Support information for:

Hierarchical anisotropy inheritance in interpenetrating polymer network hydrogels via multi-stage soft templating

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Contents

Supplementary method 1.

1-1. Preparation of Anisotropic and Isotropic Gelatin SN Hydrogels

Anisotropic and isotropic gelatin single-network (SN) hydrogels were prepared according to a previously reported method.[17] Gelatin derived from bovine skin (Type B) was dispersed in ultrapure water and dissolved by heating at 37°C to obtain a gelatin aqueous solution with a concentration of 10 wt%. The obtained gelatin solution was introduced into a reaction cell consisting of two glass substrates (10 cm × 10 cm) separated by a 2.0 mm silicone spacer. For orientation control, a polypropylene (PP) sheet–coated glass substrate was used as the template. An uncoated glass substrate was used as a control to prepare isotropic hydrogels. The reaction cell was cooled to 4°C to induce gelation. After gelation, the sheet-shaped hydrogel was removed from the cell and cut into disk-shaped samples with a diameter of 10.00 mm and a thickness of 2.00 mm. The samples were immersed in cold water to reach equilibrium swelling and stored in cold water until further measurements.

1-2. Preparation of PAAc SN Hydrogels

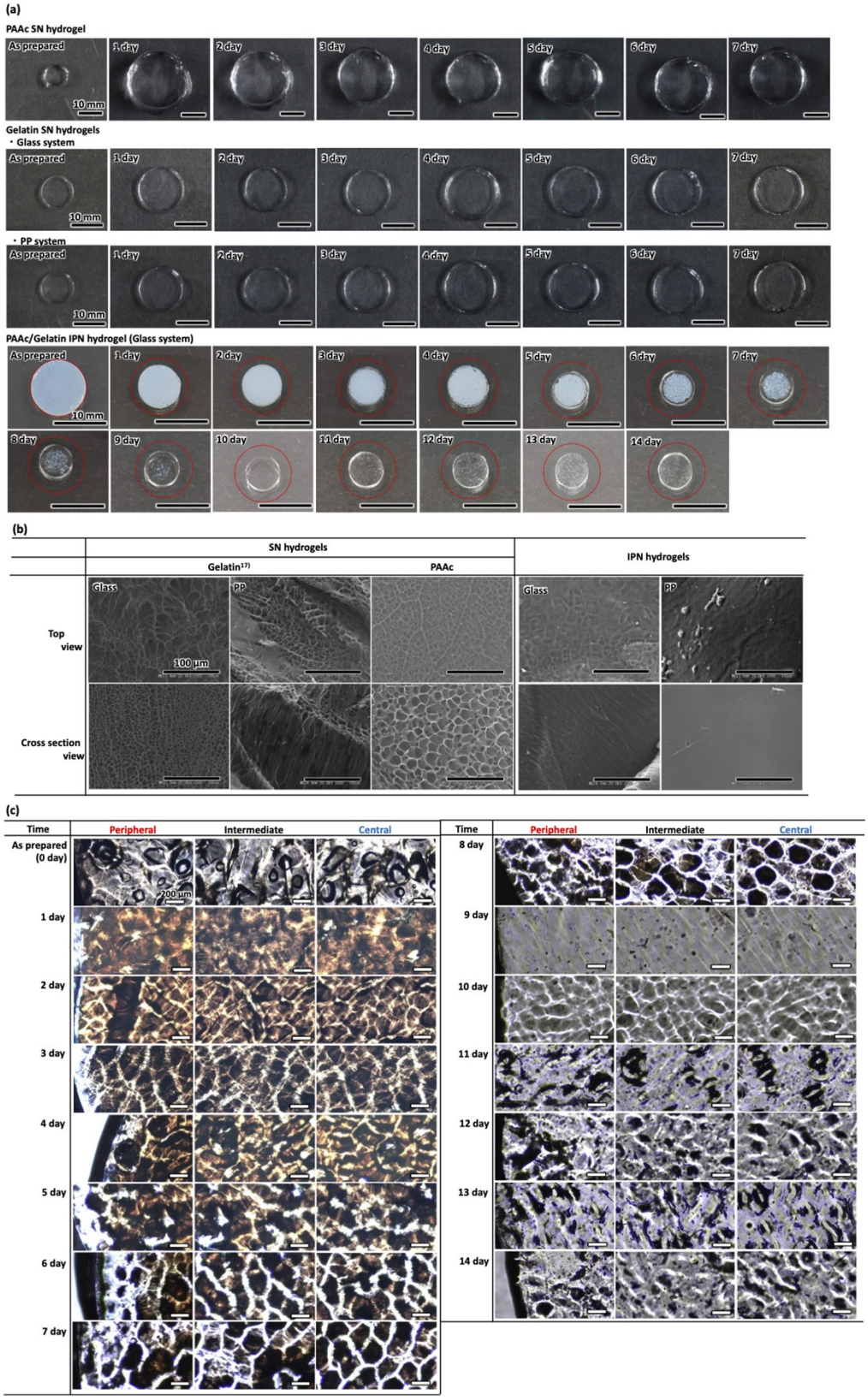
PAAc SN hydrogels were prepared by redox polymerization. AAc aqueous solutions with concentrations of 0.50, 0.75, 0.90, 1.0, and 2.0 M were first prepared. Sodium hydroxide (NaOH) was added as a neutralizing agent in an equimolar amount relative to AAc, together with N,N'-methylenebisacrylamide (MBAA, 3 mol% relative to AAc) as a crosslinker and N,N,N',N'-tetramethylethylenediamine (TEMED) as an accelerator. After complete dissolution, the solution was cooled in an ice bath, and ammonium persulfate (APS) was added as an initiator (3 mol% relative to AAc). The resulting precursor solution was poured into molds and allowed to polymerize at 4°C. The obtained hydrogels were dialyzed in ultrapure water for 7 days to remove unreacted species and reach equilibrium conditions. During dialysis, the water was replaced once per day.

1-3. Synthesis of Gelatin/PNIPAm Interpenetrating Polymer Network (IPN) Hydrogels

Gelatin/PNIPAm IPN hydrogels were prepared using a two-step method consisting of gelatin network formation (first step) followed by PNIPAm network formation (second step). First, a 10 wt% gelatin aqueous solution was prepared. N-isopropylacrylamide (NIPAm, 1.0 M), MBAA (3 mol% relative to NIPAm), and α-ketoglutaric acid (5 mol% relative to NIPAm) were added to the solution. The mixture was cooled to 4°C to induce gelation, forming an NIPAm-containing gelatin SN hydrogel. Subsequently, the sample was irradiated with ultraviolet light (365 nm) for 6 h to polymerize NIPAm and form the PNIPAm network, resulting in gelatin/PNIPAm IPN hydrogels.

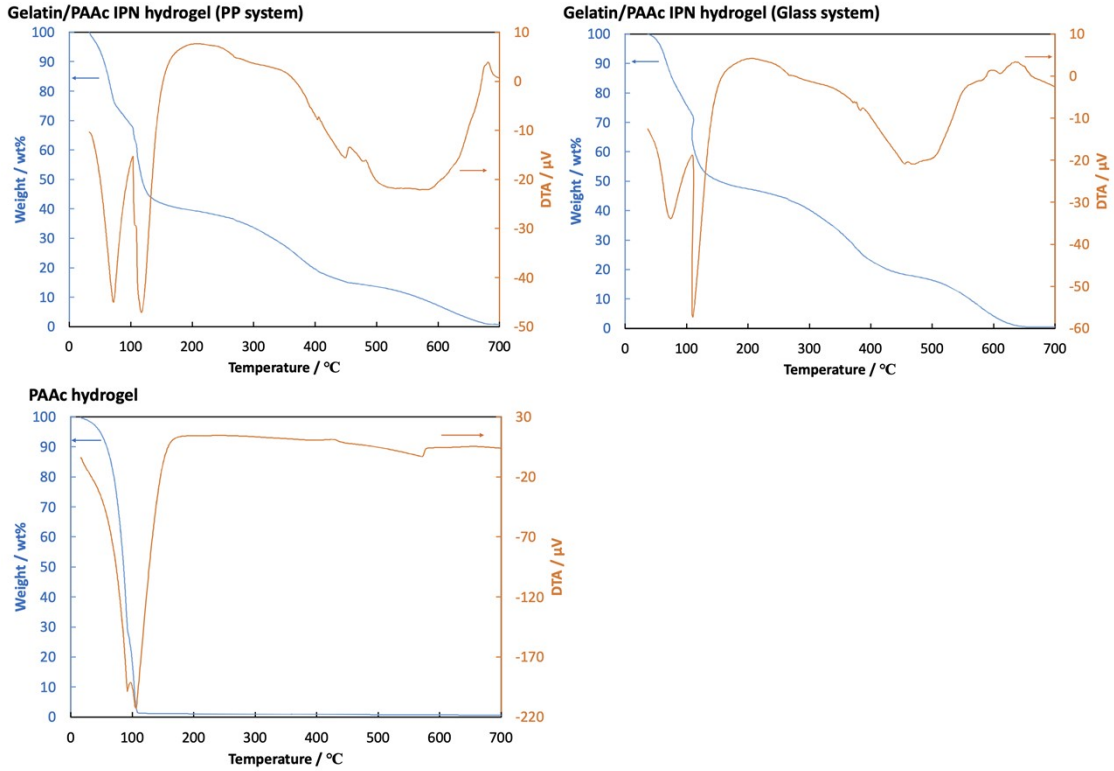
The obtained hydrogels were then dialyzed in ultrapure water for 7 days with daily water replacement.

Figure S1. Supplementary observations of the appearance and internal structures of SN and gelatin/PAAc IPN hydrogels during the dialysis process.



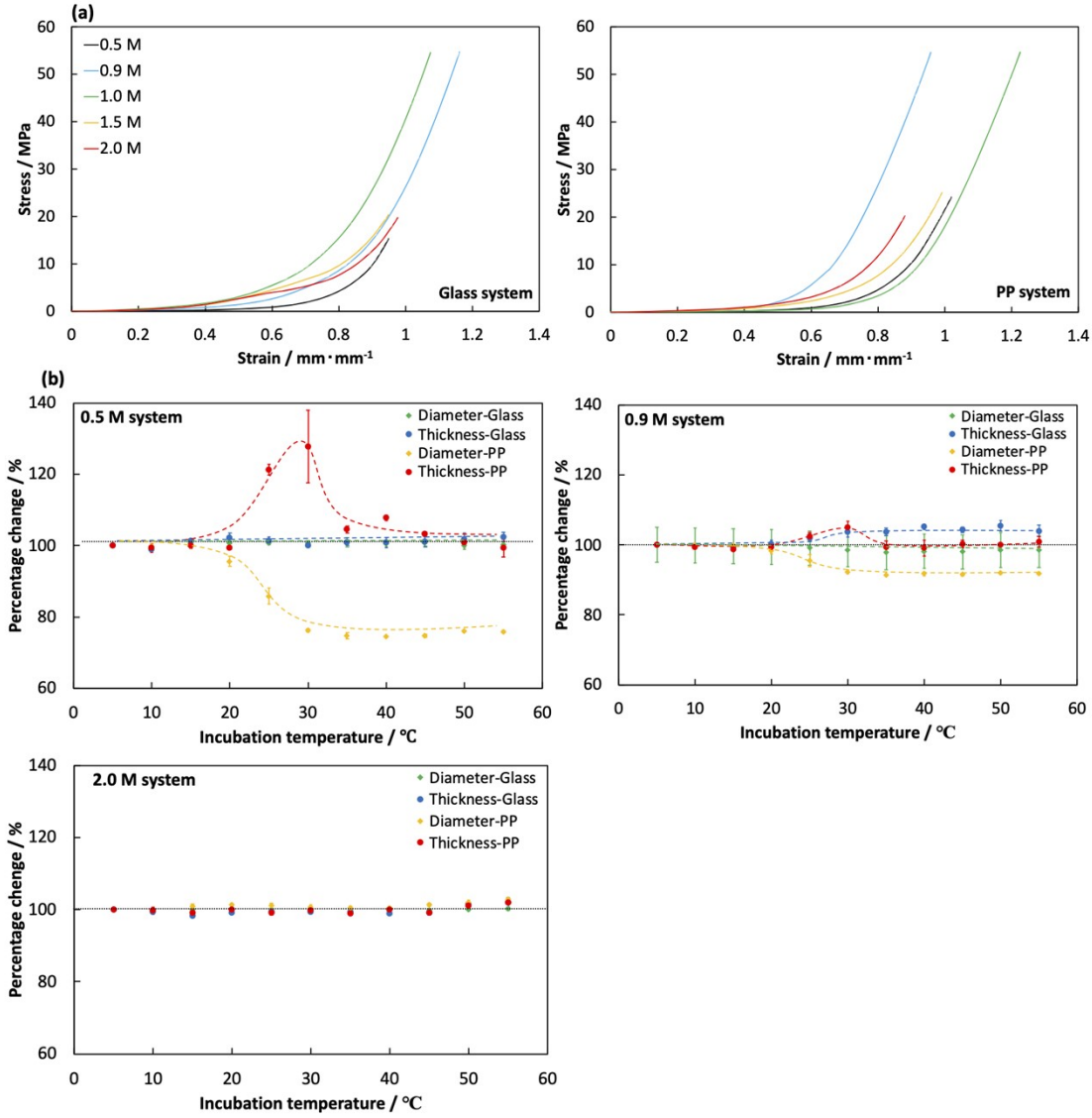
(a) Time-dependent changes in the appearance of PAAc SN hydrogels, gelatin SN hydrogels (Glass and PP systems), and gelatin/PAAc IPN hydrogels (Glass system) during dialysis. While SN hydrogels maintained transparent appearances throughout the dialysis process, the gelatin/PAAc IPN hydrogels initially exhibited turbidity and gradually became transparent over time. (b) Scanning electron microscopy (SEM) images showing surface (top view) and cross-sectional morphologies of gelatin SN, PAAc SN, and gelatin/PAAc IPN hydrogels after freeze-drying. The gelatin SN hydrogels prepared on PP substrates exhibit anisotropic structures, whereas the Glass system and PAAc SN hydrogels show relatively isotropic network morphologies. SEM images of the gelatin SN hydrogels (PP and Glass systems) were reproduced from ref. 17 with permission from the Royal Society of Chemistry. (c) Optical microscopy (OM) images showing the time-dependent structural evolution of gelatin/PAAc IPN hydrogels prepared on glass substrates during dialysis. Unlike the PP-templated system (Fig. 2c), the Glass system exhibits relatively uniform structural development across the hydrogel without a clear peripheral-to-central propagation process. Scale bars in (a): 10 mm. Scale bars in (b): 100 μm. Scale bars in (c): 200 μm.

Figure S2. Thermogravimetric–differential thermal analysis (TG–DTA) curves of gelatin/PAAc IPN hydrogels and PAAc SN hydrogels.



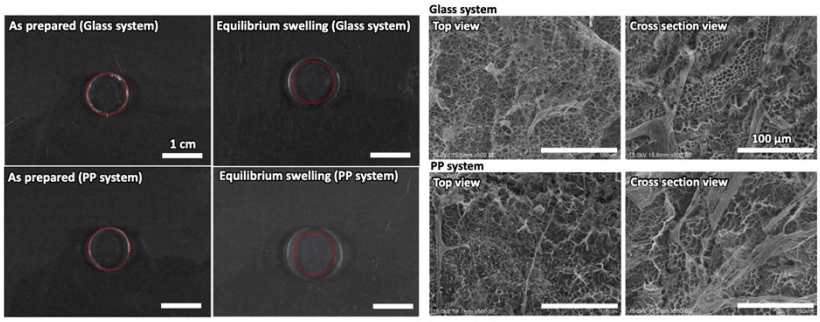
TG (blue) and DTA (orange) curves were obtained for gelatin/PAAc IPN hydrogels prepared on PP substrates (PP-templated system), gelatin/PAAc IPN hydrogels prepared on glass substrates (Glass system), and PAAc SN hydrogels. The initial weight loss observed below ~150 °C corresponds primarily to the evaporation of water contained in the hydrogels. Subsequent weight losses at higher temperatures are attributed to the thermal decomposition of polymer components. Compared with the PAAc SN hydrogel, the IPN hydrogels exhibit lower water content and higher polymer fractions, indicating the formation of densely entangled interpenetrating polymer networks.

Figure S3. Effect of AAc concentration on the mechanical properties and temperature-responsive swelling behavior of gelatin/PAAc IPN hydrogels.

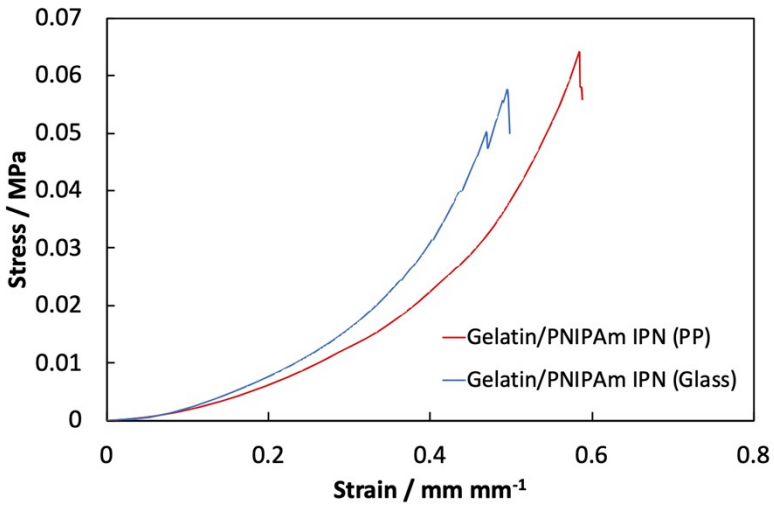


(a) Stress–strain curves of gelatin/PAAc IPN hydrogels prepared with different initial AAc concentrations (0.5–2.0 M) in the Glass and PP-templated systems. (b) Temperature-responsive dimensional changes of IPN hydrogels prepared with different AAc concentrations (0.5, 0.9, and 2.0 M). The percentage changes in diameter (*d*-axis) and thickness (*z*-axis) were measured as a function of incubation temperature for both Glass and PP-templated systems. These results demonstrate that the anisotropic swelling behavior observed in the PP-templated IPN hydrogels is most pronounced near the intermediate AAc concentration region.

Figure S4. Control experiments using gelatin/PNIPAm IPN hydrogels.



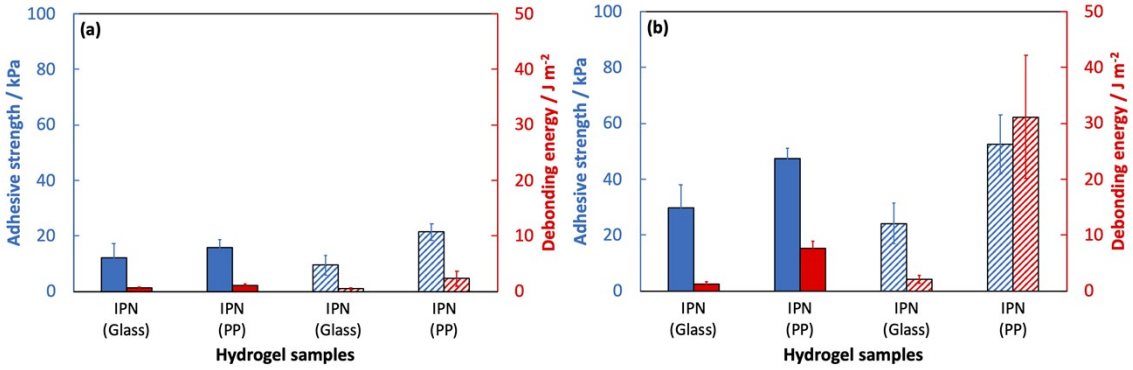
	Gelatin/PNIPAm IPN	
	PP system	Glass system
Young's modulus / MPa	0.0316 ± 0.0066	0.0478 ± 0.0089
Fracture stress / MPa	0.0538 ± 0.0094	0.0540 ± 0.0053
Fracture strain / mm mm^{-1}	0.6119 ± 0.0209	0.4665 ± 0.0213



Photographs of gelatin/PNIPAm IPN hydrogels prepared on Glass and PP substrates in the as-prepared state and after equilibrium swelling. Scanning electron microscopy (SEM) images showing the surface (top view) and cross-sectional morphologies of the corresponding hydrogels after freeze-drying. Mechanical properties of the gelatin/PNIPAm IPN hydrogels are summarized in the table, and representative stress–strain curves measured under compression are shown in the lower panel. In contrast to the gelatin/PAAc IPN hydrogels, the gelatin/PNIPAm IPN hydrogels exhibit similar structures and mechanical responses in both the Glass and PP systems, indicating that the anisotropic behavior observed in gelatin/PAAc IPN hydrogels arises from specific interactions between the gelatin network and AAc-derived PAAc network.

Figure S5. Effect of AAc concentration on the adhesion properties of gelatin/PAAc IPN hydrogels evaluated by tack tests.

	AAc conc. [M]	Gel samples	Adhesive strength [kPa]	Debonding energy [J/m^{-2}]
vs. Glass substrate	0.5	IPN (Glass)	12.04 ± 5.13	0.61 ± 0.25
		IPN (PP)	15.91 ± 2.70	1.11 ± 0.27
	2.0	IPN (Glass)	29.69 ± 8.25	1.22 ± 0.49
		IPN (PP)	47.54 ± 3.69	7.58 ± 1.41
vs. PP sheet	0.5	IPN (Glass)	9.49 ± 3.50	0.58 ± 0.17
		IPN (PP)	21.44 ± 3.05	2.32 ± 1.34
	2.0	IPN (Glass)	24.22 ± 7.34	2.05 ± 0.70
		IPN (PP)	52.64 ± 10.56	31.16 ± 11.04



Adhesive strength and debonding energy of IPN hydrogels prepared with different AAc concentrations measured on glass substrates and PP sheets. (a) IPN hydrogels synthesized with an initial AAc concentration of 0.5 M. (b) IPN hydrogels synthesized with an initial AAc concentration of 2.0 M. Blue bars represent adhesive strength and red bars represent debonding energy. Filled bars correspond to adhesion against glass substrates (vs. Glass substrate), whereas hatched bars correspond to adhesion against PP sheets (vs. PP sheet). The numerical values of adhesive strength and debonding energy are summarized in the table above the graphs.