Supporting Information

Disulfide-Based Diblock Copolymer Worm Gels: A New Wholly-Synthetic Thermo-reversible 3D Matrix for Sheet-Based Cultures

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Figure S1. Optical and fluorescence microscopy images showing PVC-lens paper composite sheet impregnated with worm gels containing 10-µm fluorescent polystyrene microspheres nine days after spotting the suspension into the zone of a PVC-lens paper composite sheet and immersing the sheet in 0.15 M PBS at 37 °C. The broken yellow lines indicate the interface between the PVC-blocked region of the mesh sheet, and the zone containing the particle-embedded worm gels.
Figure S2. Optical and fluorescence microscopy images showing PVC-mesh composite sheet impregnated with first generation worm gels containing 10-µm fluorescent polystyrene microspheres nine days after spotting the suspension into the zone of a PVC-mesh composite sheet and immersing the sheet in 0.15 M PBS at 37 °C.
Figure S3. Normalized DMF GPC chromatograms obtained for the disulfide based PGMA-PHPMA diblock copolymer, PGMA$_{54}$ macro-CTA and the disulfide based PGMA$_{55}$-DSDMA$_{0.50}$ macro-CTA obtained using refractive index detection.
Experimental Section

Synthesis and Preparation of Worm Gels

Materials

Glycerol monomethacrylate (GMA; 99.8 %) was donated by GEO Specialty Chemicals (Hythe, UK) and used without further purification. 2-Hydroxypropyl methacrylate (HPMA) and 4,4'-azobis(4-cyanopentanoic acid) (ACVA; V-501; 99 %) were purchased from Alfa Aesar (Heysham, UK). 2-Cyano-2-propyl dithiobenzoate (CPDB, 80 % as judged by $^1$H NMR spectroscopy) was purchased from Strem Chemicals (Newton, UK). Bis(2-(methacryloyloxy)ethyl disulfide monomer (DSDMA) monomer was synthesized according to a protocol reported by Rosselgong et al.$^{[1]}$ CD$_3$OD (99.8 %) and CD$_2$Cl$_2$ (99.8 %) were purchased from Goss Scientific (Nantwich, UK) and used as received. All solvents were of HPLC quality; they were purchased from Fisher Scientific (Loughborough, UK) and used as received.

Synthesis of PGMA$_{34}$ macro-CTA We weighed and combined CPDB RAFT agent (0.864 g, 3.9 mmol) and GMA monomer (25.0 g, 156.1 mmol) into a 100 mL round-bottomed flask and purged under N$_2$ for 30 min. Into the same flask, we added ACVA initiator (218.6 mg, 0.78 mmol; CTA/ACVA molar ratio = 5.0) and anhydrous ethanol (49.6 mL; previously purged with N$_2$ for 30 min), and then degassed the resulting red solution for 10 min. We then immersed the flask containing the degassed solution, and sealed and immersed in an oil bath pre-warmed at 70 °C. After 100 min, we quenched the polymerization by exposing to air, immersing in liquid nitrogen for 30 seconds followed by diluting the solution with methanol (100 mL). A final GMA conversion of 78 % was determined by $^1$H NMR analysis. The methanolic solution was precipitated into a ten-fold excess of dichloromethane. After filtering and washing with dichloromethane, we dissolved the crude polymer in water and the residual dichloromethane was evaporated under vacuum. We freeze-dried the resulting aqueous
solution overnight to yield a pink powder. $^1$H NMR analysis indicated a mean degree of polymerization of 54 for this PGMA macro-CTA. Using a refractive index detector and a series of near-monodisperse poly(methyl methacrylate) calibration standards, DMF GPC analysis indicated an $M_n$ of 14,700 g mol$^{-1}$ and an $M_w/M_n$ of 1.11 (Figure S3).

Copolymerization of DSDMA with GMA via RAFT to afford $P(GMA_{55-stat-DSDMA_{0.50}})$ We weighed CPDB RAFT agent (80 % purity; 0.192 g, 0.69 mmol), GMA monomer (5.00 g, 31.3 mmol) and DSDMA monomer (0.101 g, 0.347 mmol) into a 100 mL round-bottomed flask and purged them under N$_2$ for 30 min. We then added ACVA initiator (38.9 mg, 0.139 mmol; CTA/ACVA molar ratio = 5.0) and anhydrous ethanol (47.6 mL; previously purged with N$_2$ for 30 min) and degassed the resulting red solution for an additional 10 min. The flask was subsequently sealed and immersed in an oil bath set at 70 °C. After 18 hours, we quenched the copolymerization reaction by immersing the flask in liquid nitrogen. A final GMA conversion of 90 % was determined by $^1$H NMR analysis. Overnight storage of this ethanolic reaction solution at -25 °C caused precipitation of the PGMA$_{55}$-DS$_{0.50}$, and thus enabled us to collect the precipitate by decanting the supernatant solution containing the residual co-monomers. We dissolved the precipitate in methanol (100 mL) and then reprecipitated it into a ten-fold excess of dichloromethane. After filtering and washing with dichloromethane, we dissolved the copolymer in water and evaporated the residual dichloromethane under vacuum. We freeze-dried the resulting aqueous solution to yield a pink powder. $^1$H NMR analysis of this PGMA macro-CTA indicated a mean degree of polymerization of 55. DMF GPC analysis indicated an $M_n$ of 16,100 g mol$^{-1}$ and an $M_w/M_n$ of 1.27 (Figure S1).

Synthesis of disulfide-functionalized poly(glycerol monomethacrylate)$_{55}$-poly(2-hydroxypropyl methacrylate)$_{130}$ [0.30$P(GMA_{55-stat-DSDMA_{0.50}}) + 0.70PGMA_{34}]$-$H_{130}$ worm gel via RAFT aqueous dispersion polymerization of HPMA
We weighed PGMA\textsubscript{54} macro-CTA (2.519 g, 0.279 mmol) PGMA\textsubscript{55} -DSDMA\textsubscript{0.50} macro-CTA (1.080 g, 0.120 mmol), HPMA monomer (7.49 g, 51.94 mmol; target DP = 130; ACVA (22.3 mg, 0.080 mmol; CTA/ACVA molar ratio = 5.0) and 0.15 M PBS (44.0 g, to produce a 20% w/w aqueous solution) into a 100 mL round-bottomed flask, which we placed on ice and purged with N\textsubscript{2} for 30 min. Following this degassing protocol, we immersed the flask in an oil bath set at 70 °C. The reaction solution was stirred for 3 h before the RAFT polymerization was quenched by exposure to air. Full monomer conversion was confirmed by \textsuperscript{1}H NMR spectroscopy (complete disappearance of vinyl signals) and DMF GPC analysis indicated an \(\text{M}_n\) of 40,300 g mol\(^{-1}\) and an \(\text{M}_w/\text{M}_n\) of 1.17 (Figure S1).

*Evaluation of the Mechanical Properties of the Worm Gels via Oscillatory Rheology Studies*

We used an AR-G2 rheometer equipped with a variable temperature Peltier plate, a 40 ml 2° aluminium cone and a solvent trap to prevent evaporation of water over the time scale of the experiment. We measured loss moduli (\(G''\)) and storage moduli (\(G'\)) as a function of temperature to determine the gel strength and critical gelation temperature (CGT). We carried out the measurements for temperature sweeps at an angular frequency of 1.0 rad s\(^{-1}\) and a constant strain of 1.0 %. We increased the temperature by 1.0 °C between each measurement, allowing an equilibration time of 2 minutes in each case.

*Fabrication of PVC-Polyester Mesh Composite Sheets:* We prepared the scaffolds using a procedure modified from Simon *et al.*\cite{2} We used a craft cutter (Graphtec Craft ROBO) to cut the patterns and perforations (~3mm in diameter) in a 130 \(\mu\)m-thick sheet of polyvinyl chloride (Warp Bros); the pattern was designed in Adobe Illustrator C4 and can be provided upon request. We used a heated press to melt the sheet of PVC in between two ~90 \(\mu\)m-thick sheets of polyester mesh (McMaster-Carr); the sheets were pressed at 211 °C in three to four cycles of 10-15 seconds to form a single composite sheet (~200 \(\mu\)m thick). We placed the
composite sheets in a glass petri dish and sterilized them by autoclaving. We kept the autoclaved composite sheets in a dry, Parafilm-sealed container until use.

**Cell Culture of A549-GFP Cells:** We cultured A549-GFP cells in 1X Dulbecco’s Modified Eagle Medium (Gibco) with 10% (v/v) fetal bovine serum (HyClone), 1% (v/v) Penicillin-Streptavidin (Gibco). We maintained the cells as adherent cultures in a vented tissue culture flask (Corning) at 37°C and 5%CO₂, and passaged the cells every 4 to 5 days until use. We expressed green fluorescence protein to A549 cells (American Type Culture Collection) by transduction with Cignal Lenti GFP (Quiagen) and 5 mg/mL polybrene (Santa Cruz Biotech) as described by Mammoto *et al.*[3]

**Preparation of 3D Cell Culture:** We chilled the micropipette tips, worm gels, and composite sheets in a 4°C refrigerator at least 24 hours before seeding the cells. We detached the cells from the tissue culture flask by treatment with TrypLE™ Express (Gibco) for 5 minutes at 37°C, suspended them in media, and pelleted by centrifugation at 1,500 rpm. In a typical experiment, we prepared the suspension of cells in either cold worm gel, or cold Matrigel at a concentration of 3 × 10⁴ cells/μL suspension. We kept the suspensions, micropipette tips, and unused composite sheets in ice to avoid premature gelling of the cell suspensions. We spotted 1 μL of the cell suspension into the zones of the composite sheets, and immersed the sheets in 6-well plates containing warm media (37°C).

**Imaging of Cells in the Scaffolds:** We visualized and obtained micrographs of the A549-GFP cells cultured in the composite sheets, or in the 96-well plates using a fluorescence microscope with a CCD camera. We used a Typhoon FLA 9000 gel scanner (General Electric) at a resolution of 50 μm, and a photomultiplier tube setting of 300V to image for the intensity of GFP expressed by A549 cells in the composite sheets. We quantified the intensities of the zones from the images using Image J (NIH).

**Recovery Protocol:** We recovered the cells by incubating the composite sheets in a 6-well plate containing 1 mL/well of the recovery solution. We used cold PBS (4°C) as the recovery
solution for cells embedded in worm gels, and used warm Accumax (37°C) for cells embedded in Matrigel. We washed the cells with cold PBS, and removed the recovery solution by centrifugation at 1,500 rpm.

*Culture and Viability Assay of Recovered Cells:* We prepared suspensions of the recovered cells in media at a concentration of $3 \times 10^4$ cells/mL media. We dispensed 100 μL of the cell suspension in 96-well plates and cultured them at 37 °C and 5% CO$_2$ until measurement of viability using CellTiter-Glo® (CTG) assay. We prepared the CTG reagent as recommended by the manufacturer (Promega). Into each well, we added 100 μL CTG reagent, and incubated the samples for 20 minutes, and measured the luminescence using a PHERAstar FS microplate reader (BMG Labtech).