Percolation-induced gel–gel phase separation in a dilute polymer network

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Cosmic large-scale structures, animal flocks and living tissues can be considered non-equilibrium organized systems created by dissipative processes. Replicating such properties in artificial systems is still difficult. Herein we report a dissipative network formation process in a dilute polymer–water mixture that leads to percolation-induced gel–gel phase separation. The dilute system, which forms a monophase structure at the percolation threshold, spontaneously separates into two co-continuous gel phases with a submillimetre scale (a dilute-percolated gel) during the deswelling process after the completion of the gelation reaction. The dilute-percolated gel, which contains 99% water, exhibits unexpected hydrophobicity and induces the development of adipose-like tissues in subcutaneous tissues. These findings support the development of dissipative structures with advanced functionalities for distinct applications, ranging from physical chemistry to tissue engineering.

Liquid–liquid phase separation in dynamic living systems produces membrane-less organized structures such as nucleoli, stress granules and Cajal bodies. Liquid–liquid phase separation is an emerging challenge in life sciences and is associated with the development of several diseases, including cataracts, and neurodegenerative diseases. Phase separation is widely observed in living systems because of the presence of macromolecules. Owing to the large molar mass, mixing is not favoured in macromolecules compared to small molecules because the mixing entropy depends on the number of molecules.

Polymerization using a monomer with a lower critical solution temperature (LCST) can induce phase separation. Poly(N-isopropyl acrylamide) shows phase separation in water upon polymerization at a constant temperature slightly above LCST. Initially, the reaction mixture is transparent because the monomer is miscible with water. As the reaction proceeds and the molar mass surpasses a critical value, LCST appears and then decreases as the molar mass increases. When LCST becomes lower than the reaction temperature, micro-phase separation occurs. The micro-phase separation can be arrested by introducing a crosslinker to form a phase-separated gel. Such phase-separated gels are prepared from polymers showing a LCST.

This study identified a phase separation induced by gelation, that is, the percolation process. Throughout the percolation process, gel–gel phase separation (GGPS) occurred in a water–polyethylene glycol (PEG) binary system, whose phase separation has never been observed in a standard atmosphere. GGPS was induced from a heterogeneous structure that had been arrested through gelation at a solute concentration below a specific value; the gelation is a dissipative process, and the subsequent deswelling process formed a submillimetre scale, two...
co-continuous gel phases (Fig. 1). The hydrogel with GGPS showed unexpected hydrophobicity and induced adipose-like tissue growth when injected subcutaneously, features that were not observed in the analogous hydrogel without GGPS or in conventional PEG hydrogels.

**Formation of submillimetre filamentous structure**

Hydrogels were synthesized by coupling mutually reactive tetra-functional PEG precursors, keeping the PEG concentration (c) at approximately 10–90 g l⁻¹ at 25 °C (Supplementary Fig. 1). This AB-type molecular design inhibits intramolecular reactions including self-biting loops, while promoting intermolecular reactions to form a polymer network⁴⁸. These features enable a high reaction conversion that forms a gel (~95%), even at the lowest limit of c (~300 g l⁻¹). The gels formed by this design are called tetra-PEG gels⁴⁹–⁵¹, and their homogeneity is investigated using several techniques⁵²–⁵⁵. Currently, expansion microscopy uses this technology to achieve a high resolution with a 9.2 nm spatial error by homogeneously expanding objects using a tetra-gel⁵⁶.

The PEG precursors were miscible with water exceeding the investigated range of c (~300 g l⁻¹). Further, transparent gels were formed above the overlap concentration, c⁰ = 60 g l⁻¹ (Figs. 1 and 2a and Supplementary Fig. 2). Here, c⁰ is the concentration at which the hydrated PEG precursor domain engulfs the entire system⁵⁶. These results agree with previous results in which the LCST of the PEG–water binary system is above the boiling point of water (~130 °C)⁵⁶; thus PEG is hydrophilic. However, the gelling solution becomes cloudy as the PEG concentration decreases below c⁰ at 25 °C (Figs. 1 and 2a). This behaviour contradicts conventional ideas regarding phase separation occurring at solute concentrations above a specific value. To explain this behaviour, two representative gels (dilute-percolated and c⁰ gels) formed at c = 10 and 60 g l⁻¹, respectively, were examined. The dilute-percolated gel becomes cloudier after the gel point when the precursors percolate and form a polymer network (Figs. 1 and 2a). Despite the cloudiness, the dilute-percolated gel exhibits no distinct morphology in a submillimetre range at this point (Fig. 2b).

Immersion of the gels in solvent (water) for a week causes swelling and deswelling of the c⁰ and dilute-percolated gels, respectively (Fig. 2c). When the gels are removed from the moulds and immersed in water, the dilute-percolated and c⁰ gels deswelled and swelled, respectively, to reach equilibrium. Only the dilute-percolated gel exhibited a submillimetre-scale phase-separated structure, which was observed three dimensionally using CLSM. 400 µm x 400 µm x 200 µm volume is shown with a grid size of 50 µm.

Fig. 1 | Schematic of the processes for yielding the dilute-percolated and c⁰ gels. Tetra-functional polymeric precursors with mutually reactive functional groups are coupled under aqueous conditions. The reactive groups, maleimide (A) and thiol (B), form covalent linkages (yellow sphere). The dilute-percolated (turbid) and c⁰ (transparent) gels are formed at c = 10 and 60 g l⁻¹, respectively. The precursors are mixed under stoichiometrically balanced conditions. White turbidity appeared during the gelation in the case of the dilute-percolated gel, whereas no apparent change was observed in the c⁰ gel. After being removed from the glass vial and immersed in water, the dilute-percolated and c⁰ gels deswelled and swelled, respectively, to reach equilibrium. Only the dilute-percolated gel exhibited a submillimetre-scale phase-separated structure, which was observed three dimensionally using CLSM. 400 µm x 400 µm x 200 µm volume is shown with a grid size of 50 µm.
no morphological change (Fig. 2f, bottom). These findings confirm that the dilute-percolated gel experiences phase separation below the upper critical solution temperature (UCST), which is unusual for PEG aqueous solutions with their UCST around 300 °C (ref. 24). Below the UCST, the dilute-percolated gel shows unexpected hydrophobicity (Fig. 2d). When immersed in a suspension of hydrophobic particles, the dilute-percolated gel (Fig. 2d, top right) absorbed the hydrophobic particles, whereas the c⁺ gel (Fig. 2d, bottom right) did not. The hydrophobicity gradually increased as c decreased, that is, as there was an increase in water content (Supplementary Fig. 6). The particles were adsorbed only on the surface and not into the bulk, although the particle size (-10 µm) was ten times smaller than that of the filamentous structure. Considering the co-continuity of the structure, this result indicates that the dilute phase is also a gel state, and GGPS occurs.

This unique hydrophobic submillimetre filamentous network structure is formed by the ‘percolation below c⁺’. Another PEG gel (the c⁺-diluted gel), which has the same PEG and water composition as the dilute-percolated gel but originally formed at c⁺, showed no turbidity, submillimetre structure or hydrophobicity (Supplementary Fig. 7a). We synthesized the c⁺-diluted gel at a concentration of 60 g l⁻¹ using stoichiometrically imbalanced tetra-PEG precursors to reduce the elastic modulus and induce swelling (Supplementary Fig. 7b,c). Adjusting the ratio of AB-type prepolymers while maintaining a constant initial polymer concentration allows for precise control over the elastic modulus26. The c⁺-diluted gel was designed to swell and reach a similar equilibrium polymer concentration as the dilute-percolated gel (12 g l⁻¹). Therefore, the only difference between the c⁺-diluted and dilute-percolated gels is the gelation process. Swelling enhances the heterogeneity of polymer gels27. The absence of a submillimetre structure in the swollen c⁺-diluted gel indicates that the submillimetre structure observed in the deswollen dilute-percolated gel is not typical heterogeneity observed in conventional gels.

Formation of GGPS

We performed time-resolved small-angle X-ray scattering (SAXS) experiments to elucidate the structural change during gelation in the dilute-percolated and c⁺-gels (Fig. 3a,b). Scattering intensities (I) were normalized by the polymer concentration (I/c) and shown as a function of the scattering vector magnitude (q). Initially, for time t divided by the gel point t/tₚₓ < 1, the scattering profiles of both c⁺ and dilute-percolated gels conform to a plateau at small q, followed by a crossover to a fractal relationship I ≈ q⁻α at large q. The fractal relationship originates from the internal correlation of the PEG precursors, whose mass fractal dimension D = 2 (ref. 16). As the reaction approaches the gel point, t/tₚₓ = 1, the fractal region expands to a considerably lower q, indicating the growth of polymeric clusters. However, the fractal dimension remains D = 2 in both c⁺ and dilute-percolated gels, ruling out precipitation as the origin of the cloudiness. Precipitation occasionally competes with gelation and creates clear polymer–solvent interfaces, inducing a prominent fractal relation, I ≈ q⁻α (refs. 28, 29).

After the gel point, t/tₚₓ > 1, a significant difference is observed between the c⁺ and dilute-percolated gels. The scattering profiles of the c⁺ gel change slightly during gelation (Fig. 3b), indicating that bond formation between the PEG precursors insignificantly affects the spatial arrangement of the PEG units. The molecular representation at c > c⁺ clarifies this observation (Fig. 1; bottom)26. The scattering profiles before and after the gel point completely overlap as the precursor concentration increases (c = 2c⁺; ref. 30).

However, the dilute-percolated gel shows a unique change after the gel point (Fig. 3a). Over the entire SAXS q range, scattering intensities increase with time from the size of a polymer segment (-0.5 nm) to ten or more times the size of the precursors (-10 nm). The intensity increment of the dilute-percolated gel in the q range for intra-precursor distribution (q > 0.05 Å⁻¹) was markedly different from that of the c⁺ gel, where intensities were constant over time. Increased scattering

**Experimental verification of GGPS**

The filamentous structure was thermally stable (Figs. 2f,f). The dilute-percolated gel shrunk, the cloudiness decreased and the submillimetre-scale structure vanished when the temperature increased to 90 °C (Fig. 2e, Fig. 2b, top and Supplementary Fig. 5). Shrinkage at elevated temperatures has been observed for conventional PEG hydrogels, which reflects the increased hydrophobicity of PEG23. However, decreasing the temperature from 90 to 25 °C made the dilute-percolated gel cloudy, and the filamentous structures grew (Supplementary Video 5). In the temperature range, the c⁺ gel shows

![Image](https://example.com/image1)

**Fig. 2** | Clouding during gelation and thermodynamically stable submillimetre-scale phase-separated structure. **a** Temporal changes in turbidity with gelation of the dilute-percolated (grey line) and c⁺ (black line) gels. The reaction time t is normalized by the gel time tₚₓ (550 and 140 s for dilute-percolated and c⁺ gels, respectively). **b** CLSM images of inner structure (left) and particles absorbed on gel’s surface (right) of the dilute-percolated (top) and c⁺ (bottom) gels before swelling/deswelling (scale bars, 100 µm). **c** Time evolution of the swelling ratio (degree of swelling, Q) of the dilute-percolated (blue triangles) and c⁺ (black circles) gels. Error bars represent the standard deviation of the mean obtained from three samples (n = 3). The ratio of 1 is marked with a dashed line. **d** CLSM images of the inner structure (left) and particles absorbed on the surface (right) of the dilute-percolated (top) and c⁺ (bottom) gels after swelling/deswelling (scale bars, 100 µm). **e** The swelling ratios of the dilute-percolated (blue triangles) and c⁺ (black circles) gels decreased when the temperature was changed from 25 °C to 90 °C and then back to 25 °C. Error bars represent the standard deviation of the mean obtained from three samples (n = 3). **f** CLSM images of the dilute-percolated (top) and c⁺ (bottom) gels during the temperature change (scale bars, 100 µm).
units. Notably, the gel point is still far from equilibrium, and an additional crosslinking reaction occurs because the conversion at the gel point is only approximately 60%, eventually increasing to 95% (ref. 19). The crosslinking reaction occurs efficiently in the condensed area, enhancing local elasticity. This positive response is driven by (1) the continuous dissipation of chemical energy and (2) matter exchanges with the condensed and sparse phases. According to SAXS (Fig. 3), the dissipative structure was permanently frozen during gelation. In an open environment enabling macroscopic volume changes, local condensation occurred and formed a distinct interface as observed by CLSM (Fig. 3). The dense phase may become denser to minimize the interfacial energy, causing GGPS. Consequently, the homogeneous gel structure would become destabilized, and uneven co-continuous structures would emerge and grow to submillimetre scales (Supplementary Videos S5 and 6), leading to the separation of the phases in a homogeneous miscible gel system.

**Biological response with hydrogels**

The extracellular matrix in living systems is analogous to the dilute-percolated gel in terms of opaqueness and mesoscale structure15. To demonstrate, disc-shaped hydrogels in the equilibrium swollen state were implanted subcutaneously in rats, and the differences in biological response with the gels were investigated over 14 days (Fig. 4). We first assessed degradation by performing in vivo images of the fluorescence-labelled hydrogels (Fig. 4a). Only the dilute-percolated gel showed a gradual decay of the fluorescence signal overall, as quantified by analysing the radiance efficiency relative to the day 0 signal (Fig. 4b). The relative fluorescence of the dilute-percolated gel reached approximately 50% at day 14, whereas the reduction for the c* gel was subtle. These results suggest that some biological response of the two gels differs. Gross findings for rats with the dilute-percolated gel further highlighted the biological response; the dilute-percolated gel was attached with interfaces with living tissue (Fig. 4c, Supplementary Fig. 8a and Supplementary Video 7). This is unique for the dilute-percolated gel, but not for the c* gel (Supplementary Video 8). We further elucidated biological responses regarding hydrogel degradation and subsequent tissue substitution via Masson trichrome staining, hematoxylin staining and immunostaining (Fig. 4d,e and Supplementary Figs. 8b and 9). The staining shows that adipose-like tissue was abundant in the region where the dilute-percolated gel initially existed, whereas the dilute-percolated gel remained at the limited area at day 14 (Fig. 4d and Supplementary Fig. 8b). These results suggest that not only did the volume of the dilute-percolated gel decrease, but that subsequent substitution to adipose tissue also occurred. To further investigate the tissue substitution mechanism, we focused on macrophages because (1) innate immune cells recognize molecules with hydrophobicity10 and (2) macrophages are involved in the degradation of PEG-based hydrogels by secreting reactive oxygen species16. Immunohistochemistry revealed CD68-positive cells in the tissues surrounding the residual dilute-percolated gel and inside the substituted regions (Fig. 4e, top left images), which was further supported by high magnification images (Supplementary Fig. 9). These results suggest that macrophages attack and induce hydrogel degradation, leading to tissue substitution. The formation of adipose-like tissues was further characterized by the immunohistochemistry of peroxisome proliferator-activated receptor (PPARγ), a master regulator of adipocytes (Supplementary Fig. 10). In addition, the adipose tissue contained a CD31-positive blood vessel structure (Fig. 4e, top right images). However, CD68- and CD31-positive cells were restricted in the vicinity of the implanted c* gel; no blood vessel formation was observed in the area (Fig. 4e, bottom images). The inert nature of the c* gel is consistent with previous experiments on PEG-based hydrogels17. Therefore, only the dilute-percolated gel dissolves in vivo and is substituted into an adipose tissue containing the vascular structure. This behaviour may be caused by the hydrophobicity that regulates protein migration and cell adhesion. This study reports...
PEG-hydrogel-based cell migration and tissue substitution without any specific bioactive motifs (for example, cell adhesion peptides (RGD sequences)) or apparent degradable segments.

**Outlook**

We demonstrated that the hydrophilicity and phase behaviour of gels can be affected by network formation. Previously, these properties were considered to be solely determined by the chemical structure. PEG hydrogels are typically hydrophilic at room temperature, displaying a LCST of 130 °C and a UCST of 300 °C. However, our dilute-percolated PEG gel exhibited hydrophobic behaviour at room temperature and a UCST below 90 °C. Our study demonstrates that other dilute-percolated gels may possess unexpected physical properties based on their chemical structures.

The dilute-percolated PEG gel possesses a cell-interactive filamentous structure similar to the extracellular matrix found in living systems. PEG is a cell-inert synthetic polymer, and numerous PEG-based biomedical products are listed in the Food and Drug Administration's Drug Master Files. Development of biomedical products using other synthetic polymers with unknown biosafety is unadvised because of rigorous biosafety regulations. Therefore, the cell-interactive PEG gel may enable the development of PEG-based biomedical products, such as scaffolds for regenerative medicine.
The following aspects make it challenging to fully comprehend GGPS: (1) the size of the characteristic structure varies across four orders of magnitude, from 10 to 10^4 nm and (2) the time required for structure formation varies, taking less than an hour for gelation, approximately a day to complete the gelation reaction and up to a week to form filamentous structures. Unveiling such phenomena that cover a wide range of length and time scales is a laborious task. Despite these obstacles, our findings point to a paradigm shift, demonstrating that the percolation process can drastically alter the physical properties of gels, previously believed to be solely determined by their chemical structure. This study explored GGPS in a PEG–water binary system, which may inspire discussions and advancements, particularly in materials science, soft matter physics and biology.

Online content
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Methods
Preparation of PEG hydrogels
Four-armed PEGs (weight-averaged molecular mass $M_\text{w} = 10,000 \text{ g mol}^{-1}$) functionalized with sulfhydryl (tetra-PEG–SH) and maleimide (tetra-PEG–MA) were purchased from NOF. The PEG hydrogels were prepared by dissolving fixed amounts of tetra-PEG–SH and tetra-PEG–MA in citrate phosphate buffer (CPB). Equal amounts of these precursors were subsequently mixed and immediately poured into a mould. The gelation reaction was conducted at 25 °C for 24 h prior to the experiments. The overlapping concentration $c$ of the PEG precursors was approximately 60 g l$^{-1}$ and the hydrogels prepared at 10 g l$^{-1}$ and 60 g l$^{-1}$ were referred to as dilute-percolated and dense gels, respectively. Hydrogels prepared using the following concentrations ($c$) were investigated and characterized based on the normalized concentration $c/\langle c \rangle: c = 10, 15, 20, 30, 40, 50, 60, 75$ and 90 g l$^{-1}$ ($c/\langle c \rangle = 0.16, 0.25, 0.33, 0.50, 0.66, 0.83, 1.0, 1.25$ and 1.5, respectively). CPB (50 mM) with pH 3.8 was used to prepare hydrogels with concentrations of ≥40 g l$^{-1}$, whereas CPB (50 mM) with pH 5.0 was used for hydrogels with lower concentrations (≤30 g l$^{-1}$).

UV–visible spectra
The precursors of the samples were mixed and poured into plastic cells with an optical length of 10 mm. The transmittance at wavelength $\lambda = 400 \text{ nm}$ was measured using a UV–visible spectrophotometer (V-670, JASCO) at 25 °C every 5 s for 48 h. The turbidity was estimated by normalizing the absorbance with the polymer concentration (grams per litre).

Optical observation, swelling behaviour and turbidity of hydrogels
The precursors were mixed and poured into Teflon moulds (diameter, 15 mm; height, 7 mm) and allowed to sit in an incubator for 24 h at 25 °C to enable gelation. The gel samples were carefully removed from the moulds and subsequently immersed in distilled water at 25 °C. Optical imaging and turbidity analysis of the hydrogels were performed, using macroscopic observations of the gel samples. The degree of swelling ($Q$) was calculated from the diameter of the as-prepared gels ($d_0$) and swollen gels ($d_s$) using the following formula: $Q = (d_s/d_0)^3$. Time-wise, $Q$ was evaluated by an M165C optical microscope (Leica Camera AG). The relative turbidity was estimated based on the background intensity $T_\text{b}$ and the hydrogel intensity $T_\text{h}$ was calculated using ImageJ software.

Rheological analysis of hydrogels
The PEG precursors were poured into the attached double cylinder geometries of the rheometer (MCR302, Anton Paar). The time-dependent changes in storage modulus ($G'$) and loss modulus ($G''$) were measured with a shear amplitude $\gamma = 1\%$ and an oscillating frequency $\omega = 10 \text{ Hz}$ at a constant temperature of 25 °C.

SAXS measurements
SAXS measurements were performed at the High Energy Accelerator Research Organization, KEK (Ibaraki, Japan). A lab-sourced small-angle instrument (NANOPIX, Rigaku) with a wavelength of 0.154 nm and the BL-6A beamline of the Photon Factory with a wavelength of 0.15 nm were used. The sample-to-detector distance was 1.30 m and 2.54 m, respectively. A 1-mm-thick gel sample was placed in a custom-made planar cell and sealed with two 30-µm-thick glass windows. In the gelation processes, scattering patterns were obtained every 5 min. The scattered intensities were circularly averaged to obtain one-dimensional intensity profiles, which were then corrected for incident beam flux, sample absorption, sample thickness, exposure time and cell and solvent scattering using a custom-made data reduction package (Red2D; https://github.com/hurxl/Red2D) within a scientific data analysis software package (Igor Pro 8, WaveMetrics). The intensity was plotted as a function of the magnitude of the scattering vector $q$. Glassy carbon (National Institute of Standards and Technology) and silver behenate (Nagar Science) standards were used for calibration. All measurements were conducted at ambient temperature (−25 °C).

Confocal laser scanning microscopy
CLSM images were obtained to visualize the mesoscale phase separation of the hydrogels. Fluorescence-labelled PEG was first prepared to facilitate CLSM. Briefly, 1.0 g tetra-PEG–SH was dissolved in 20 ml distilled water and stirred for 10 min at ambient temperature. Further, 1 mg Alexa Fluor 488 C5 maleimide (Thermo Fisher Scientific) was dissolved in 1 ml dimethyl sulfoxide (DMSO), and 128.6 µl of this solution (0.0125 equiv. versus tetra-PEG–SH) was added to the tetra-PEG–SH solution. We assumed that the reaction between tetra-PEG–SH and Alexa Fluor 488 C5 maleimide proceeds completely and that one of 8,000 thiols reacts with a fluorescent molecule, which hardly influences the gelation reaction and hydrophilicity of the formed gel. The mixture was allowed to sit for 3 h at ambient temperature and subsequently dialysed against distilled water for 3 h to remove unreacted molecules. Then, the sample was freeze-dried to obtain tetra-PEG–SH partially functionalized with Alexa Fluor 488 as a faint yellow powder (yield, 950 mg).

Gel precursors prepared using fluorescently labelled tetra-PEG–SH and tetra-PEG–MA were poured into a Teflon mould (diameter, 15 mm; height, 7 mm) and allowed to sit for 24 h at 25 °C to enable gelation. The prepared hydrogels were carefully removed from the mould and immersed in distilled water at 25 °C for 7 days. The CLSM images were obtained with a Leica TCS SP8 using a long working distance, water immersion objective lens, HCXIRPO1 × 25/0.95 W (Leica Microsystems) for Fig. 1 and Supplementary Videos 1–6. The three-dimensional images were rendered after alignment, stitching and deconvolution using the Huygens software package (Scientific Volume Imaging). Other CLSM images were taken with a Zeiss LSM 800 using a water immersion objective lens, C-Apochromat ×10/0.45 W (Carl Zeiss).

Particle adsorption to hydrogels
Gelling solution was poured into a Teflon mould (diameter, 15 mm; height, 7 mm) and incubated for 24 h at 25 °C. Further, Fluoresbrite YG Carboxylate Microspheres (diameter, 10 µm; Polysciences) were suspended in water to obtain 0.1 w/v particle solution. Prepared gel samples before and after removal from the mould were immersed in the particle solution and incubated for 24 h at 25 °C. The CLSM images were obtained using the LSM 800 set-up.

Temperature analysis of the gels
Variations in the mesoscale phase separation with temperature were examined by immersing the hydrogels in distilled water for 24 h and exposing them to various temperatures (25 °C and 90 °C). Optical and CLSM imaging as well as turbidity analysis were performed as described above after 24 h at a specific temperature.

Preparation and characterization of $c^*$-diluted gels
Gelling solution (60 g l$^{-1}$), which was prepared at different proportions ($r$) of 0.5, 0.4, 0.3, 0.2 and 0.18, where $r = \text{[tetra-PEG–SH]/([tetra-PEG–MA + tetra-PEG–SH])}$, was poured into a Teflon mould (diameter, 15 mm; height, 7 mm) and incubated for 24 h at 25 °C. The gel samples were carefully removed from the moulds and subsequently immersed in distilled water at 25 °C. The optical images, swelling behaviour, inner structure of hydrogels and adsorption of particles were probed using the methods mentioned above. The concentration after swelling ($C_{\text{sw}}$) was calculated as $C_{\text{sw}} = 60/Q$.

In vivo study on rats
All animal experiments were performed according to the protocol approved by the Animal Care and Use Committee of the University of Tokyo (approval number P19-115). Twelve-week-old female Wistar rats weighing 170–200 g were used in this study. The rats were housed and...
and 5.0 mg kg⁻¹ butorphanol was intraperitoneally administered to and sterile conditions. For general anaesthesia, a combination anaesthesia provided with laboratory rat chow and water ad libitum and exposed to a 12 h light/dark cycle at room temperature (22 °C).

Subcutaneous implantation of hydrogels
PEG precursors for dilute-percolated and G* gels were poured into a rectangular silicon mould (15 mm × 35 mm; height, 3 mm) and incubated at room temperature for 24 h. The obtained hydrogels were removed from the mould, immersed in phosphate-buffered saline and incubated at room temperature for 7 days. The hydrogel samples were cut into disc-shaped hydrogels (diameter, 10 mm; height, 3 mm).

All surgical procedures were conducted under general anaesthesia and sterile conditions. For general anaesthesia, a combination anaesthetic composed of 0.3 mg kg⁻¹ medetomidine, 4.0 mg kg⁻¹ midazolam and 5.0 mg kg⁻¹ butorphanol was intraperitoneally administered to each rat at 0.3 ml per 100 g of body weight. Epilation of the skin at the surgical site was conducted before the incision. A 4 cm skin incision was made in the epilated back right side, and subcutaneous tissue was exposed. After making space for the hydrogel by separating the subcutaneous tissue from fascia, the G* gel or dilute-percolated gel was gently implanted in each rat (n = 3). After the operation, the skin was closed with skin staples, and 5 mg kg⁻¹ enrofloxacin was administered intramuscularly for antibiotic prophylaxis. The rats were killed at 14 days after the surgical procedure and subjected to histological analysis or immunohistochemistry. For macroscopic gross appearance analysis, hydrogels implanted in rats were carefully harvested, and some deposits adhering to the gel surface were removed.

In vivo images
IRDye 800CW Maleimide (SCRUM) was dissolved beforehand in super dehydrated DMSO (FUJIFILM Wako Pure Chemical) to obtain a 1 mM solution. Tetra-PEG–SH was dissolved in CPB to obtain 10 or 60 g l⁻¹ gelling precursors. The solution of IRDye 800CW Maleimide was added to the prepared PEG solution (1 vol%), and the mixture was incubated at room temperature for 5 min. Subsequently, tetra-PEG–MA was dissolved in CPB to obtain 10 or 60 g l⁻¹ gelling precursors. Equal volumes of two PEG precursors at the same concentration were mixed and poured into a rectangular silicon mould followed by incubation at room temperature for 24 h. Thereafter, the solution was immersed in phosphate-buffered saline after removal from the mould, incubated at room temperature for 7 days and finally cut into disc-shaped hydrogels. The hydrogels were implanted subcutaneously and monitored (excitation at 745 nm and emission at 800 nm) by the IVIS Spectrum imaging system (Caliper). The sample size was set to three in order to minimize the number of animals used.

Histological analysis
The rats were killed 14 days after surgery, and the skin including the subcutaneous tissues and hydrogel was harvested and fixed in 4% paraformaldehyde buffered with phosphate-buffered saline (pH 7.4) at 4 °C for 1 day. The samples were embedded in paraffin and cut into 5 µm sagittal slices. The tissue specimens were stained with Masson trichrome staining according to standard protocol. For immunohistochemistry, sections were incubated with antibodies against CD68 (1:500; ab125212, Abcam), CD31 (1:500; sc-376764, Santa Cruz Biotechnology) and PPARγ (1:250; ab209350, Abcam). For visualization, simple stain MAX-PO(R) (Nichirei Bioscience) was used.

Statistical analysis and reproducibility
For the turbidity, degree of swelling and particle adhesion, and in vivo degradation studies, three replicates were prepared for each condition. For the in vivo study, three mice were used for each gel. Values are expressed as mean ± standard deviation. For the CLSM, SAXS, rheology and in vivo staining studies, representative results with reproducibility are shown.

Reporting summary
Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability
Data generated or analysed during this study are included in the main and Supplementary Information files. Further data are available from the corresponding authors upon request. Source data are provided with this paper.

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Author contributions
All authors contributed to writing the paper and discussing the results. Experiments were planned, conducted and analysed by S.I., Y.I., T.U. and I.F. Discussions regarding the mechanism of GGPS involved S.I., N.S., T.K. and T.Sakai. CLSM observation was performed by S.I. and Y.O. Animal experiments were performed by S.I. and Y.I.; N.S. and T.Sakai. conceived the idea and supervised the project.

Competing interests
T.S. is an inventor on a patent application (WO2020027016) that describes the use of this hydrogel with GGPS.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41563-023-01712-z.

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| ☐ | ☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection | Image J |
|-----------------|---------|
| Data analysis   | Excel 365, Image J, Igor Pro 8 |

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The data that support the findings of this study available in a publicly accessible repository.
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| Reporting on sex and gender | N/A |
|-----------------------------|-----|
| Population characteristics  | N/A |
| Recruitment                 | N/A |
| Ethics oversight            | N/A |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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- Life sciences
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical methods were used to determine sample size. Experiments in biologically independent triplicates were performed for statistical analyses, according to the standard scientific conventions in the research field, and to reach a conclusion using the smallest possible sample size. |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | No data were excluded from the analyses.                                                                                                                                                           |
| Replication | Experiments were independently performed three times. All replication attempts were reproduced successfully.                                                                                  |
| Randomization | The experimental animals were allocated randomly.                                                                                                                                                 |
| Blinding | Investigators were not blinded to group allocation in animal experiments. This is because different hydrogels implanted subcutaneously in rats were easily recognized during sample collections even if the investigators were blinded to group allocation. Except for the in vivo experiments, the investigators were blinded in the analysis. |

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Materials & experimental systems

- n/a
- Antibodies
- Eukaryotic cell lines
- Palaeontology and archaeology
- Animals and other organisms
- Clinical data
- Dual use research of concern

Methods

- n/a
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Antibodies

- Antibodies used: anti-CD68 antibody (1:500; ab125212, Abcam), anti-CD31 antibody (1:500; sc-376764, Santa Cruz Biotechnology), and anti-PPARγ antibody (1:250; ab209350, Abcam)

Validation

- We used standard antibodies validated by the manufacturers. All antibodies were purchased from commercial vendors.
Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

| Laboratory animals | Twelve-week-old female Wistar rats weighing 170–200 g were used in this study. |
|---------------------|---------------------------------------------------------------------------------|
| Wild animals        | The study did not involve wild animals.                                          |
| Reporting on sex    | Only female rats were used.                                                      |
| Field-collected samples | The study did not involve samples collected from the field.                  |
| Ethics oversight    | All animal experiments were performed according to the protocol approved by the Animal Care and Use Committee of the University of Tokyo. |

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