x).

References

1 T. Canal and N. A. Peppas, *J. Biomed. Mater. Res.*, 1989, **23**, 1183-1193.
2 B.-H. Lee, B. Li and S. A. Guelcher, *Acta Biomater.*, 2012, **8**, 1693-1702.