Supporting Information

# Self-curing super-stretchable polymer/microgel complex coacervate gels without covalent bond formation

Shanglin Wu,*[a] Mingning Zhu,[a] Dongdong Lu,[a] Amir H. Milani,[a] Qing Lian,[a]

Lee A. Fielding,[a] Brian R. Saunders *[a]

[a]*School of Materials, University of Manchester, MSS Tower, Manchester, M13 9PL, UK*

Matthew J. Derry,[b] Steven P. Armes [b]

[b]*Department of Chemistry, The University of Sheffield, Dainton Building, Brook Hill, Sheffield, South Yorkshire, S3 7HF, UK*

Daman Adlam [c] and Judith A. Hoyland [c, d]

[c]*Division of Cell Matrix Biology and Regenerative Medicine, Faculty of Biology, Medicine and Health, University of Manchester, Oxford Road, Manchester, M13 9PT, UK*

[d]*NIHR Manchester Biomedical Research Centre, Central Manchester Foundation Trust, Manchester Academic Health Science Centre, Manchester, M13 9WL, UK*

## 1. Materials and Methods

### 1.1. Materials

Ethylacrylate (EA, 99%), methacrylic acid (MAA, 99%), divinylbenzene (DVB, 80%), ammonium persulfate (98%), sodium dodecyl sulphate (SDS, 99%), potassium phosphate dibasic (98%), phosphate buffered saline (PBS), $CaCl_2$ (≥ 98.0 %), $NaNO_3$ (99%) were purchased from Sigma-Aldrich. Branched polyethylenimine (PEI) with molecular weights of 0.60, 10 and 70 kD were purchased from Polysciences, Inc. All materials were used as received.

### 1.2. Methods

**1.2.1. Microgel synthesis.** The synthesis of anionic microgel nanoparticles was performed using the seed-feed emulsion polymerisation. SDS (1.80 g) was added to water (518 mL) in a reactor with a mechanical stirrer. The solution was purged with nitrogen for 30 min and heated to 80 °C. Comonomer solution (31.5 g) containing EA (66.0 wt%), MAA (32.7 wt%) and DVB (1.3 wt%) was prepared and transferred to the reactor. The seed formation was proceeded by addition of $K_2HPO_4$ (3.15 g of a 7.0 wt% solution) and APS (10 g of a 2 wt% solution). After 30 min, comonomer (218.5 g) mixture was then fed into the reactor at a uniform rate (2.4 g $min^{-1}$) over a period of 90 min. The copolymerisation proceeded for a further hour and was finally quenched in an ice bath. The product was extensively dialyzed with water. The final MG was concentrated to 17.2 wt% by rotary evaporation at room temperature and stored at 5 °C before use.

**1.2.2. PEI/MG gel preparation.** Ultrahigh purity deionized water was used in all experiments. The following gives an example of preparation of PEI/MG(50-0.67). MG dispersion (1.20 mL, 17.2 wt%) pH ~ 4 – 5 was transferred to a vial. PEI solution (10 kD) was diluted to 17.2 wt% with water and the desired quantity (0.80 mL) injected into the MG dispersion. The mixture

was mechanically stirred and vigorously mixed by hand using a spatula to form a white (Pre-gel). The solution pH of this pre-gel was 7.3. The pre-gel was further mechanically mixed using a kneading method similar to "dough making" for a further 5 min until it reached a smooth, uniform state. The gel was then sealed by two pieces of glass slides and clips with a hollow mold. These molds consisted of either an O-ring (inner diameter = 19 mm and wall diameter = 2.5 mm) or a PTFE hollow cylinder (inner diameter = 12 mm and height = 12 mm). Parafilm was used to completely encapsulate the molds to ensure that no potential evaporation during heating occurred. The gel was heated at the required temperature for 20 hr. Other gel MRs were prepared by varying the PEI:MG ratio (from 0.40 to 1.0) and molecular weight of PEI. The total volume of initial pre-gel was 2.0 mL. In the $Ca^{2+}$ stiffening experiments, PEI/MG(50-1.5) gels were immersed in saturated aqueous $CaCl_2$ solutions for various times.

**1.2.3. Swelling and adhesive experiments.** During the gel preparation step, six prepared PEI/MG (50-0.67) gels were separately placed into different pH buffers. Universal pH indicator was added to each buffer solution. The gels were allowed to swell for 2 days. The change of color and weight was recorded. The adhesive ability was examined by a flip experiment with a glass slide. The gel at pH 7.4 were selected for adhering to various materials: rubber, plastic, steel, Teflon, glass and fresh porcine skin.

**1.2.4. Self-healing experiment.** The disc-like samples were separated into two parts by cutting through the middle line. The gels were then re-joined together with some drops of water along the cutting line. They were sealed again and placed for 24 h at room temperature to allow healing process. The self-healed ability was quantified by uniaxial tensile measurement.

**1.2.5. Physical measurements.** *Z*-average diameter ($d_z$) and zeta potential ($\zeta$) data were measured at 25 °C using a Malvern Zetasizer Nano ZS instrument. The $d_z$ values were calculated using the Stokes-Einstein equation. The carboxylic acid content and p$K_a$ for the MGs

were determined via potentiometric titration using a Mettler Toledo DL15 titrator. TEM samples were prepared by drop-casting MG dispersions (10 μL, 0.001 wt.%) onto copper grids (300 mesh) coated with holey carbon film. MGs were stained with uranyl acetate at room temperature overnight. TEM studies were performed using an FEI Tecnai 12 BioTwin instrument operating at 100 kV. SEM samples were freeze-dried and coated with Au/Pd prior to imaging to prevent sample-charging. All SEM studies were conducted using a FEI Quanta 650 FEG-SEM instrument operating at 20 kV. FTIR spectra were collected using a Nicolet 5700 ATR-FTIR spectrometer (128 scans, 2 $cm^{-1}$ resolution). Dynamic rheology measurements were performed on a Discovery HR-3 Hybrid rheometer, with a parallel plate geometry and 1800 μm gap. The diameter of test gels was 15.5 mm. The strain amplitude and oscillation frequency sweeps were conducted at a frequency of 1 Hz and a strain of 1%, respectively. The storage modulus ($G'$) and loss modulus ($G''$) were recorded simultaneously at 25 °C. Mechanical properties were assessed using an Instron series 5569 instrument. Rectangular and cylinder shapes were prepared for tensile and compression tests, respectively. Uniaxial tensile measurements were conducted at a strain rate of 0.055 $s^{-1}$, while uniaxial compression studies utilized a strain rate of 0.015 $s^{-1}$. Compression experiments were ceased at ~ 84% strain to avoid damage to the instrument and gels had still not fractured under such conditions. The cyclic tensile test was performed on both tensile and compression measurements. Each sample was allowed to rest for 5 min between each successive cycle unless otherwise stated. The elastic modulus was obtained from the linear part of the stress-strain graph in the low strain region. Engineering stress and strain are reported in this study. The adhesive strength was characterized by lap shear testing. Glass and Teflon substrates (75 mm × 25 mm × 1 mm) were cleaned using sonication in water and ethanol. Excess fat from porcine skins (75 mm × 25 mm × 1 mm) was removed using a scalpel and the skin cleaned with water and soap and rinsed well with water. All samples were cut into the size of 15 mm × 15 mm before attachment. The gel was evenly

placed on one substrate surface, then two substrate surfaces were joined together by overlapping the gel for 10 s. The adhesive strength was obtained from the failure point of the gel under uniaxial loading.

**1.2.6. Small-angle X-ray scattering and model fitting.** Unless otherwise stated the gels studied using SAXS were immersed in aqueous saturated $CaCl_2$ solution for 30 s prior to measurement. SAXS data were collected using a laboratory SAXS instrument (Xeuss 2.0, Xenocs, France) equipped with a liquid gallium MetalJet X-ray source (Excillum, Sweden, wavelength $\lambda$ = 0.134 nm), two sets of motorized scatterless slits for beam collimation and a Dectris Pilatus 1M pixel SAXS detector (sample-to-detector distance 5.088 m). SAXS patterns were recorded over a $q$ range of 0.003 $Å^{-1} < q < 0.13$ $Å^{-1}$, where $q = (4\pi \sin\theta)/\lambda$ is the length of the scattering vector and $\theta$ is one-half of the scattering angle. A cell comprising two mica windows (each of 25 μm thickness) separated by a 2 mm polytetrafluoroethylene spacer used as a sample holder for liquid samples and unstretched gels, whereas stretched gels were secured across a metal A-frame. In all cases, data were collected over 5 min and averaged where multiple acquisitions were recorded. Data were reduced (normalization and integration) using the Foxtrot software package supplied with the instrument and further analyzed (background subtraction and data modelling) using Irena SAS macros for IgorPro[1].

Data collected for 1.0% w/w and 17% w/w aqueous dispersions of MG particles in their collapsed state were successfully fitted to a two-population model of homogeneous spheroids plus Gaussian polymer chains, where the total scattering intensity, $I(q)$, is represented by:

$$I(q) = \frac{d\Sigma}{d\Omega}(q)_s + \frac{d\Sigma}{d\Omega}(q)_c \qquad \text{(S1)}$$

where $\frac{d\Sigma}{d\Omega}(q)_s$ is the scattering cross-section per unit sample volume of homogeneous spheroids and $\frac{d\Sigma}{d\Omega}(q)_c$ is the scattering cross-section per unit sample volume of Gaussian polymer chains. Specifically, $\frac{d\Sigma}{d\Omega}(q)_s$ is represented by:

$$\frac{d\Sigma}{d\Omega}(q)_s = NS_{PY}(q)\int_0^\infty g_{Gauss}(R)|F(qR)|^2 dR \quad \text{(S2)}$$

where $N$ is the number of scatterers, $S_{PY}(q)$ is the hard-sphere interaction structure factor based on the Percus-Yevick approximation[2], $g_{Gauss}(R)$ is their Gaussian size distribution function and $F(qR)$ is the particle form factor. Specifically, $g_{Gauss}(R)$ is expressed as:

$$g_{Gauss}(R) = \frac{1}{\sigma_R\sqrt{2\pi}} e^{-\frac{(R-\bar{R})^2}{2\sigma_R^2}} \quad \text{(S3)}$$

where $\bar{R}$ is the mean radius of the particles and $\sigma_R$ is the standard deviation of the size distribution. The particle form factor, $F(qR)$, is expressed as:

$$F(qR) = \frac{4}{3}\pi R^3 \Delta\xi \left(3\frac{sin(qR) - qRcos(qR)}{(qR)^3}\right) \quad \text{(S4)}$$

where $\Delta\xi$ is the X-ray scattering contrast. $\frac{d\Sigma}{d\Omega}(q)_c$ is represented by:

$$\frac{d\Sigma}{d\Omega}(q)_c = \varphi(\Delta\xi)^2 V_{mol} F_{mol}(q) \quad \text{(S5)}$$

where φ is the volume fraction of polymer, $V_{mol}$ is the total molecular volume and Δξ is the excess scattering length density of the polymer [$\Delta\xi = \xi_{pol} - \xi_{H_2O}$ = 2.46 x $10^{-10}$ $cm^{-2}$], where the scattering length density of the polymer (PMAA), $\xi_{pol}$ = 11.88 x $10^{-10}$ $cm^{-2}$ and the scattering length density of water, $\xi_{H_2O}$ = 9.42 x $10^{-10}$ $cm^{-2}$. The generalized form factor for a Gaussian polymer chain, $F_{mol}(q)$, is given by[3]:

$$F_{mol}(q) = \left[\frac{1}{\upsilon U^{1/(2\upsilon)}}\gamma\left(\frac{1}{2\upsilon},U\right) - \frac{1}{\upsilon U^{1/\upsilon}}\left(\frac{1}{\upsilon},U\right)\right] \quad \text{(S6)}$$

where the lower incomplete gamma function is $\gamma(s,x) = \int_0^x t^{s-1}\exp(-t)dt$ and $U$ is the modified variable:

$$U = (2\upsilon + 1)(2\upsilon + 2)\frac{q^2 R_g{}^2}{6} \quad \text{(S7)}$$

Here $\upsilon$ is the extended volume parameter and $R_g$ is the radius of gyration. Thus, these two fitting parameters are used for $F_{mol}(q)$.

**1.2.7. Live/Dead and MTT assays.**

T/C28a2, immortalised human chondrocyte cells, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Gibco) and antibiotic/antimycotic (Sigma-Aldrich, UK) at 37 °C in a humidified 5% $CO_2$ atmosphere. Cells were seeded at a density of 5 x $10^5$ onto 35 mm glass bottom dishes (VWR International) containing 5 mm discs of gel (40 mg) and cultured up to 72h with daily media changes. Viability of cells adjacent to the gels was determined at 24, 48 and 72 hour time-points versus gel-free controls by live/dead assay (Life Technologies, UK). Images were obtained with an Olympus BX51 fluorescence microscope and a Lietz Diavert inverted phase contrast light microscope. For quantitative assay, the T/C28a2 cells were seeded at a density of 5 x $10^4$ per well onto 24 well plates containing 0.4 μm cell-culture inserts (BD Biosciences) and allowed to adhere overnight before exposure to 15 mg of gel via the insert (n = 3). Cell viability was determined by MTT assay (Sigma-Aldrich) using a FLUOstar OMEGA plate reader.

## 2. Legend for Supplementary Movie

**Movie S1**

**Stretching PEI/MG(50-0.80)**

The rod-like gel sample is stretched by hand and finally breaks when stretched to more than eight times its original length.

## 3. Supplementary Figures

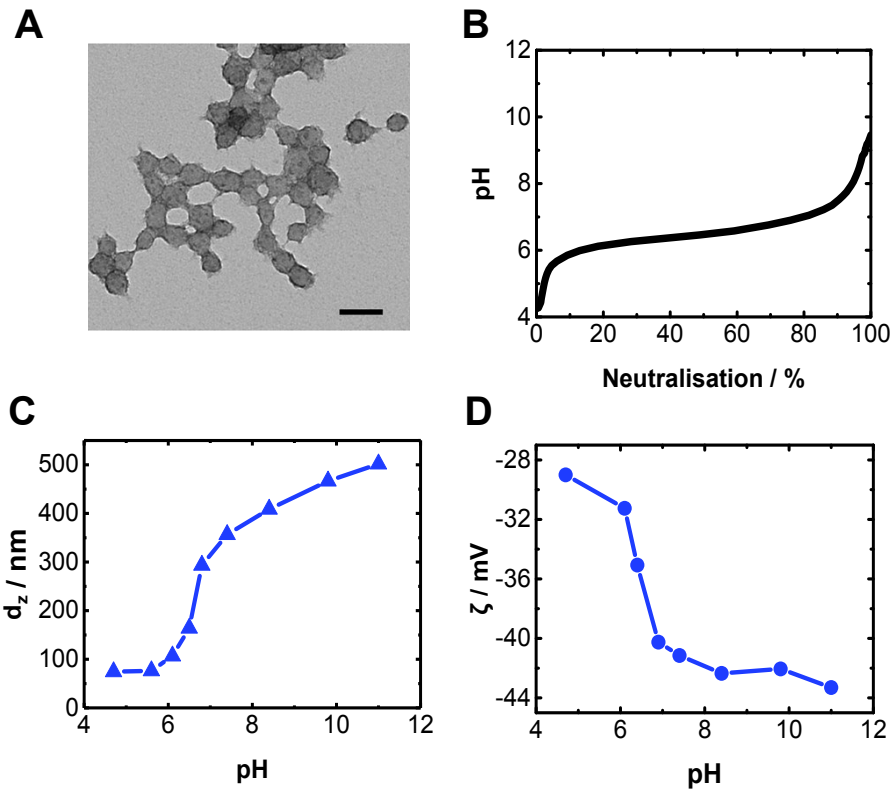


**Figure S1 Characterization of the microgel (MG).** (**A**) Representative TEM image of MGs. Scale bar: 100 nm. (**B**) Potentiometric titration data. (**C**) Dynamic light scattering (DLS) variation of the z-average diameter ($d_z$) with pH. (**D**) Zeta potential of MG particles at different pH.

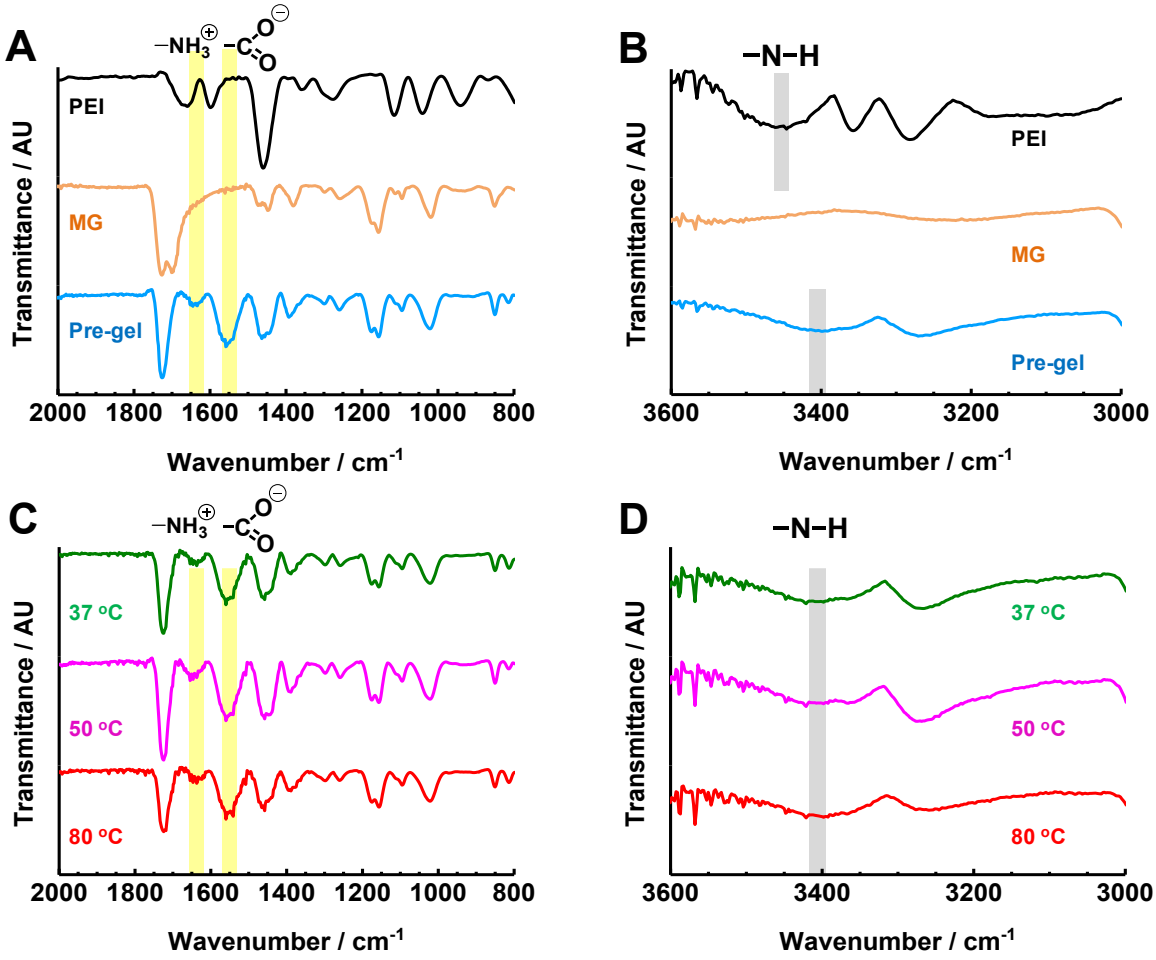


**Figure S2. FTIR spectra for various systems.** Comparison of the FTIR spectra for (**A** and **B**) PEI, MG and Pre-gel (PEI/MG(0.67)) as well as (**C** and **D**) PEI/MG(T-0.67) gels heated at various temperatures. The spectra for the pre-gel and PEI/MG(T-0.67) gels show new bands due to ionic groups (indicated) and a shift of the N-H group. The pH of the gels was 7.3.

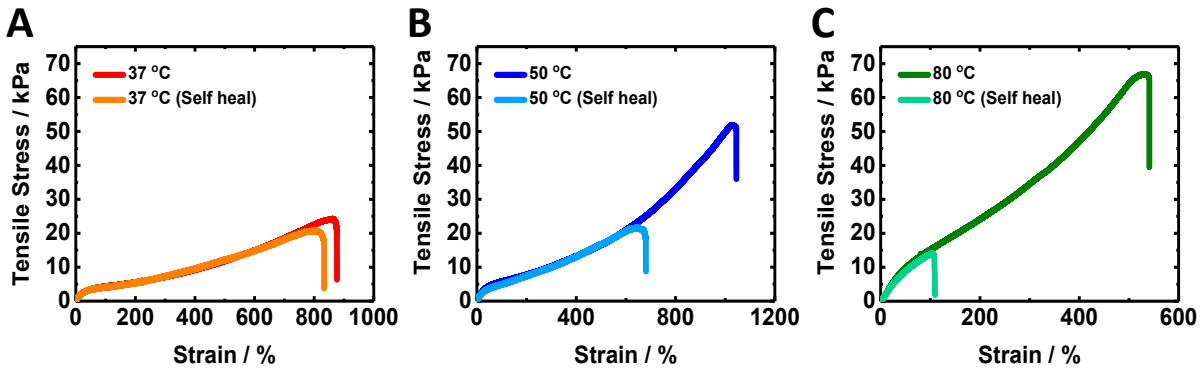


**Figure S3. Self-healing properties for the gels measured using uniaxial strain.** The uniaxial tensile test showed the extent of self-healing for the PEI/MG(0.67-T) gels cured at (**A**) T = 37, (**B**) 50 and (**C**) 80 °C. For the self-healing samples, the gels were interfaced for 1 day prior to measurement. The control (as made) gels were stored for a total of 2 days before tensile measurement. For the PEI/MG(0.67-37) gel the additional day of storage resulted in a modest decrease of the breaking strain.

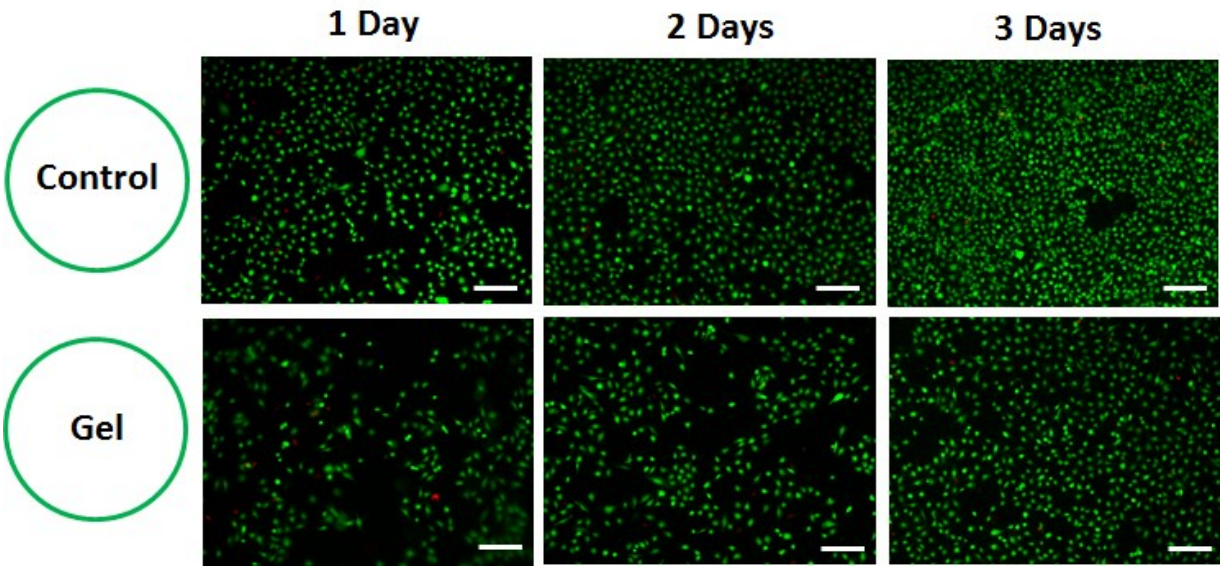


**Figure S4. Live/Dead cell assay for chondrocytes in the absence and presence of gel.** The gel was PEI/MG(50-0.50) and the time points are shown. The scale bar is 200 μm.

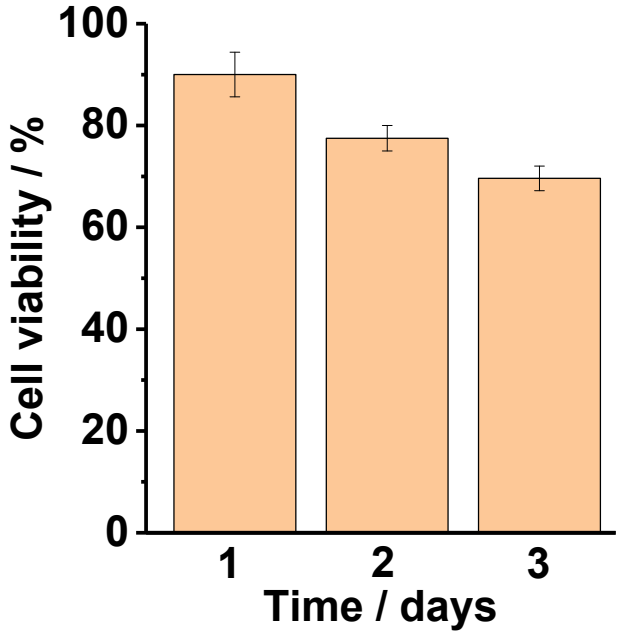


**Figure S5. Cell viability of chondrocytes in the presence of gel.** The gel was PEI/MG(50-0.50) and the time points are shown. Cell viability was calculated from the MTT assay.

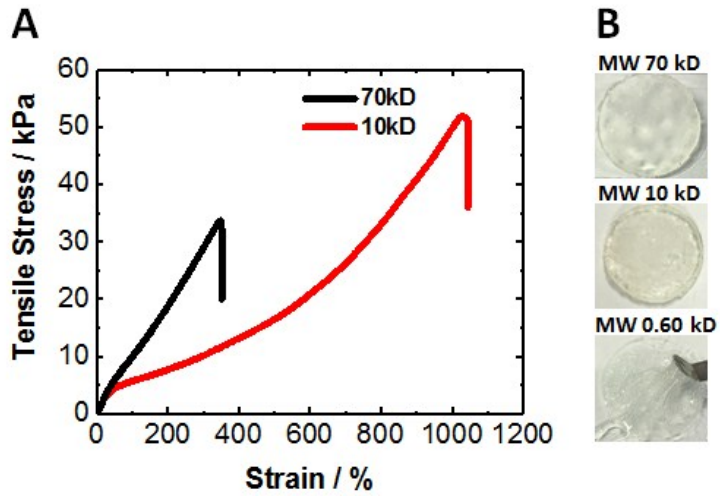


**Figure S6. Effect of PEI molecular weight on gel mechanical properties.** (**A**) Uniaxial tensile stress-strain data for PEI/MG(50-0.67) gels prepared with different PEI molecular weights. (**B**) Digital photographs showed the appearance of the PEI/MG(50-0.67) gel. The mixture of the 0.60 kD branched PEI and MG remained highly viscous without forming a hydrogel network.

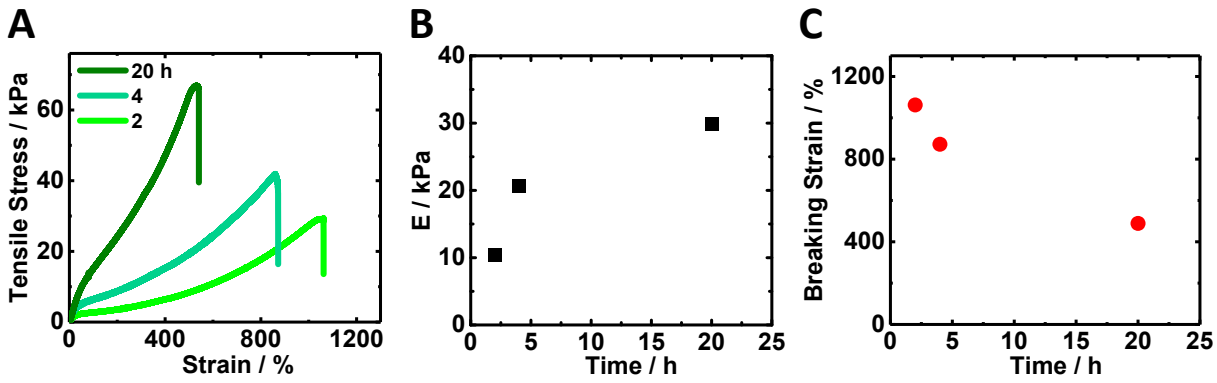


**Figure S7. Effect of annealing time on gel mechanical properties.** (**A**) Tensile stress-strain data measured for PEI/MG(80-0.67) gel heated for various times (shown). The gels were heated at 80 °C. The modulus (**B**) and breaking strain (**C**) increased and decreased, respectively, with increasing annealing time.

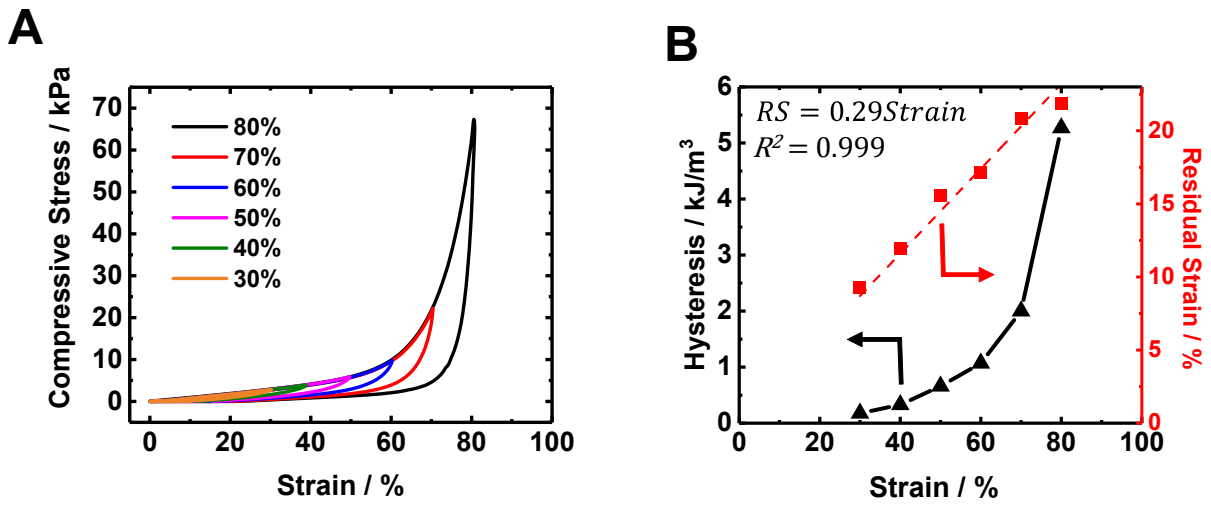


**Figure S8. Cyclic variable strain uniaxial compression data for PEI/MG(50-0.67) gel.** (**A**) The cyclic compression test was conducted at six different strains. (**B**) Hysteresis and residual strain from the gels obtained from (A).

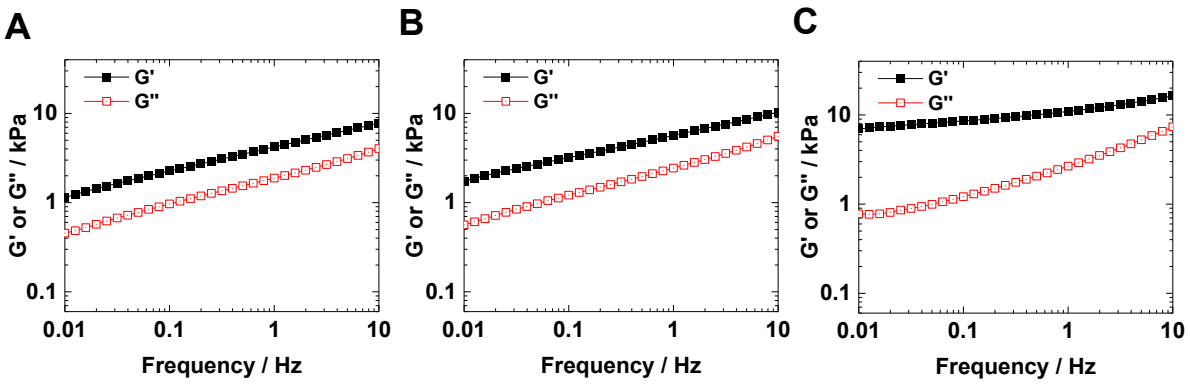


**Figure S9. Frequency-sweep dynamic rheology measurements for PEI/MG(T-0.67) gels.**

The temperatures used were (**A**) 37 °C, (**B**) 50 °C and (**C**) 80 °C.

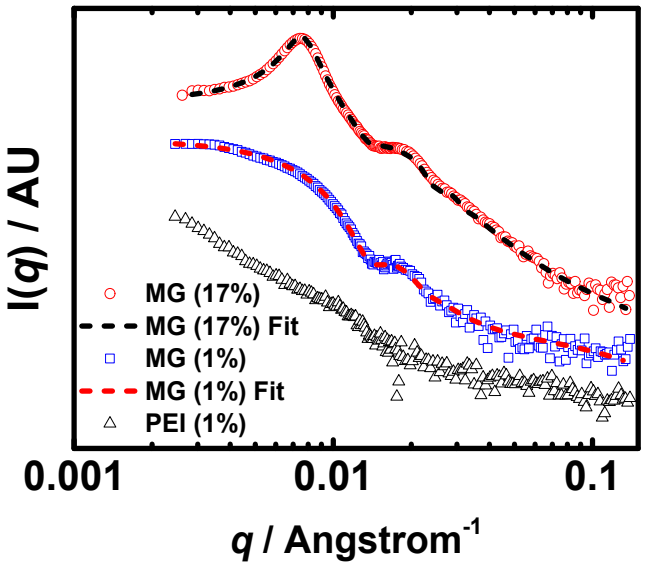


**Figure S10**. **1D SAXS profiles for MGs and PEI.** The dashed black lines represent fits to a combined spheroid plus Gaussian coil model. A structure peak is present for the concentrated MG dispersion. The concentrations for each system (wt.%) are shown. Fitting data to models described above indicated a mean MG particle core diameter of 64 nm with PEI chains with an $R_g$ of 1.6 nm. At 17 wt.%, the mean centre-to-centre MG particle separation distance was 87 nm.

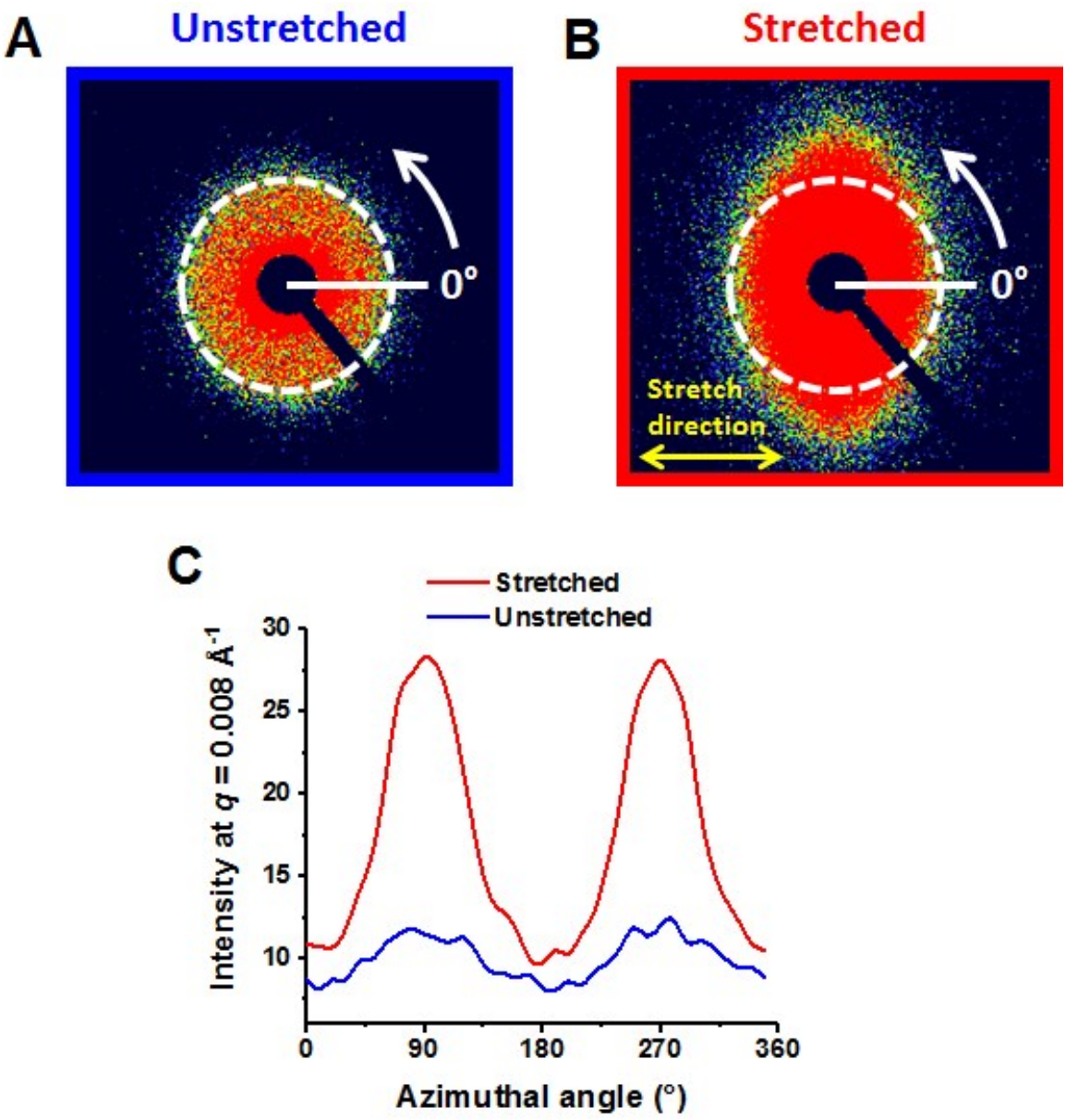


**Figure S11**. **SAXS data for a stretched PEI/MG gel showing anisotropic behavior.** 2D SAXS patterns for (**A**) unstretched and (**B**) stretched (155% strain) PEI/MG(50-0.67) gels after 30 s $CaCl_2$ treatment. The latter was used to enhance X-ray scattering contrast. (**C**) Azimuthal profiles at $q$ = 0.008 Å$^{-1}$ for each 2D SAXS pattern (see dashed circle at $q$ = 0.008 Å$^{-1}$ for reference). Note that the weak anisotropy observed in the unstretched gel arises due to the sample preparation step of pressing the gel between two mica disks.

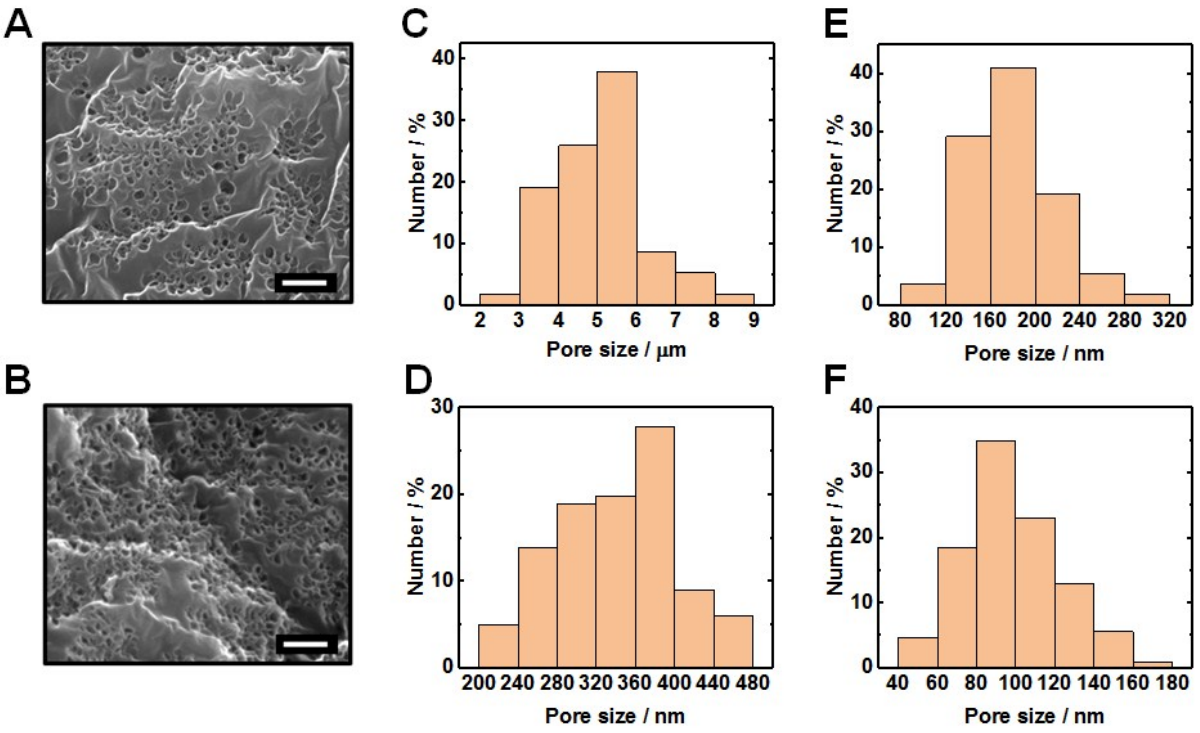


**Figure S12. SEM and pore size distributions for freeze-dried PEI/MG(T-0.67) gels.** SEM images are shown for (**A**) PEI/MG(50-0.67) and (**B**) PEI/MG(80-0.67) gels. Scale bars: 1 μm. Pore size distributions are shown for (**C**) PEI/MG(0.67) pre-gel, (**D**) PEI/MG(37-0.67), (**E**) PEI/MG(50-0.67) and (**F**) PEI/MG(80-0.67). The average pore sizes were 5.06 ± 1.11 μm, 0.34 ± 0.06 μm, 0.178 ± 0.037 μm and 0.098 ± 0.025 μm, respectively.

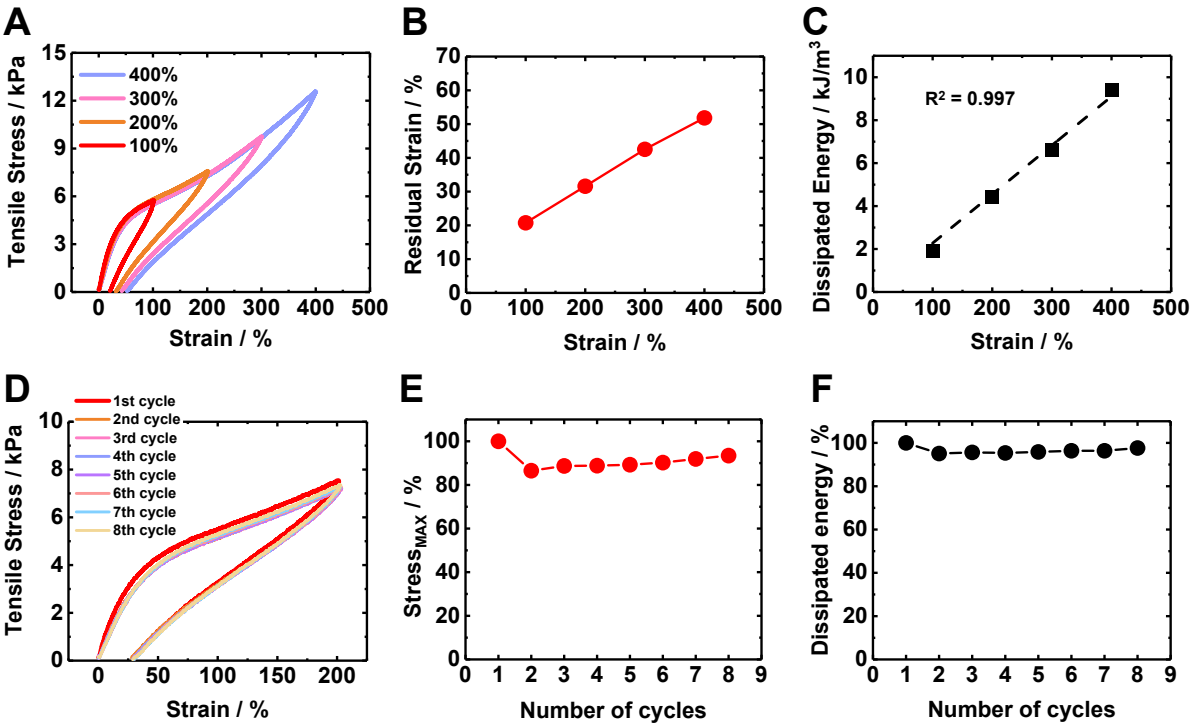


**Figure S13. Cyclic uniaxial tensile data for the gels.** (**A**) The cyclic loading of PEI/MG(50-0.67) gel was subjected to four different strain (100%, 200%, 300%, 400%). (**B**) The residual strain and (**C**) dissipated energy were acquired from the hysteresis loop and plotted against the applied strain. (**D**) Multiple cyclic loading of PEI/MG(50-0.67) conducted for eight runs. (**E**) The percentage change of maximum stress at 200% and (**F**) dissipated energy were recorded for every loading-unloading cycle.

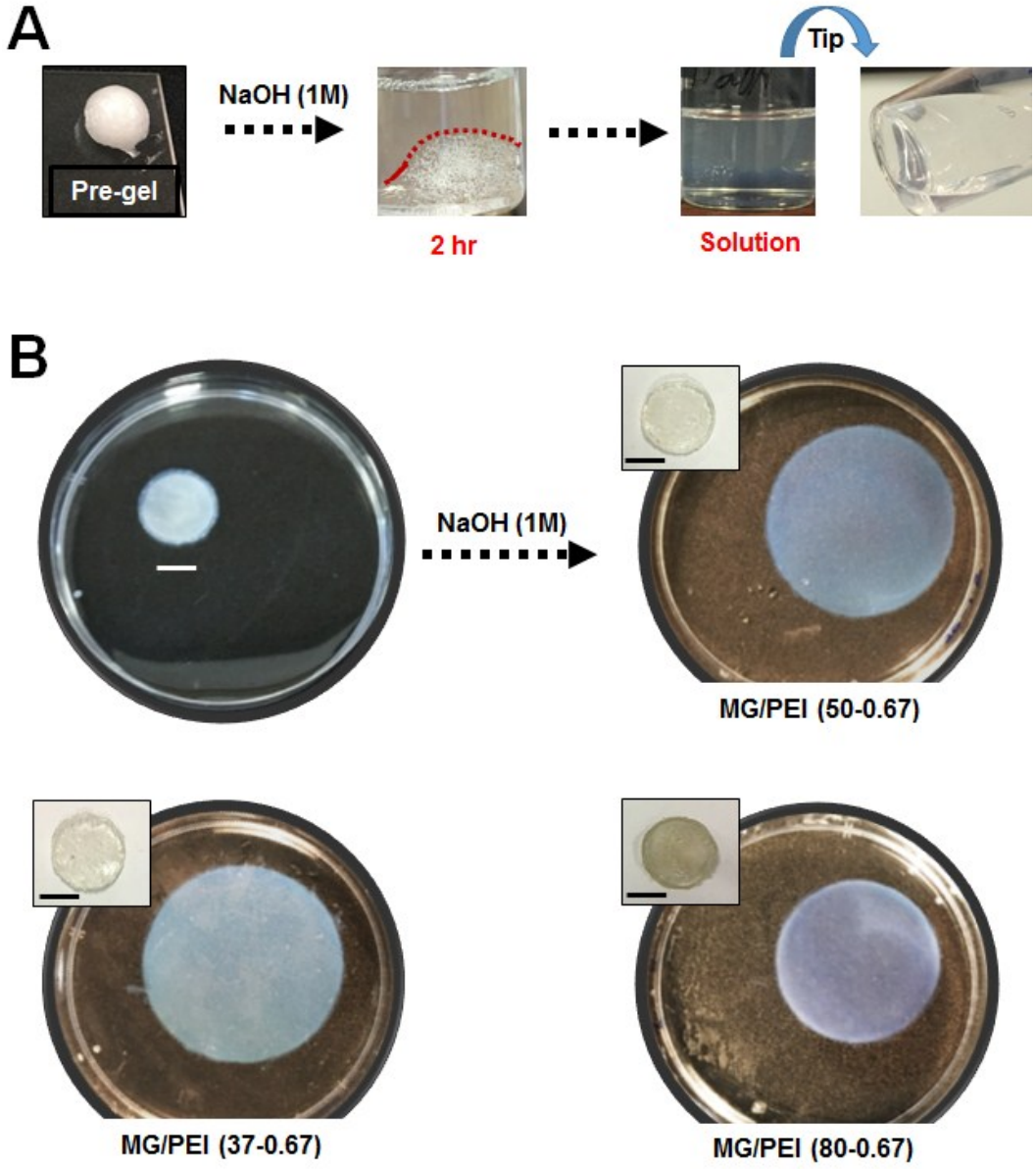


**Figure S14. Effect of strong alkaline solution on the pre-gels and T-gels.** The (**A**) pre-gels and (**B**) T-cured gels were placed in aqueous NaOH (1.0 M) for 2 days. The pH was ~ 14. Whilst the PEI/MG(0.67) pre-gel dissolved and formed a solution (A), the T-gels swelled but did not dissolve (B). Scale bar 10 mm.

## 4. Supplementary Figures

**Table S1.** Mechanical properties with different mass ratios for PEI/MG(50-MR) gels.

| MR[a)] | E [kPa][b)] | Tensile strength [kPa] | Strain at break [%] | Toughness [MJ/m$^3$] |
|---|---|---|---|---|
| 0.40 | 39.0 ± 5.4 | 64.0 ± 10.3 | 909 ± 69 | 0.267 ± 0.040 |
| 0.50 | 36.4 ± 5.9 | 59.6 ± 2.8 | 955 ± 51 | 0.262 ± 0.025 |
| 0.67 | 15.5 ± 2.2 | 45.4 ± 7.0 | 1015 ± 18 | 0.194 ± 0.029 |
| 0.80 | 11.4 ± 1.0 | 20.3 ± 1.8 | 790 ± 37 | 0.072 ± 0.007 |
| 1.00 | 5.5 ± 0.9 | 12.3 ± 1.2 | 728 ± 27 | 0.041 ± 0.004 |

[a)]PEI-to-MG mass ratio; [b)] Young's modulus

**Table S2.** Mechanical properties of PEI/MG(T-0.67) gels prepared at different temperatures.

| Gel | E [kPa][a)] | Tensile strength [kPa] | Strain at break [%] | Toughness [MJ/m$^3$] |
|---|---|---|---|---|
| PEI/MG(80-0.67) | 29.8 ± 2.8 | 62.5 ± 5.2 | 489 ± 41 | 0.152 ± 0.022 |
| PEI/MG(50-0.67) | 15.5 ± 2.2 | 45.4 ± 7.0 | 1015 ± 18 | 0.194 ± 0.029 |
| PEI/MG(37-0.67) | 12.4 ± 0.2 | 19.8 ± 1.2 | 1122 ± 79 | 0.104 ± 0.004 |

[a)] Young's modulus.

**Table S3.** Comparison of mechanical properties for PEI/MG(50-0.67) gels at different $Ca^{2+}$ equilibration times.

| Time [min] | E [MPa][a)] | Tensile strength [MPa] | Strain at break [%] | Toughness [MJ/m$^3$] |
|---|---|---|---|---|
| 0.5 | 0.30 ± 0.06 | 0.09 ± 0.02 | 351 ± 49 | 0.223 ± 0.035 |
| 10 | 3.00 ± 0.08 | 0.39 ± 0.02 | 152 ± 33 | 0.472 ± 0.063 |
| 60 | 27.0 ± 1.3 | 3.1 ± 0.2 | 95 ± 21 | 2.56 ± 0.46 |
| 180 | 33.5 ± 2.5 | 3.4 ± 0.1 | 89 ± 17 | 3.03 ± 0.44 |

[a)] Young's modulus

## 5. References

1. J. Ilavsky and P. R. Jemian, *J. Appl. Crystallog.*, 2009, 42.
2. D. J. Kinning and E. L. Thomas, *Macromolecules*, 1984, **17**, 1712-1718.
3. B. Hammouda, *Probing Nanoscale Structures - The SANS Toolbox, National Institute of Standards and Technology*, 2008.