Nerve cells decide to orient inside an injectable hydrogel with minimal structural guidance

Supplementary materials and methods

Materials

Six armed, star-shaped poly(ethylene oxide-*stat*-propylene oxide) (star-PEG) consisting of 80% ethylene oxide and 20% propylene oxide was provided by CHT R. Beitlich GmbH. Pyridine (anhydrous , 98%, Sigma-Aldrich), toluene (anhydrous, 98%, Sigma-Aldrich), acrylic anhydride (≥90%, Polyscience), diethyl ether (≥ 95,5%, Sigma-Aldrich) and dichloromethane (anhydrous, ≥99,8%, Sigma-Aldrich) were used as received.

2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959), poly(ethylene glycol) (PEG-OH 200 g/mol), and fluorescein o-acrylate were purchased from Sigma-Aldrich, and dimethyl sulfoxide (DMSO) from VWR. Bifunctional perfluoropolyether-urethane methacrylate (Solexis Fluorolink MD700) was supplied by Acota Ltd., 2-Hydroxy-2-methyl-1-phenyl-propan-1-one (Irgacure1173) by BASF, polyethylenterephthalat (PET) film (50 µm thickness) by Goodfellow GmbH, concentrated hydrogen peroxide by VWR, and polyvinylpyrrolidone (PVP) MW 360000 g/mol, concentrated sulfuric acid, acetone, and 2-propanol by Sigma-Aldrich. Photomasks of silica wafers were designed using KLayout software (version 0.23.10) and wafers were processed by AMO GmbH with i-line stepper.

As superparamagnetic iron oxide nanoparticles (SPIONs), anionic-coated, water based dispersion (EMG 700) was provided by Ferrotec Europe GmbH. Suprapur nitric acid (65 %) and hydrochloric acid (30 %), supplied by Merck, were used for sample ionization for iron quantification. For labeling of fibrinogen the Pierce NHS-Rhodamine Antibody Labeling Kit was purchased.

Dow Corning Sylgard 184 polydimethylsiloxane (PDMS) was purchased from MAVOM GmbH, and Tissue-Tek® O.C.T. compound (Sakura® Finetek) from VWR.

For cell experiments Dulbecco's Modified Eagle Medium, RPMI Media 1640, PBS by Lonza, TrypLE™ Express Enzyme, and Antibiotic-Antimycotic (100X) were supplied by gibco Thermo Fisher Scientific, fetal bovine serum by Biowest, thrombin from human plasma by Sigma-Aldrich, human fibrinogen by Milan Analytica, factor XIII (Fibrogammin 1250) by CLS Behring, recombinant human β-Nerve Growth Factor by PeproTech. For staining, Triton-X 100 was provided by Sigma-Aldrich, 4',6-diamidino-2-phenylindole and paraformaldehyde byAppliChem, Alexa Fluor 594 Phalloidin by abcam, neuronal class III (Tuj1)

monoclonal antibody mouse-derived by BioLegend, Rhodamine (TRITC) goat-derived anti mouse antibody by Sanbio.

Synthesis and analysis of 3kDa 6-arm star-PEG-acrylate

The functionalization of 6-armed poly(ethylene oxide-*stat*-propylene oxide) with acrylate groups (star-PEG-A) was performed according to literature (*1*). In short, hydroxyl-terminated sP(EO-*stat*-PO) (3kDa) (101.80 g, 0.204 mol OH groups) was dried at 80°C for 20 h. Subsequently, toluene (750 mL) and pyridine (24.2 g, 0.306 mol, 1.5 eq) were added to the solution. Acrylic acid anhydride (33.38 g, 0.265 mol, 1.3 eq) was added dropwise to the solution at room temperature. After stirring for 24 h, toluene was removed under reduced pressure, the residue was taken up in dichloromethane, and the polymer was purified by precipitation in cold diethyl ether (5 times).

Degree of functionalization was determined by ^{1}H NMR analysis with a Bruker DPX-400 FT NMR spectrometer (400 MHz). Results are reported as follows: chemical shift d (ppm) (multiplicity, number of protons, assignment). TMS (δ=0.0 ppm) was used as internal standard. Chemical shifts are reported to the nearest 0.01 ppm. Yield: 72.3 g (64%). Degree of functionalization: 98 %. ^{1}H NMR (CDCl$_3$): □ (ppm) = 1.08 (d, 3H, -CH$_3$ PEG), 1.20 (d, 3H, PEG-OCH$_2$CH(CH_3)OCOCHCH$_2$), 3.20-3.80 (m, PEG backbone), 4.20-4.30 (m, 2H, PEG-CH$_2$CH_2OCOCHCH$_2$), 4.94-5.16 (m, 1H, PEG-CH$_2$CH(CH$_3$)OCOCHCH$_2$), 5.74-5.84 and 6.27-6.42 (m, 2H, PEG-OCOCHCH_2), 6.00-6.12 (m, 1H, PEG-OCOCHCH$_2$).

Molecular weights (M_n and M_w) and dispersity values (Đ = Mw/Mn) were determined by size exclusion chromatography (SEC) as M_n= 2200 g/mol; M_w=2300 g/mol; Đ = 1.1. SEC analysis was carried out with dimethylformamide (DMF, HPLC grade, VWR). DMF-SEC was performed using an Agilent 1100 system equipped with a dual RI-/Visco detector (ETA-2020, WGE). The eluent contained 1g/L LiBr (≥99%, Sigma Aldrich). The sample solvent contained traces of water (HPLC grade, VWR) as internal standard. One pre-column (8x50 mm) and four PSS Gram gel columns (8x300 mm) were applied at a flow rate of 1.0 mL/min at 40°C. The diameter of the gel particles measured 10 µm, the nominal pore width were 30, 10^2, 10^3 and 3000 Å. Calibration was achieved using narrowly distributed PEG standards (PSS Mainz). Results were evaluated using the PSS WinGPC UniChrom software (version 8.1).

Preparation of microgel pre-polymer solution

10 wt/vol% of the photoinitiator 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959) was dissolved in DMSO and added to the required amount of star-PEG-A in a molar ratio of 1% acrylates. 20 mg/mL fluorescein o-acrylate was added for fluorescent imaging. For water-based microgel precursor solutions, components were dissolved in 70 vol% ethanol.

After stirring for 4 h, the pre-polymer solution was used pure or diluted in a non-reactive polymer diluent (200 g/mol PEG-OH, 3000 g/mol star-PEG-OH, 18000 g/mol star-PEG-OH) or water. Surface tensions were measured with a tensiometer (DSA 100, Krüss).

Fabrication of microgels with mold-based soft lithography

Before usage, silica wafers were cleaned by 20 min incubation in acidic piranha solution (3:1 sulfuric acid to hydrogen peroxide), washing in water, and 10 min sonication in acetone and isopropanol. Mold-based soft lithography methodology was derived from literature (2). In short, perfluoropolyether (PFPE) molds were prepared by pouring the pre-polymer with 1 w% Irgacure 1173 onto an inverse-patterned silica wafer and UV-curing for 120 min under a constant nitrogen stream. Via a supportive PET-sheet, PFPE-molds were peeled off the wafer. The microgel pre-polymer solution was cast onto the mold and spread via a sacrificial PET-sheet. Upon removal of PET, molds were placed within a nitrogen atmosphere and UV-cured for 60 min. Excess gel was cut off and the mold was placed into a 50 wt/vol% PVP layer and tightly pressed. The PVP glue layer was dried for 4-5 days at 40 °C, followed by removal of the mold. Microgels were dispersed in water and purified by centrifugation at 4500 g for 10 min and re-dispersion in water, which was repeated two times.

Preparation and characterization of star-PEG-A hydrogel disks

A PDMS layer of 1 mm thickness was punched to form 12 mm round cavities, into which the pre-polymer solution was inserted. The cavity was enclosed by a flat PDMS layer. The pre-polymer solution was gelled by UV-curing for 1 h in nitrogen atmosphere inside a glovebox (InerTec AG). Gel disks were washed three times in water for 30 min to extract the non-reactive polymer diluent and non-incorporated SPIONs. Swelling degrees were determined by weighing the hydrogel disks at room temperature in water swollen state and after drying for 48 h at 40 °C. For mechanical characterization, hydrogel disks were subjected to unconstrained compression at room temperature using a DMA device (Q800 DMA, TA Instruments) equipped with a submersion clamp. The elastic modulus was calculated from the slope of the linear region of the stress-strain curves, obtained by applying a force ramp of 1 N/min towards the samples.

For cryo-FE-SEM, swollen gels were frozen in liquid ethane for 1 min and transferred into liquid nitrogen. Hydrogel cross-sections were cut inside the FE-SEM pre-chamber and samples were visualized at 1 kV and 1 µA with FE-SEM SU4800 (Hitachi Ltd. Corporation) after 4 min sublimation (Fig. S5) and another 6 min (Fig. S6) down to -80 °C. Microgel dispersions were loaded onto rivet sample holders and correspondingly frozen and visualized after 10 min sublimation. For cryo-sections, hydrogels were embedded in O.C.T. gel inside 10 x 10 x 5 mm cryo molds and frozen in liquid nitrogen. A cryotome (Leica Camera AG) was used to polish the sample and cut sections of 10 µm thickness, which were

collected onto glass slides. For FE-SEM imaging, samples were sputtered with gold/palladium (Sputtercoater EM ACE600, Leica) before FE-SEM analysis with 5 kV and 5 µA.

Dispersion and quantification of SPIONs

Before usage, SPIONs were ultrasonicated 5 min on ice at 0°C. The amplitude was set to 10 % of maximum. The wave function was modulated on a staircase function with the frequency of 1s and a treatment interval of 1 s to 1 s break. The non-reactive polymer diluent was mixed with the desired amount of SPIONs in water, making up 5 vol% of the dispersion, and ultrasonicated with the same parameters. Star-PEG-A with photoinitiator and fluorescein o-acrylate was blended and ultrasonicated for 1 min. The final dispersion was stored on ice and used for molding within 30 min. For quantification of SPION retention, a high amount of SPIONs (5.44 mg/mL) was dispersed in the pre-polymer solutions to achieve detectability. Gels of 25 µL volume were cured for 120 min with UV in nitrogen atmosphere and afterwards washed for at least 3 times 30 min in water. They were dried at 40 °C for 48 h and ionized for elemental analysis via first nitric acid (65 %) treatment and second hydrochloric acid (30 %) in microwave. The ICP-atom emission spectrometer Plasma 400 (Perkin Elmer) was used for iron quantification with a reliable quantification limit of 50 µg/L. For the release studies, SPION-loaded hydrogels were incubated in 1 mL PBS at 37 °C and the supernatant was replaced by fresh PBS at day 1, 7, 14, and 28 for measurement. TEM measurements of microgels were performed by molding 400 µg/mL SPIONs in 10 wt/vol% star-PEG-A, diluted in PEG-OH 0.2 kDa into 1 x 1 x 10 µm cavities. After curing and harvesting, microgels were purified as above and placed onto carbon-coated cupper grids (PLANO, CF300-CU). Microgels were dried for 24 h at 40 °C and visualized by the STEM SU9000 (Hitachi Ltd. Corporation) in TEM mode at 30 kV.

Analysis of microgel orientation

For orientation analysis, purified microgels were counted with a Neubauer counting chamber and the concentration was adjusted to $5 \cdot 10^5$ microgels/mL. Aliquots of 10 µL were pipetted onto small glass pieces, which were placed in magnetic inserts of 100 or 300 mT magnetic fields (LUM GmbH). To determine the longest possible orientation time, microgels were pre-aligned, followed by a shift of the magnetic field of 90°. The orientation rotation was recorded by bright field microscopy time-lapse experiments with 1 image/s. The videos were imported into ImageJ and the dominant orientation and coherency were measured with the plugin OrientationJ. The stagnation of the relative coherency is applied for determination of the orientation time. When the relative coherency (related to maximum coherency) changes less than 0.1 % per second within an interval of 5 s, the 5th point (second 5) was considered as the moment of alignment (see red marks in Fig. S10a).

To fix the microgel position and orientation, they were re-dispersed in PBS and mixed with fibrinogen (4 mg/mL in final gel). After 15 min activation at 37 °C of the enzyme solution, consisting of factor XIII (fibrogrammin, 4 U/mL in final gel), thrombin (0,125 U/mL in final gel), and calcium chloride in HEPES buffer (5 mM in final gel), both components were mixed and microgels were aligned in magnetic inserts for 20 min at 37 °C. To label the fibrin, 20 µg/mL of the applied fibrinogen was labeled with the NHS-Rhodamine Antibody Labeling Kit (Thermo Scientific) according to the manufacturer's protocol. Images were acquired by laser scanning confocal microscopy and the relative distribution of microgel orientation was determined by OrientationJ (Distribution function, Fourier Gradient, Gaussian window 1 pix, 70 % minimum coherency, 2 % minimum energy according to Rezakhaniha et al. (3)) with 15° binning.

Cell culture

Cell culture experiments were carried out with L929 mouse-derived fibroblasts or isolated dorsal root ganglions (DRGs) from day 10 chicken embryos. Fibroblasts were cultured in a basal medium consisting of DMEM, supplemented with 10 % fetal bovine serum and 1 % antibiotics/antimycotics, at 37 °C, 5 % CO_2 and 95 % humidity. Microgels required more rigorous washing steps to be applicable for cell experiments, consisting of acetone washing for 2 h before harvesting and 2 x ethanol, 2 x water, 2 x medium after harvesting.

For MTS cell viability analysis, equivalently washed hydrogels with SPIONs were incubated 24 h in medium, which was applied onto cells (5000 cells/well in 96 well plate) after one day cultivation. At day 0, 2, or 5 the medium/extract was removed and 20 µL MTS reagent with 100 µL fresh medium/extract were added to the cells. MTS signals after 3 h were used for survival and proliferation rate determination.

To assure sterility while applying a magnetic field for composite hydrogels, glass bottom PDMS wells were prepared (5 mm inner diameter, 8 mm outer diameter) by treating upper surfaces of PDMS and glass with oxygen plasma for 10 s with 250 W (41 mL/min oxygen flow) and attaching PDMS and glass tightly. These custom-made wells were autoclaved before cell experiments. Magnetic inserts were built by cutting and bending stainless steel stripes to separate two cylindrical magnets (10 mm diameter, 3 mm thickness, N42, supermagnetic.de; Fig. S11b). The magnets were glued to the insert by 2K metal glue and disinfected by 70 % ethanol and 30 min UV irradiation prior to use. The magnetic field in between the magnets was determined to be 130 mT by measurements with a magnetometer (Koshava 5, wuntronic).

For orientation of microgels with fibroblasts, washed microgels were counted with a Neubauer chamber and the concentration was adjusted accordingly (microgel volume corresponding to PFPE mold cavity volume). Microgels in media were mixed with fibrinogen and the activated enzyme solution (same conditions as

described above, 8 mg/mL fibrinogen). 30 µL liquid dispersion was inserted per PDMS well, which was standing in the middle of the magnetic insert (most homogenous part of the magnetic field). The filled wells were kept for 10 min inside the inserts, followed by 10 min at 37 °C to assure complete gelation. Afterwards, 1.5 mL media was added and fibroblasts were cultivated for 2 days.

For orientation of microgels with dorsal root ganglia (DRG), fertilized chicken eggs from top-class brown laying hens (bruteiershop.de) were incubated for 10 days at 37 °C and a humidity of 40 – 50 %. DRGs were isolated and stored in Hank's Balanced Salt Solution (HBSS) supplemented with 6 g/L glucose until use. For cultivation of DRGs in composite hydrogels, the composite precursor solution was prepared as before, but with a lower fibrinogen concentration (4 mg/mL). After or before pipetting into a glass-bottom PDMS well, DRGs were quickly placed in the middle of the non-crosslinked microgel-fibrin solution with a Ti #5 forceps (Plano GmbH), while being positioned inside the magnetic field. After 10 min in the magnetic field, gels were incubated for 10 min at 37 °C, and 1.5 mL of basal medium was added, consisting of RPMI, supplemented with 10 % fetal bovine serum and 1 % antibiotics/antimycotics and 20 ng/mL β-Nerve Growth Factor (NGF). DRGs were cultivated at 37 °C, 5 % CO_2 and 95 % humidity for 5 days and after 2 days media was exchanged. In the case of single primary neurons, DRGs were incubated for 30 min in Trypsin/HBSS solution, followed by mechanical dissociation with Pasteur pipettes. The cell suspension was subjected to a 2 h panning step to separate the neurons from the other more adherent cells, followed by collection and counting. We confirmed high purity of neurons by staining against F-actin and β-tubulin (data not shown). 500 neurons per µL were added to 30 µL liquid dispersion (microgels with fibrin precursor solution), which was inserted per PDMS well, standing in the middle of the magnetic insert (most homogenous part of the magnetic field). The filled wells were kept for 10 min inside the inserts, followed by 10 min at 37 °C to assure complete gelation. Afterwards, 1.5 mL media (DMEM), supplemented with 20 ng/mL β-NGF, was added and neurons were cultivated for 7 days.

Staining of cells

Cells were first washed 3 times 30 min with PBS and fixed with 4 % paraformaldehyde for 60 min, followed by 2 times washing with PBS for 30 min. 0.1 % Triton-X 100 was added and incubated for 60 min. Samples were washed 2 times with PBS again for 30 min and then blocked for 60 min with 1 % BSA. Afterwards, antibodies were added (1:250 Tuj1 monoclonal antibody mouse-derived for nerve cells, 1:1000 Alexa Fluor 594 Phalloidin for fibroblasts) and incubated overnight, followed by 3 times washing for 30 min with PBS. In the case of neuronal staining, the secondary antibody (1:100 Alexa Fluor 633 goat-derived anti mouse antibody) was added, incubated for 4 h and washed as before. In case of nucleus staining, DAPI was added, incubated for 1 h and washed 3 times with PBS for 30 min. Samples were visualized with laser

scanning confocal microscopy (SP8 Tandem Confocal, Leica Microsystems Inc.), using a photodiode 405 (DAPI), argon laser adjusted to 488 nm emission (fluorescein in microgels), diode pumped solid state laser 561 nm (Alexa Fluor 594), or Helium-neon laser 633 nm (Alexa Fluor 633).

Analysis of cell orientation

To determine the distribution of orientation, the OrientationJ Distribution function was applied, using the Fourier Gradient, a Gaussian window of 1 pix (fibroblasts) or 4 pix (DRGs), a minimum coherency of 20 %, and a minimum energy of 2 % (fibroblasts) or 5 % (DRGs) (*3*). Counts per degree were related to the sum of all counts, providing the relative frequency. Single cell analysis was performed with the OrientationJ Measure function, where single cells were marked and their orientation was determined. No cell accumulations/overlaps were added to the measurements. Coherencies below 20 % were filtered out to assure high enough quality of the determined orientation (*4*). Afterwards, cell orientation was related to the microgel orientation. In the case of DRG neurite outgrowth analysis, neurite extension was quantified starting from the full white circles depicted on the figures (Fig. 4C, Fig. 5A).

Statistical analysis

Statistical analysis was performed with OriginPro 2016G. Depending on the number of groups, a one-way (Fig. 3a) or two-way (Fig. 2c, 2d, 3d, 5c) ANOVA was executed with *post-hoc* Bonferroni comparison for evaluation of statistical significance between groups (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). Data shown as mean average with error bars indicating the standard deviation.

Supplementary references

1. M. C. Lensen *et al.*, Micro- and Nanopatterned Star Poly(ethylene glycol) (PEG) Materials Prepared by UV-Based Imprint Lithography. *Langmuir* **23**, 7841-7846 (2007).
2. K. P. Herlihy, J. Nunes, J. M. DeSimone, Electrically driven alignment and crystallization of unique anisotropic polymer particles. *Langmuir* **24**, 8421-8426 (2008).
3. R. Rezakhaniha *et al.*, Experimental investigation of collagen waviness and orientation in the arterial adventitia using confocal laser scanning microscopy. *Biomech Model Mechanobiol* **11**, 461-473 (2012).
4. K. Mandal, I. Wang, E. Vitiello, L. A. C. Orellana, M. Balland, Cell dipole behaviour revealed by ECM sub-cellular geometry. *Nat Commun* **5**, (2014).

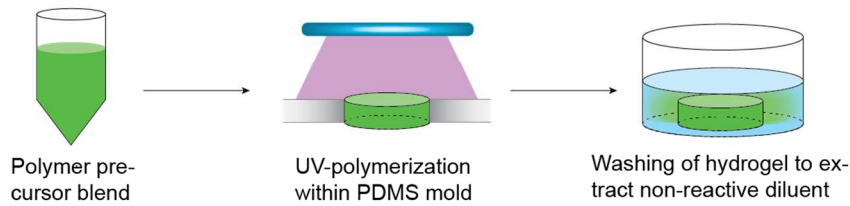# Supplementary figures

Supplementary Figure 1
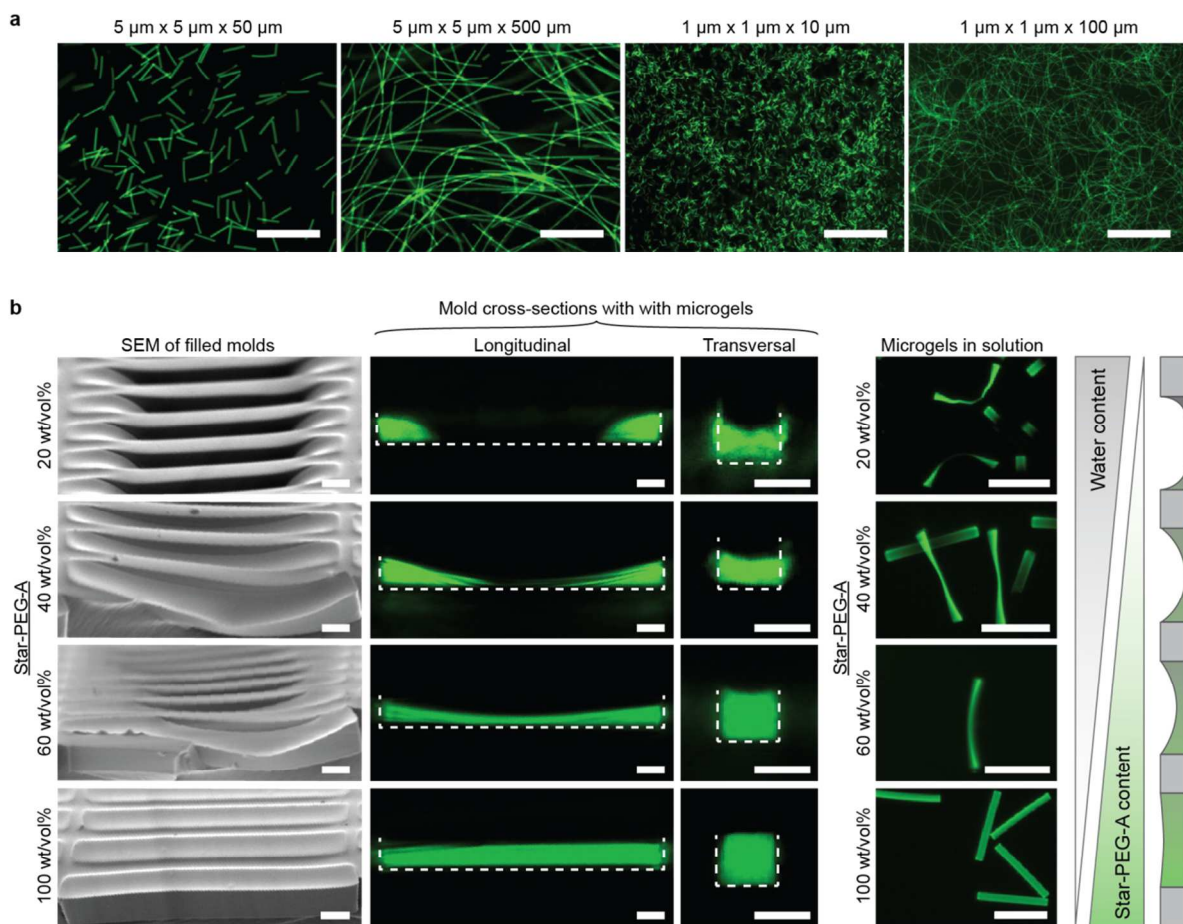
Supplementary Figure 1: Influence of pre-polymer properties on molding. **a,** Molding 100 wt/vol% star-PEG-A enabled molding of polymeric particles in various geometries. **b,** By diluting star-PEG-A with water down to 20 wt/vol%, microgels obtained a convex shape, as water evaporates, leaving only star-PEG-A behind, visible by SEM of filled molds or fluorescent microgels in mold cross-sections. Cavities are outlined by dotted white line. Scale bar is 50 µm in A and C and 5 µm in B. Green: fluorescein.

Supplementary Figure 2
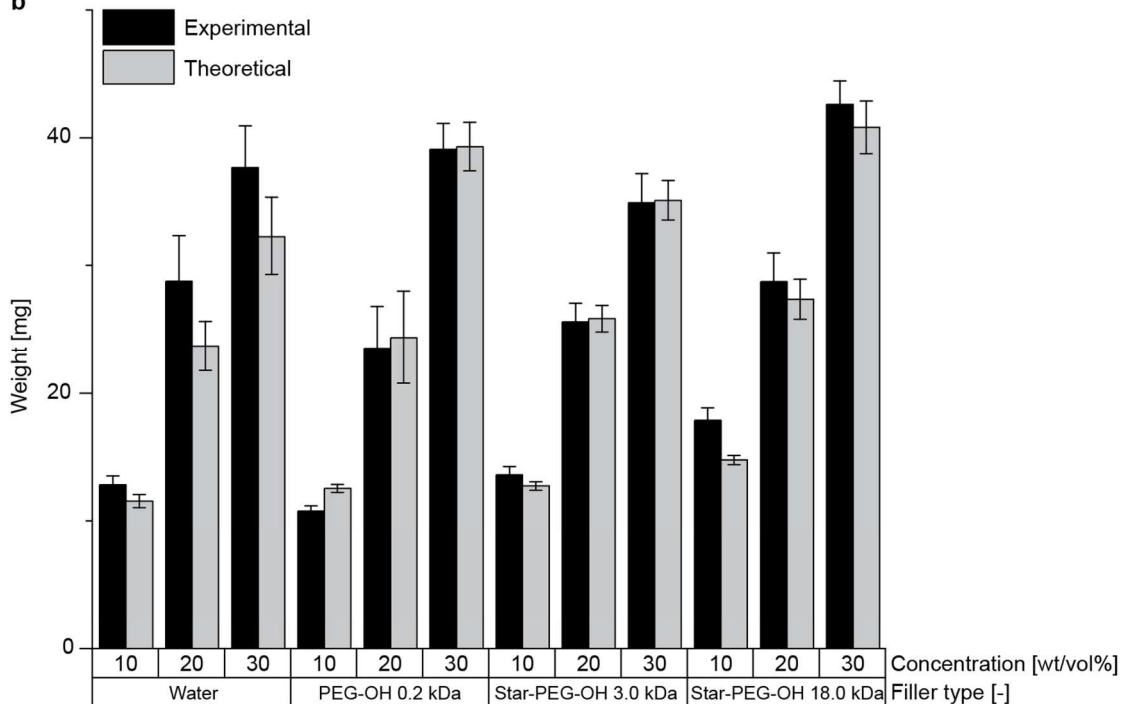
Supplementary Figure 2: Extraction of PEG-diluent. **a,** Procedure of hydrogel preparation, curing, and extraction. **b,** Comparison between experimental and theoretical dry weight of hydrogel disks after washing repeatedly 3 times for 30 min. Hydrogels consisted of 10, 20, or 30 wt/vol% star-PEG-A mixed with water, PEG-OH 0.2 kDa, star-PEG-OH 3.0 kDa, or star-PEG-OH 18.0 kDa. 3-way ANOVA showed no significant difference between experimental and theoretical weight, as the p-value is greater than 0.05, confirming complete removal of the non-reactive polymer diluent.

Supplementary Figure 3
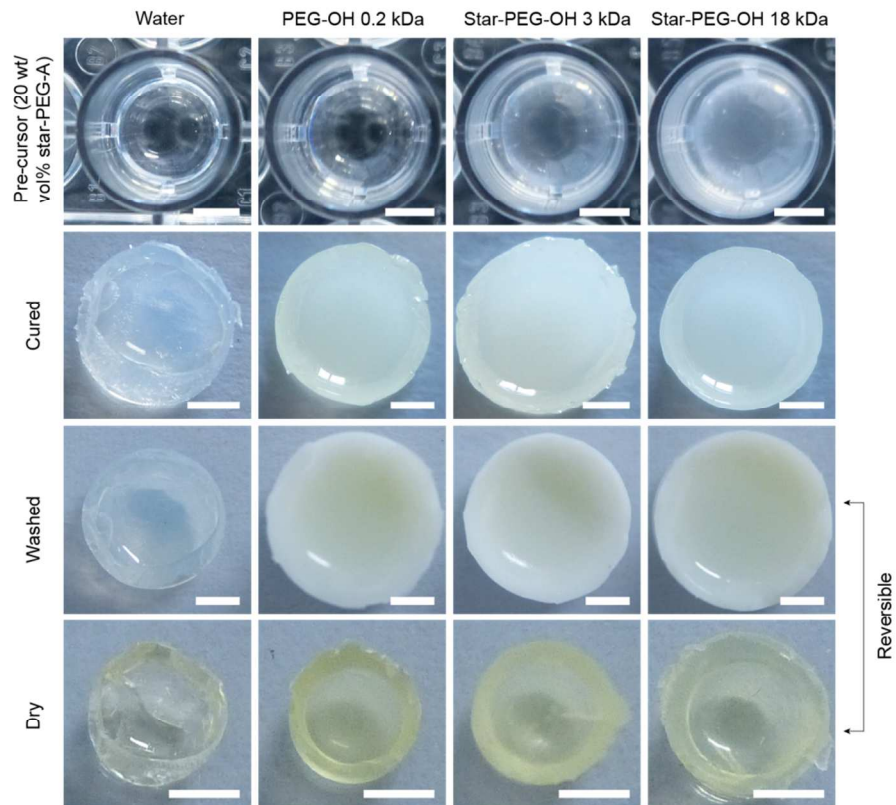
Supplementary Figure 3: Pictures of 20 wt/vol% star-PEG-A mixed with water or different non-reactive PEG-based diluents as pre-cursor solution, after curing, washing and drying. Changes in hydrogel opacity between water-swollen and dry state were reversible. All Scale bars are 2 mm.
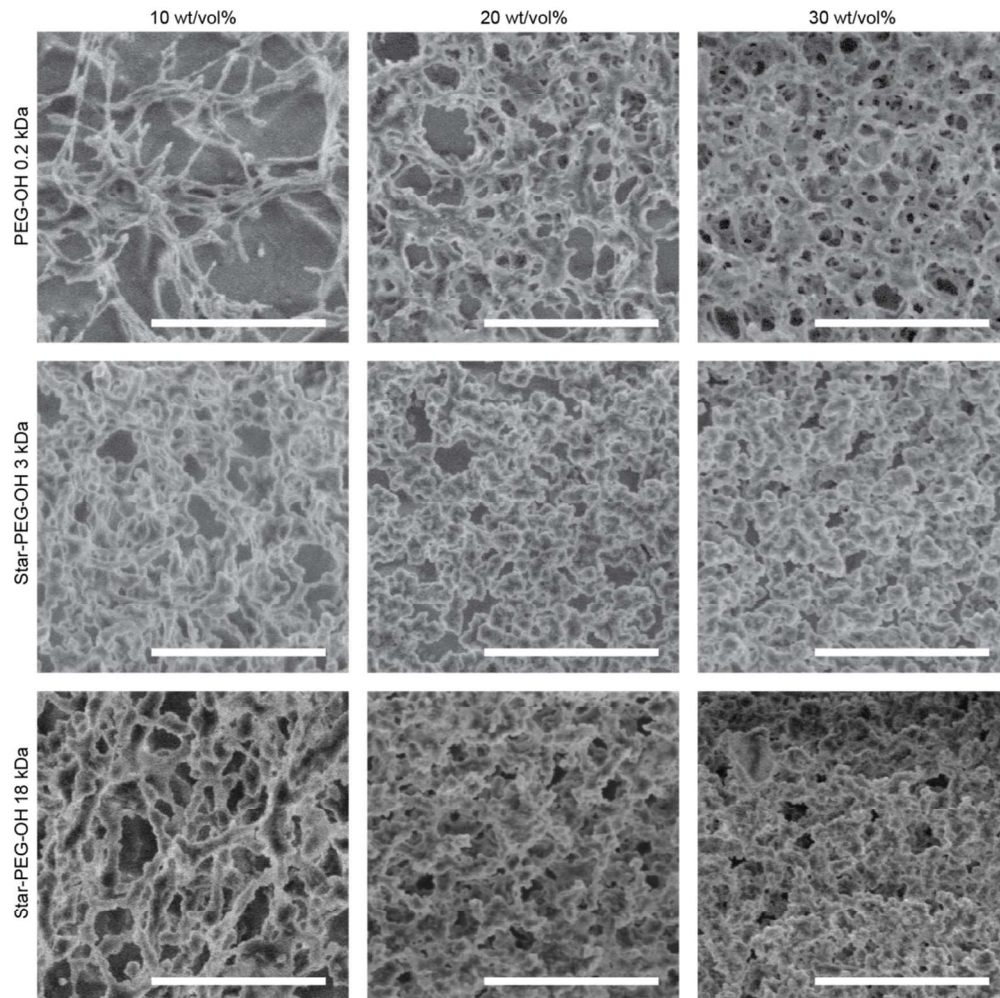
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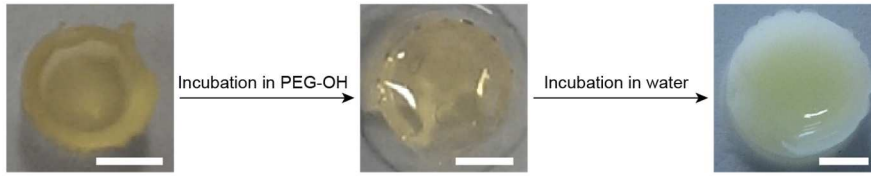
Supplementary Figure 4: Swelling of a dry 20 wt/vol% star-PEG-A hydrogel in 200 Da PEG-OH did neither lead to a volume increase, nor to a loss of transparency, whereas water did. All scale bars are 2 mm.
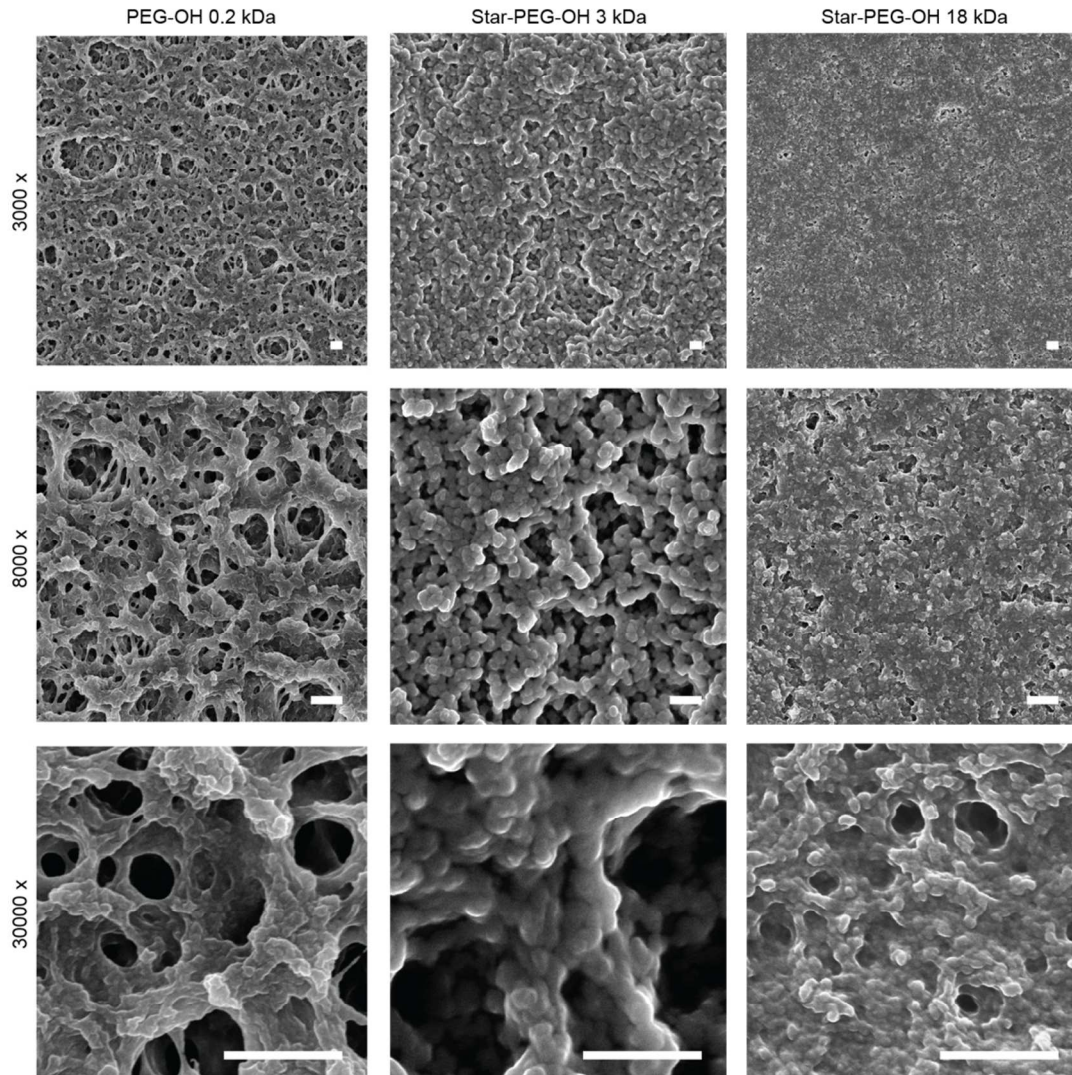
Supplementary Figure 5
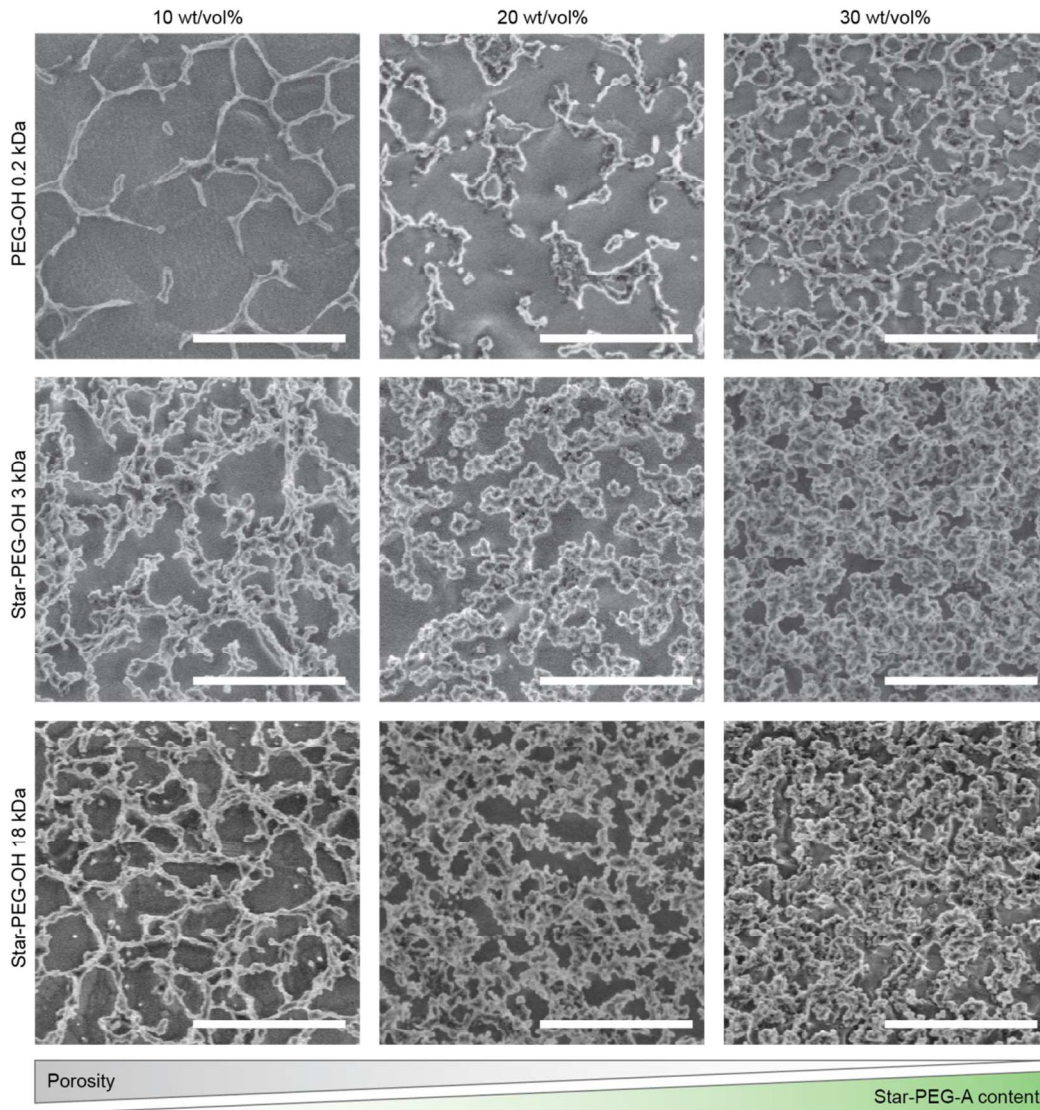
Supplementary Figure 5: Cryo-FE-SEM images of hydrogel cross-sections after 4 min sublimation time. Star-PEG-A contents of 10, 20, or 30 wt/vol% were achieved by diluting with different non-reactive PEG-OH components. Hydrogels were cured, washed and frozen in liquid ethane. Frozen hydrogels were cut inside the FE-SEM pre-chamber to visualize cross-sections. The pore size reduced upon an increasing star-PEG-A content. All scale bars are 5 µm.

Supplementary Figure 6

Supplementary Figure 6: Cryo-FE-SEM images of hydrogel cross-sections after 4+6 min sublimation time. Star-PEG-A contents of 10, 20, or 30 wt/vol% were achieved by diluting with different, non-reactive PEG-based diluents. Hydrogels were cured, washed and frozen in liquid ethane. Frozen hydrogels were cut inside the FE-SEM pre-chamber to visualize cross-sections. Different gel morphologies were observed. The 200 Da PEG-OH diluent led to a mesh structure at 20 and 30 wt/vol% star-PEG-A, whereas both star-PEG-OH diluents showed a coarse globular structure at these concentrations. At 10 wt/vol% star-PEG-A these morphological differences were not visible, however, star-PEG-OH diluents had a reduced pore size in comparison to 200 Da PEG-OH. All scale bars are 4 μm.
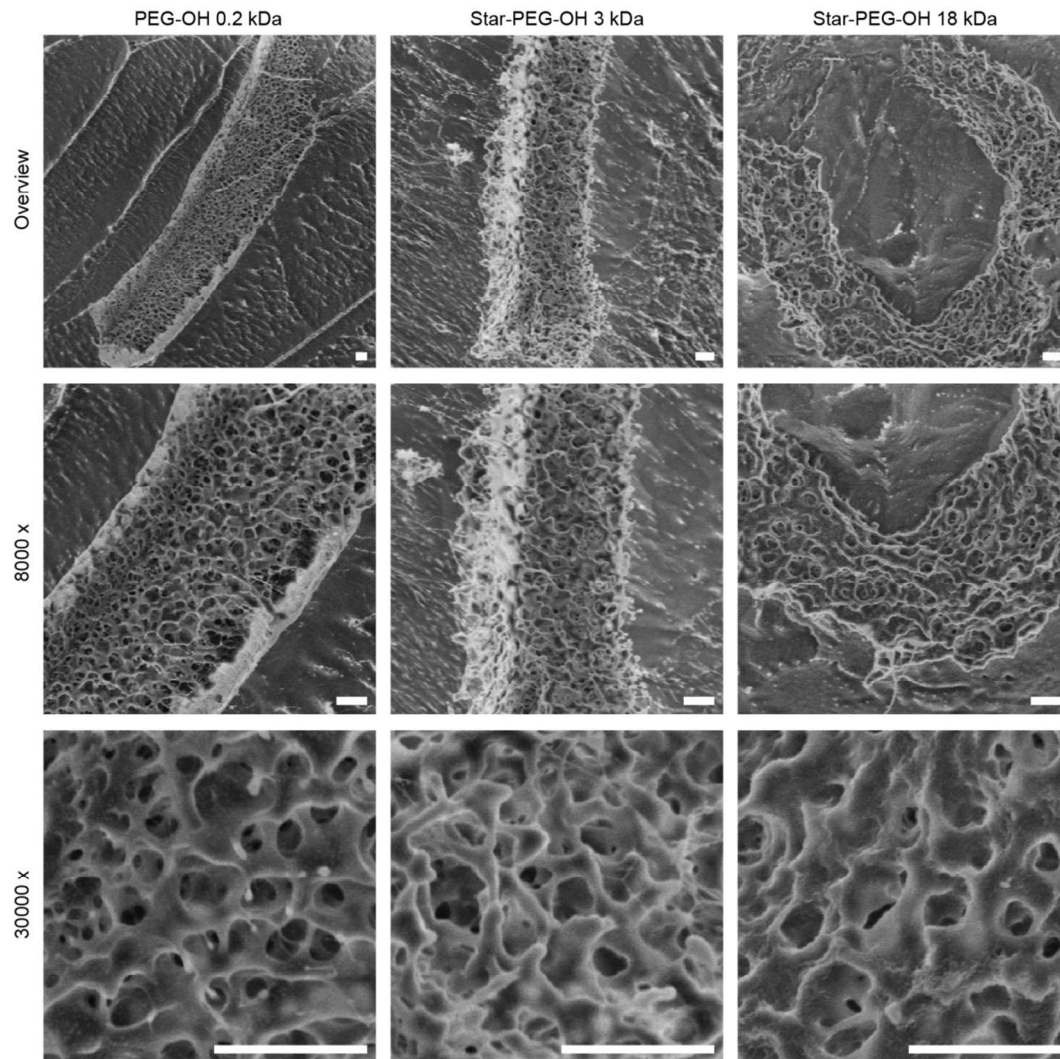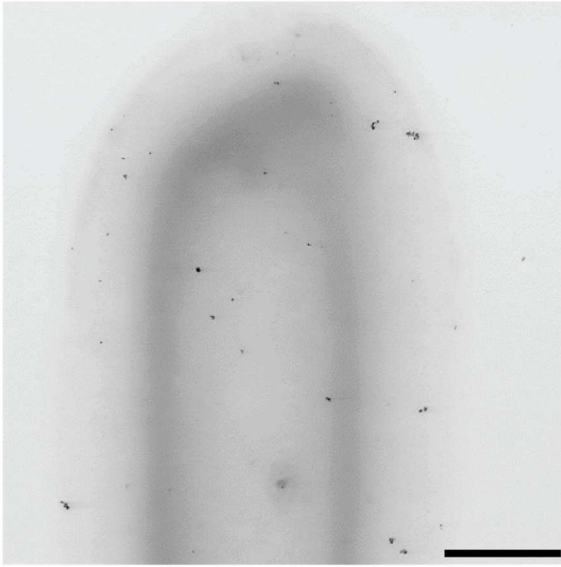
Supplementary Figure 7

Supplementary Figure 7: FE-SEM images of 20 wt/vol% star-PEG-A hydrogel cryo-cuts blended with different non-reactive PEG-based diluents. Hydrogels were cured, washed and frozen within OCT gel by dipping into liquid nitrogen. Gels were cryo-sectioned and 10 µm thick sections were collected on glass slides. Samples were dried to remove all remaining water and sputtered before FE-SEM visualization. Similar observations regarding the difference in hydrogel morphology as in Fig. S6 can be made. All scale bars are 1 µm.

Supplementary Figure 8

Supplementary Figure 8: Cryo-FE-SEM images of 20 wt/vol% star-PEG-A microgels prepared by diluting star-PEG-A with different non-reactive PEG-diluents. Microgels were visible after 10 min sublimation time. The heterogeneous structure of large hydrogels is translated to the microgels, as these inherited similar microporous morphologies. All scale bars are 1 μm.
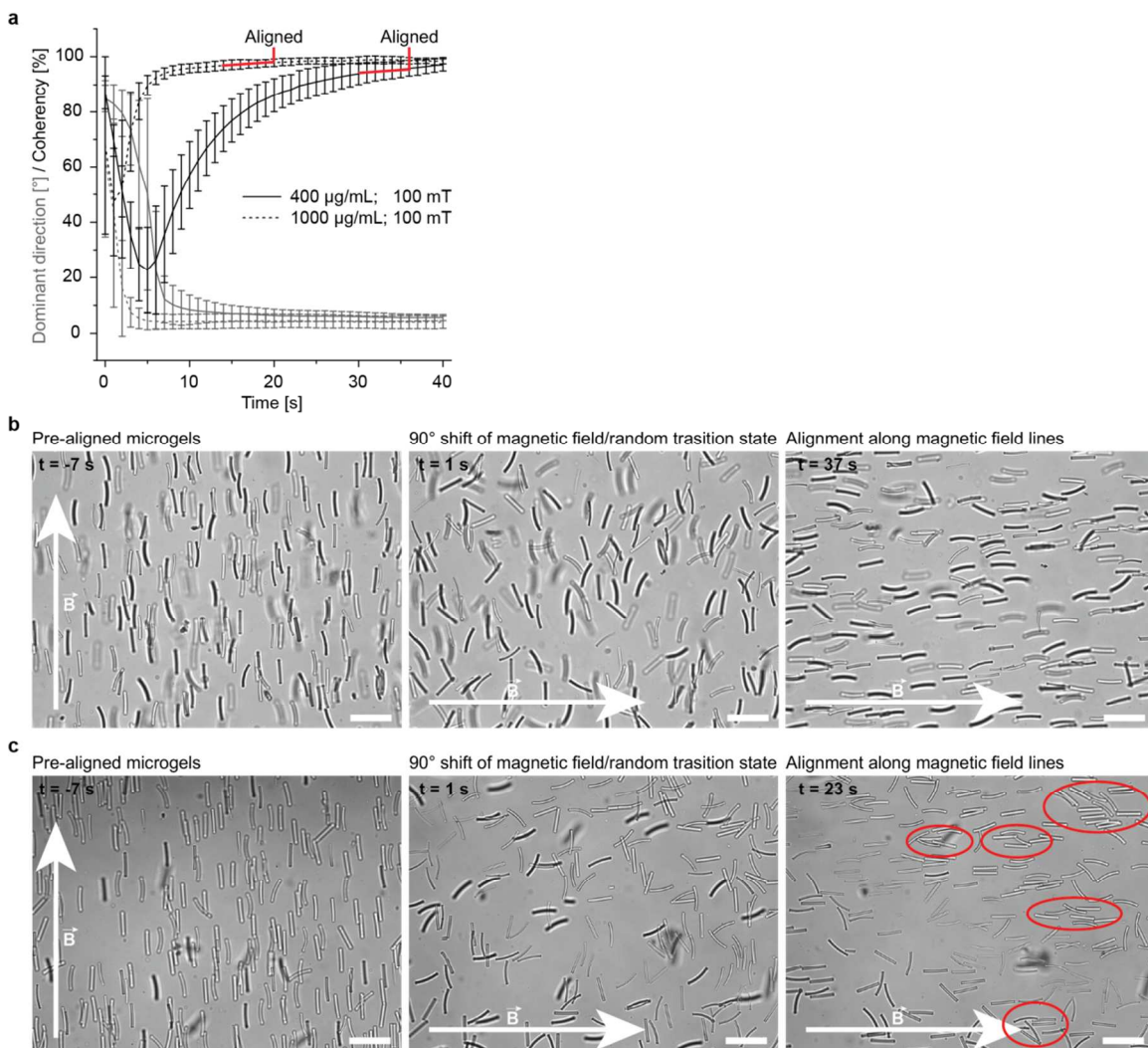
Supplementary Figure 9

Supplementary Figure 9: TEM of 1 x 1 x 10 µm³ microgel, loaded with 400 µg/mL SPIONs, revealed low aggregation and well overall dispersion. As the microgels were in the dried state, adsorbed to the TEM grid, microgels extended, causing larger dimensions than in dispersion. Scale bar is 1 µm.
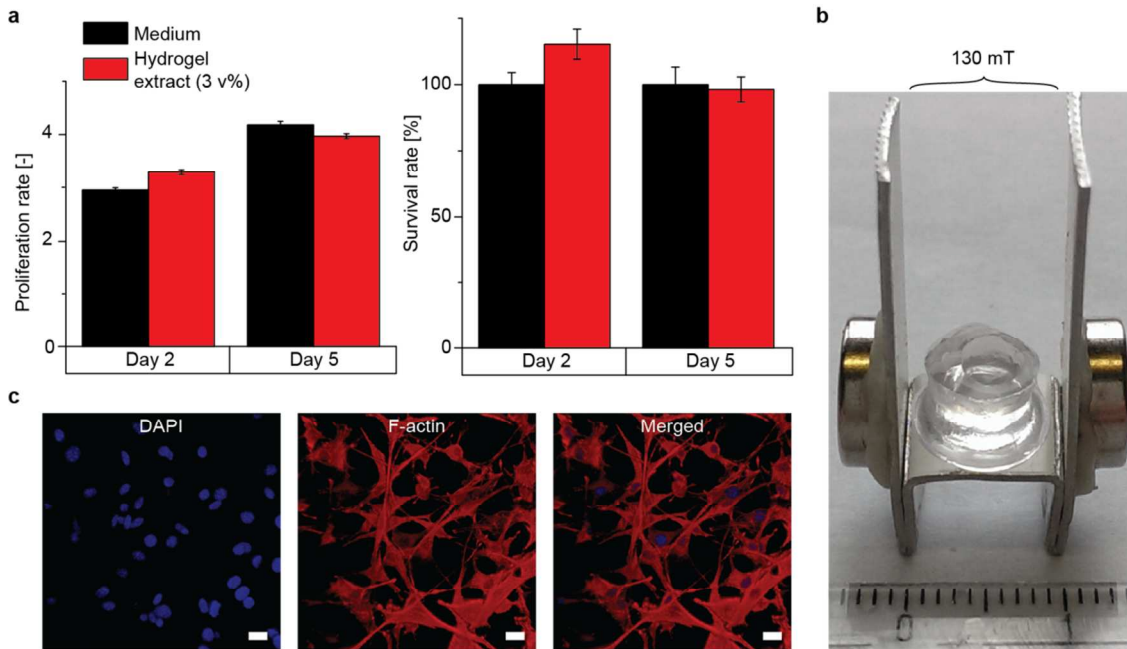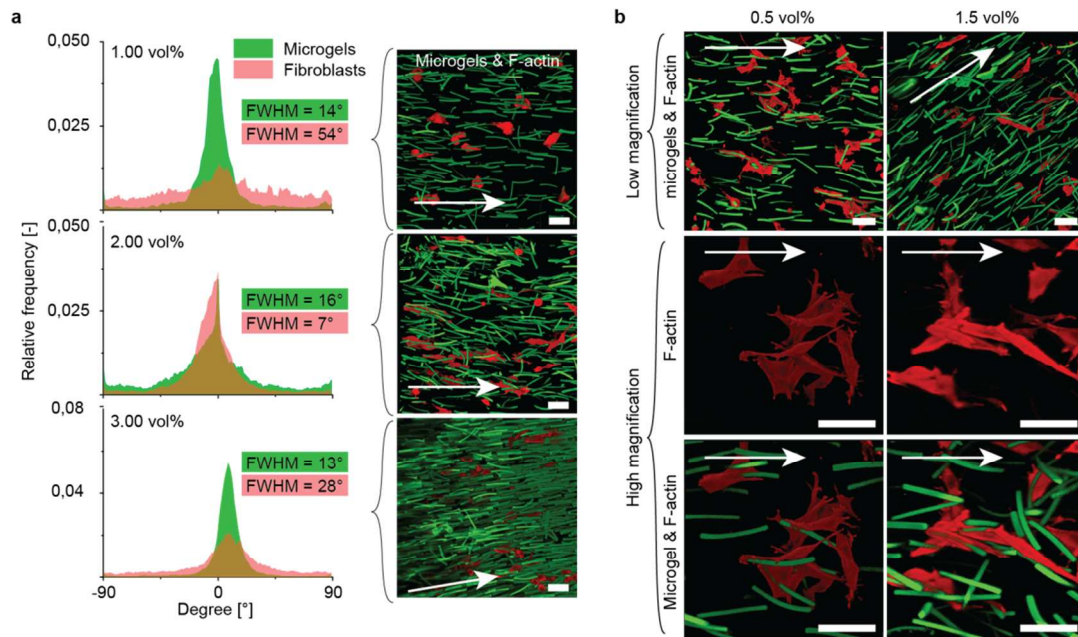
Supplementary Figure 10

Supplementary Figure 10: Set-up for orientation analysis of aligning magnetoceptive microgels. **a,** Dominant direction and relative coherency of direction over 40 s in a 100 mT magnetic field, determined by OrientationJ. When the relative coherency changed less than 0.1 % per second over a period of 5 s, microgels were considered as aligned (see red marks), which was applied to determine orientation times. **b,** Microgels were doped with 400 µg/mL SPIONs and the response in a 100 mT field was recorded. First, microgels were pre-aligned to be able to determine the maximum time necessary for microgel alignment. After a random transition state, the microgels aligned within the magnetic field until after 37 s, no further improvement (change in coherency) was quantified according to the method. **c,** 1000 µg/mL SPIONs were incorporated into the microgels and the response in a 300 mT field was recorded. Despite the fast response, the coherency of the alignment direction was instable due to stronger microgel interaction (aggregation; red circles) and movement. White arrows indicate the magnetic field direction. All scale bars are 100 µm.

Supplementary Figure 11

Supplementary Figure 11: Optimization of the cell experiments. **a,** Proliferation and survival rate of fibroblasts, which were either cultivated in cell media (black) or in media extract, which was incubated for 24 h in washed and purified 20 wt/vol% star-PEG-A hydrogel in a volume content of 3 vol% (with PEG-OH 0.2 kDa as diluent, 400 µg/mL SPIONs, in red). **b,** Small magnetic inserts (130 mT) were built to align microgels in a glass bottom PDMS well. **c,** 500 cells/µL (composite) hydrogel and 2 days cultivation time were found optimal for visualizing the fibroblast behavior. Sale bars are 20 µm.

Supplementary Figure 12

Supplementary Figure 12: a, Distribution of microgel and fibroblast (F-actin) orientation in the case of 1.0, 2.0, and 3.0 vol% microgels, showing cell orientation parallel to the magnetic microgel orientation for 2.0 and 3.0 vol% but not for 1.0 vol%. **b,** Ability of composite hydrogel matrix to align fibroblast cell growth. Fibrin hydrogels were doped with 0.5 and 1.5 vol% microgels, which were aligned in a magnetic field of 130 mT. Pre-mixed fibroblasts extended along the longitudinal microgel axis (green: fluorescein), visible by the stretched F-actin filaments (red: Alexa Fluor 594 phalloidin), depending on the microgel concentration. Scale bars in **a** are 20 μm and in **b** 50 μm.

Supplementary Table 1

Interfacial tension between different polymeric solutions and air.

Sample [-]	Mean interfacial tension [Nm/m]
Star-PEG-A organic	36,78 ± 0,26
Star-PEG-A aqueous	38,43 ± 0,26
Star-PEG-OH 3 kDa	39,48 ± 0,15
10 wt/vol% star-PEG-A, diluted in water	45,34 ± 0,32
20 wt/vol% star-PEG-A, diluted in water	43,01 ± 0,48
30 wt/vol% star-PEG-A, diluted in water	41,44 ± 0,53
10 wt/vol% star-PEG-A, diluted in 3 kDa star-PEG-OH	38,99 ± 0,15
20 wt/vol% star-PEG-A, diluted in 3 kDa star-PEG-OH	38,15 ± 0,54
30 wt/vol% star-PEG-A, diluted in 3 kDa star-PEG-OH	38,87 ± 0,24

Supplementary Movie 1

Orientation movement of rod-shaped, magnetoceptive microgels. Microgels (5 x 5 x 50 µm³) are doped with 400 µg/mL SPIONs and the response towards 100 mT is recorded. Scale bar is 100 µm.

Supplementary Movie 2

Interaction of rod-shaped, magnetoceptive microgels. Microgels (5 x 5 x 50 µm³) are doped with 1000 µg/mL SPIONs and the response towards 300 mT is recorded. Scale bar is 100 µm.

Supplementary Movie 3

Confocal image of single nerve cells in Anisogel with 1 vol% microgels. Green: fluorescein-labeled microgels; red: β-tubulin-stained nerve cells (Alexa Fluor 633).