Materials

Four-arm hydroxyl functionalized PEG (Mn 10,000 g mol−1) was purchased from JenKem Technology USA Inc. (Allen, TX, USA). Nitrous acid depolymerized low molecular weight heparin (LMWH) was purchased from Celsus (Cincinnati, OH, USA). N-(2-aminoethyl)maleimide, trifluoroacetate salt (AEM), 1-hydroxybenzotriazole hydrate (HOBT), 2-(N-morpholino)ethanesulfonic acid (MES), 3-mercaptopropionic acid (MP), *p*-toluenesulfonic acid monohydrate (PTSA), and light mineral oil were purchased from Sigma-Aldrich (St. Louis, MO, USA). N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl), and all other reagents and materials were purchased from Fisher Scientific unless noted (Pittsburgh, PA, USA). Proton nuclear magnetic resonance (1H NMR) spectra were acquired under standard quantitative conditions at ambient temperature on a Bruker DRX-400 NMR spectrometer (Billerica, MA). The spectra of all purified compounds were recorded in either deuterated chloroform or deuterium oxide.

Synthesis of PEG-Thiol

The synthesis of thiolated four-arm PEG was performed as previously reported.[15] In short, PEG (1meq.), 3-mercaptopropionic acid (40meq.) and PTSA (0.4meq.) were dissolved in toluene. Under a flow of nitrogen the reaction was refluxed with stirring for 48hrs. Water was collected by using a Dean Stark trap. Toluene was removed under reduced pressure and the polymer was precipitated 3 times in cold ether. The polymer was reduced by dissolving 1meq polymer in methanol with DTT (1meq) and triethylamine (1meq) under nitrogen for 5hours. The finished reaction was acidified with trifluoroacetic acid (1.1meq) and precipitated in ether and rinsed with 2-propanol then hexane. Functionality was determined via ^{1}H-NMR spectroscopy and was ~4 (>95%) for all derivatives. All products were stored under Argon or vacuum at room temperature to maintain the reduced state of the thiol during experiments.

Synthesis of Maleimide-functionalized LMWH.

LMWH molecular weight was determined by size exclusion chromatography (SEC) using previous described methods. The SEC system comprised of a Waters 515 HPLC pump (Milford, MA, USA), two Waters Ultrahydrogel (7.8 × 300 mm) columns in series, a Waters 2414 refractive index detector, a Waters 2996 photodiode array detector and a Precision Detectors light scattering unit (Bellingham, MA, USA). The number average molecular weight was determined to be 8,300 g mol-1.

The synthesis of maleimide-functionalized heparin was performed as previously described with slight modification to reactant quantities to control the extent of modification.59 Briefly, 500mg LMWH (0.06mMol) was dissolved with 103mg HOBT (0.67mMol), 103mg AEM (0.67mMol) and 103mg EDC·HCl (0.54mMol) dissolved in 50ml of 0.1M MES pH 6.0 (Scheme 2). The reaction proceeded overnight at room temperature with stirring. The product was purified by dialysis (MWCO 1000) against 1M NaCl solution and de-ionoized water. The freeze-dried sample was characterized via 1H-NMR indicating a degree of functionalization of 2.6.

Experimental methods

Preparation of multiple particle tracking microrheology samples

Solutions of four-arm star poly(ethylene glycol) (PEG) end functionalized with thiol (f=4) and maleimide functionalized low molecular weight heparin (LMWH, f=2) were prepared in 0.1M phosphate buffered saline (PBS, 150 mM NaCl, pH 6.0). Prior to the initiation of the gelation reaction, fluorescently labeled probe particles (diameter 2a=1.04±0.02 μm, Polysciences, Inc.) were dispersed in the LMWH solution at a concentration of 0.054% solids per volume. The gelation reaction is initiated by mixing the LMWH and PEG precursor solutions. The hydrogel

forms in a polydimethylsiloxane (PDMS) chamber (6 mm diameter by 800 µm height cylinder, Dow Corning) and remains submerged in 1 × phosphate buffered saline (pH=7.4, Invitrogen) at 37°C. The hydrogel degrades via the hydrolysis of the ester linkage between the PEG and maleimide-functionalized cross-linker.

Multiple particle tracking microrheology

Samples were monitored over 33 days with measurements taken three times a day using multiple particle tracking microrheology. Data was collected in the center of the hydrogel and approximately 200 µm from the bottom of the gel. An inverted microscope (Axiovert 200, Carl Zeiss, Inc.) was used to visual the probe particles at a magnification of 63X (water immersion objective, NA 1.2, 1X optovar, Carl Zeiss, Inc.). The total image magnification gives a resolution of 0.246 µm/pixel. Data was used collected at 30 frames per second and an exposure time of 1 ms using video microscopy (Phantom v5.1, Vision Research Inc.). These acquisition parameters were chosen to minimize the affects of static and dynamic error on the collected data[17]. Static and dynamic particle tracking errors were first identified by Savin and Doyle[17]. Static error refers to error in determining the actual position of a static particle. This error is strongly influenced by the signal to noise of the equipment. Dynamic error refers to the smearing of a particle position if the shutter of the camera is open too long. Dynamic error can be minimized by minimizing the amount of time the shutter is open. These errors must be carefully balanced because shortening the exposure time allows less light into the system which should increase the signal to noise, thereby, increasing the static error[17]. All reported particle motion in the manuscript is above the noise floor of the equipment. Each movie was analyzed using standard particle tracking algorithms[16].

Bulk rheology

Samples were gelled in situ by co-injection of the separately dissolved functionalized materials onto the rheometer peltier plate. An AR-2000 rheometer (TA Instruments) was used to measure oscillatory shear properties at 37°C with maximum shearing amplitude of 1.0%. A 20mm 1°56" cone plate geometry with a 33µm truncation requiring 40µL of solution was used in all experiments. Time sweeps were performed at a constant 6 rad s^{-1} while frequency sweeps were done over a logarithmic scale from 0.1 rad s^{-1} to 100 rad s^{-1}. The rheometer peltier plate was chilled to 25°C for alkyl thiol PEG functionalities and 4°C for thiophenyl PEG functionalities before co-injection of materials. Time sweep data collection and a 3 minute temperature ramp to 37°C was initiated once materials were injected onto the stage. Light mineral oil was applied to the perimeter of the sample to prevent evaporation over the course of the experiment. Greatest storage moduli were obtained when using 1.2x excess functional maleimides over thiols.

The loss in modulus by degradation was monitored by oscillatory rheology by swelling the perimeter of the hydrogel with reducing or non-reducing buffers. Hydrogels were formed as described above with slight modification to the procedure. Before adding the solutions to the peltier plate a polyvinylchloride (PVC) tube with a height of 7mm, inside diameter of 35mm and an outside diameter of 42mm was pre-coated with a thin film of vacuum grease on the bottom and positioned above the geometry by a ring stand and clamp. After the cross-linked hydrogel had reached equilibrium the rheometer bearing was locked to prevent disruption of the hydrogel while the mineral oil was washed away three times with hexanes. The PVC tube was then firmly pressed into the peltier plate creating a water tight seal with the vacuum grease. The volume between the gel and cylinder walls was filled with 2ml of buffer (50mM phosphate, 150mM NaCl, 10mM or 10µM GSH at pH7.4) and capped with mineral oil to prevent evaporation. Because of

the reduced volume a higher buffer concentration was used to maintain a constant pH throughout the experiment. Data points were collected every 5 minutes over 5 days.