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Electronic Supplementary Information

Biodegradable S-nitrosothiol tethered multi-block polymer for nitric oxide delivery

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S1. Polymer Synthesis

(Refer Fig. 1 in the manuscript for the synthetic scheme)

S1a. Poly(LA/GL/HMPA) (1)

L-lactide (1.7 g, 85% w/w), glycolide (0.2 g, 10% w/w), HMPA (0.1 g, 5% w/w) and stannous octoate (0.01 g, 5% w/w of total polymer) were mixed with 2 mL dry dichloromethane (DCM) in a polymerization tube under nitrogen. The solvent was removed under vacuum (between 100-150 torr). Nitrogen and vacuum were then applied alternatively several times to remove any residual moisture before heating the reaction to 70 °C in an oil bath. Vaccum was again applied for 30 min at 70 °C and then replaced with nitrogen and heated to 120 °C to perform the polymerization in the melt phase. After 24 h, the polymerized material was cooled to room temperature and crystallized using a dichloromethane-diethylether mixture (1:20) to remove any unreacted monomers. Yield 1.67 g (83.5%). ^{1}H NMR ($CDCl_3$) (Fig. S1): δ 5.17 (m, -O-C**H**(CH_3)-CO-), 1.56 (d, -O-CH(C**H**$_3$)-CO-), 4.58 – 4.92 (m, -O-C**H**-CO-), 4.18 – 4.40 (m, -O-C**H**$_2$-C-) and 1.27 (s, -CH_2-C(C**H**$_3$)-).

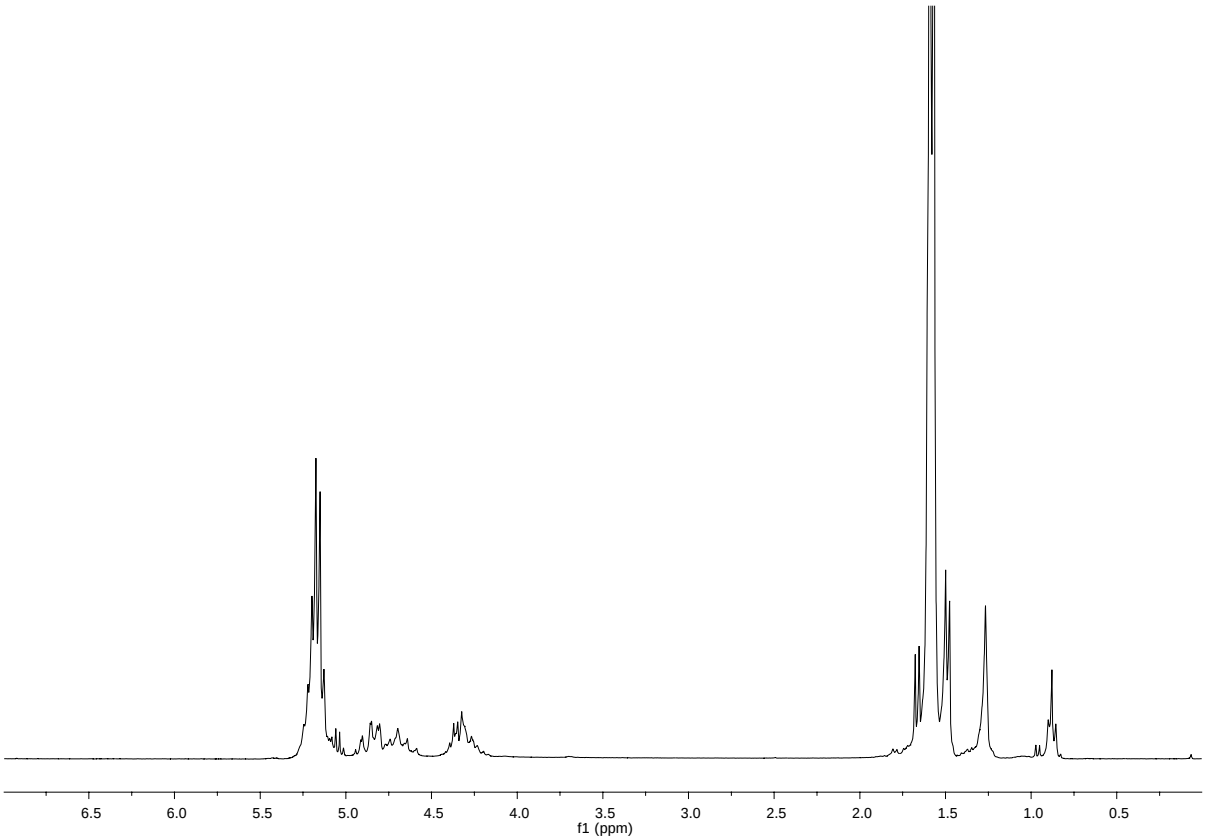

Fig. S1 ^{1}H NMR of poly(LA/GL/HMPA) (polymer (**1**)) in $CDCl_3$

S1b. Poly(LA/GL/HMPA)-cysteamine (3)

The carboxyl functionalized intermediate (**1**) (1.6 g, 0.58 mmol equivalent to COOH group) and N-hydroxysuccinimide (NHS, 0.17 g, 1.46 mmol, 2.5 molar eq.) were mixed together in 6.5 mL anhydrous dimethylformamide (DMF, 4 vol.) under an N_2 atmosphere. A solution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl, 0.28 g, 1.46 mmol, 2.5 molar eq.) in DMF (9.5 mL, 6 vol.) was slowly added using a pressure-equalized addition funnel. The solution was maintained at 20 °C for 24 h to complete the activation of all available carboxyl groups.

Cysteamine hydrochloride (0.34 g, 2.92 mmol, 5 molar eq. of COOH) was dried separately in 20 mL anhydrous DMF under vacuum for 2 h to remove any moisture and then mixed with triethylamine (0.47 g, 4.68 mmol, 1.6 molar eq. of cysteamine hydrochloride). The resulted neutralized cysteamine was slowly charged into the NHS activated polymer solution under N_2 and further stirred for 24 h. The polymer solution was then concentrated under vacuum to remove the DMF and the crude product was precipitated out by adding excess diethylether and dried under vacuum.

The crude product was then redissolved in DCM (25 vol.) and washed two times with saturated sodium chloride solution (5 vol.) to remove excess cysteamine and to hydrolyze any unreacted NHS ester groups. The clear DCM extract was separated and stirred with 50 mg dithiothreitol (DTT) for 30 min to reduce any possibly formed disulfide bonds. The DCM extract was again washed two times with saturated sodium chloride solution (5 vol.), dried over anhydrous sodium sulfate and filtered through celite to remove any undissolved particles. The product was isolated after distilling the solvent off and crystallized using excess diethylether. Yield 0.96 g (60%). ^{1}H NMR ($CDCl_3$) ((Fig. S2)): δ 3.09 (m, -NH-C**H**$_2$-CH_2-) and 2.64 (m, -NH-CH_2-C**H**$_2$-).

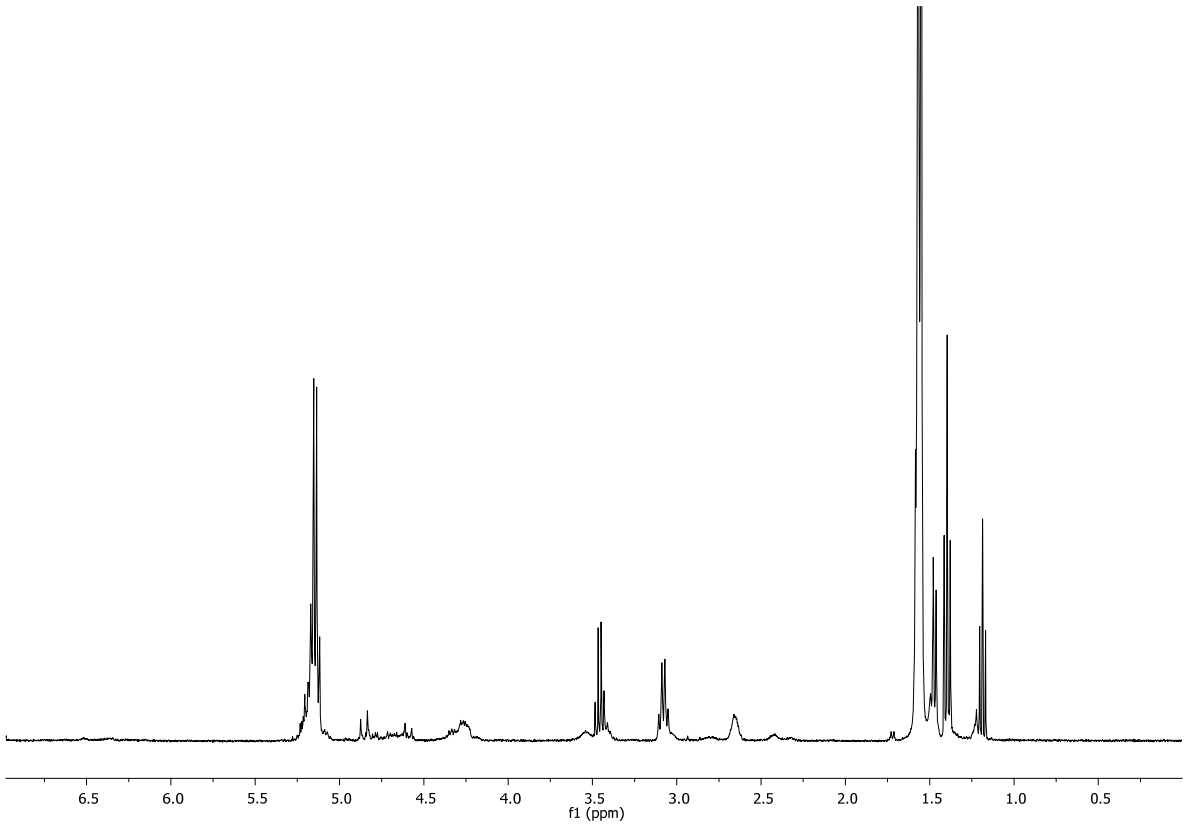

Fig. S2 ^{1}H NMR of poly(LA/GL/HMPA)-cysteamine (polymer (**3**)) in $CDCl_3$

S1c. S-nitrosated poly(LA/GL/HMPA)-cysteamine (4)

A 50 mg sample of poly(LA/GL/HMPA)-cysteamine (**3**) was dissolved in a 2 mL dichloromethane-methanol (1:2) mixture in an amber colored EPA vial (Fisher Scientific, NJ). In a separate vial, a solution of 8.4 mg t-butylnitrite (4 molar eq., pre-treated with 10% w/v disodium ethylenediamine tetraacetate dehydrate (EDTA-disodium salt)) in a 1 mL dichloromethane-methanol (1:2) mixture was prepared and added into the polymer solution while protected from direct exposure to light. The solution was stirred at 20 °C for 4 h and then concentrated under vacuum to isolate the S-nitrosated product.

S2. Optimization of the S-nitrosation reaction

Optimization of the S-nitrosation reaction was performed by monitoring the UV absorption maximum observed at 333 nm (Fig. S3) using a Nicolet Evolution 300 UV–Vis spectrophotometer (Thermo Electron Co., MA). Fig. S3 shows a representative spectrum of the S-nitrosothiol product. Experiments were performed at 2, 4, 6 and 8 h following the same reaction conditions and molar ratios. Sample solutions with 2.5 mg/mL concentrations were prepared in a dichloromethane-methanol (1:2) solution and the absorption spectra were recorded. As shown in Fig. S4, the maximum absorbance indicates that the maximum conversion is obtained at 4 h. Further reaction time resulted in deteriorating yield. Consequently, further experiments were performed at 4 h, the optimized reaction time for S-nitrosation.

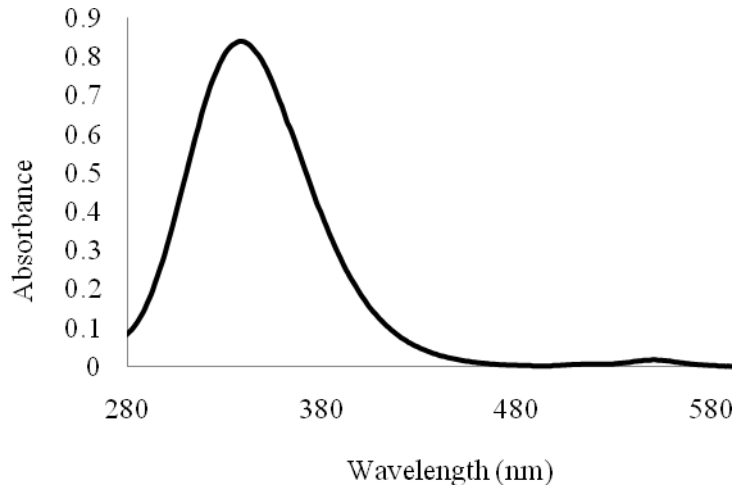

Fig. S3 UV spectrum of S-nitrosated polymer (**4**)

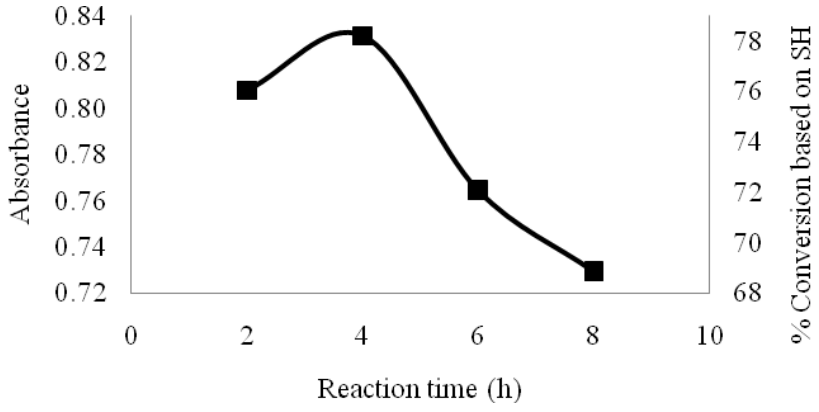

Fig. S4 S-nitrosation reaction monitoring using UV absorption at 333 nm

S3. Molecular weight measurements

Gel permeation chromatography (GPC) analyses of the polymers were carried out using a Waters University 1500 GPC instrument equipped with four 5 mm PL gel columns (Polymer Laboratories, CA). Samples were analyzed at 40 °C with chloroform as the eluent at a flow rate of 1.0 mL/min. Poly(methyl methacrylate) (PMMA) standards were used as the molecular weight standards. Chromatograms were processed with Waters Empower software (version 2002) and the number-average molecular weight (Mn) and polydispersity index (PDI) of samples were calculated relative to PMMA standards. Samples were analyzed in triplicate and the Mn and PDI values are reported as the average and standard deviation of each sample.

Table S1 Average Mn and PI of polymers (**1**) and (**3**)

Polymer	Mn	PDI
Polymer (**1**)	4400±700	1.39±0.06
Polymer (**3**)	5500±700	1.49±0.04

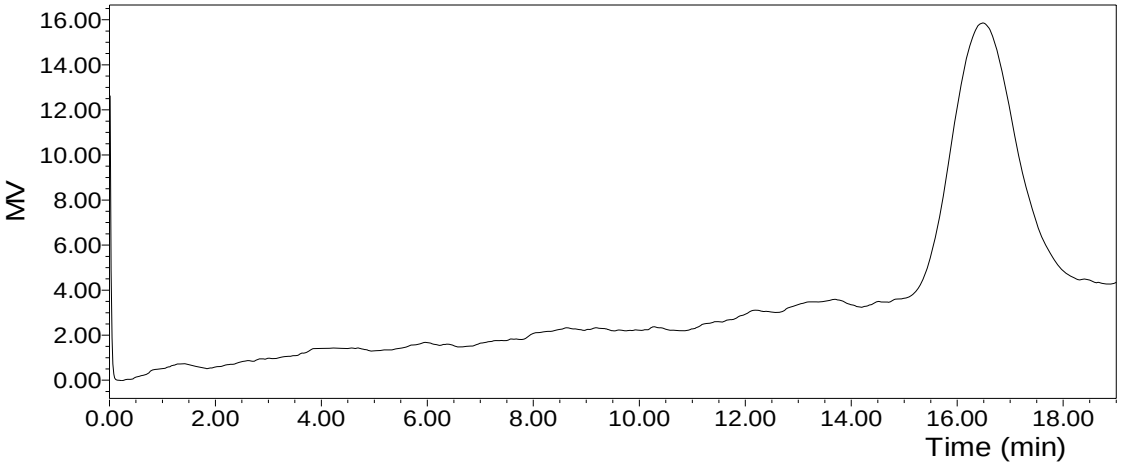

Fig. S5 GPC flow curve of polymer (**1**) in chloroform

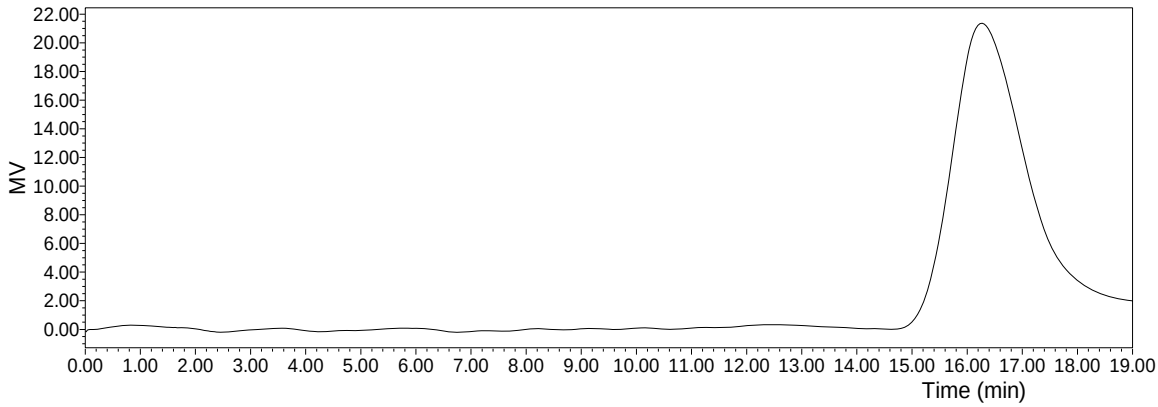

Fig. S6 GPC flow curve of polymer (**3**) in chloroform.

S4. Thermal analysis

S4a. TGA measurements

TGA of the polymer samples were performed using a TGA 2950 (TA Instruments, DE) at a heating rate of 10 °C/min to 500 °C. Scans were performed in triplicate and the average and standard deviation of the T_d for poly(LA/GL/HMPA) (polymer (**1**)) and poly(LA/GL/HMPA)-cysteamine (polymer (**3**)) was determined to be 219±1 and 246±3 °C respectively.

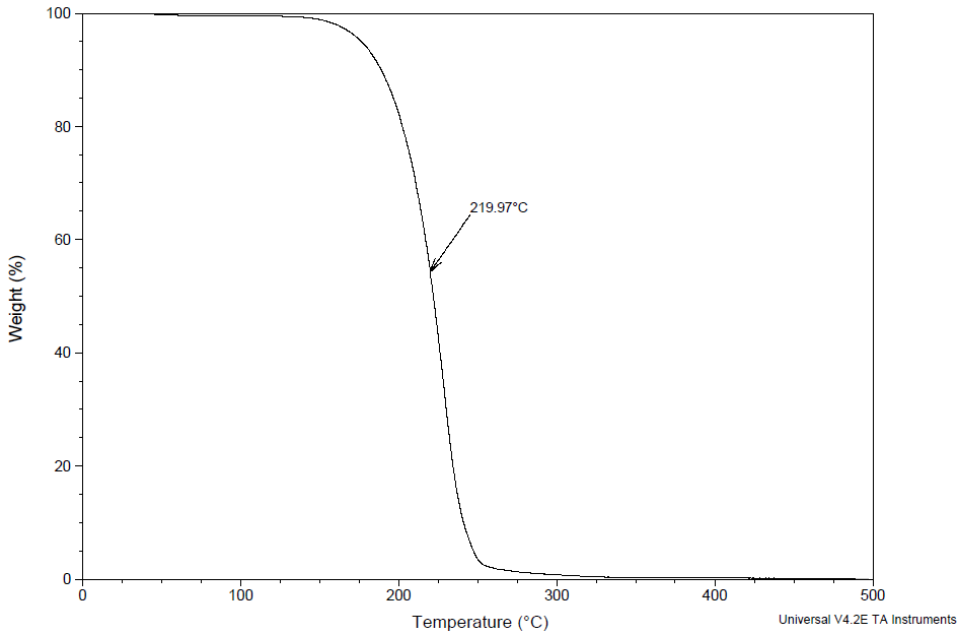

Fig. S7 A representative TGA thermogram of polymer (**1**)

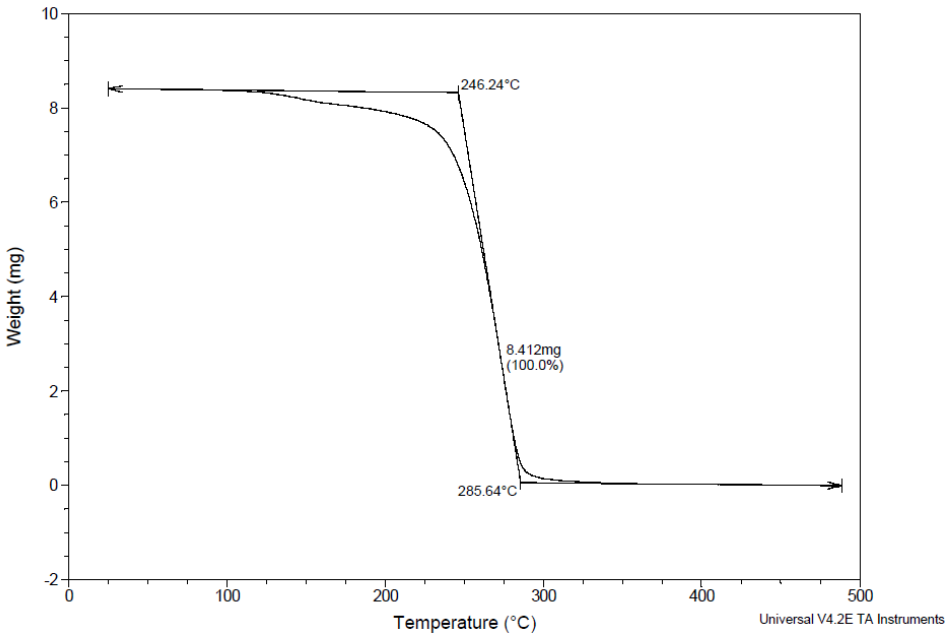

Fig. S8 A representative TGA thermogram of polymer (**3**)

S4b. DSC measurements

DSC experiments were performed on a TA Instruments DSC model 2920 using sealed hermetic aluminum pans. In the first cycle, samples were heated up to 200 °C at a rate of 10 °C/min followed then heated isothermally for 2 minutes and subsequently cooled to 25 °C at the same rate. In the second cycle, after an initial isotherm at -10 °C for 2 min, samples were heated to 150 °C at a rate of 10 °C/min for recording the glass transition temperature. Samples were analyzed in triplicates and the average and standard deviation of the glass transition temperature (T_g) for polymer (**1**) and polymer (**3**) were found to be 40.5±0.5 °C and 25.0±0.4 °C respectively.

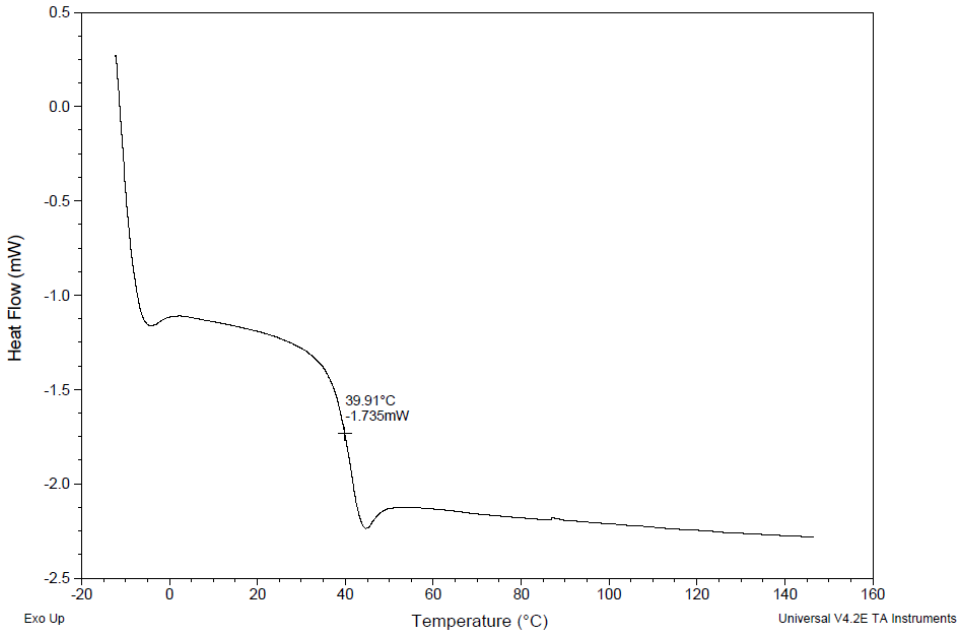

Fig. S9 A typical DSC thermogram indicating the glass transition temperature of polymer (**1**)

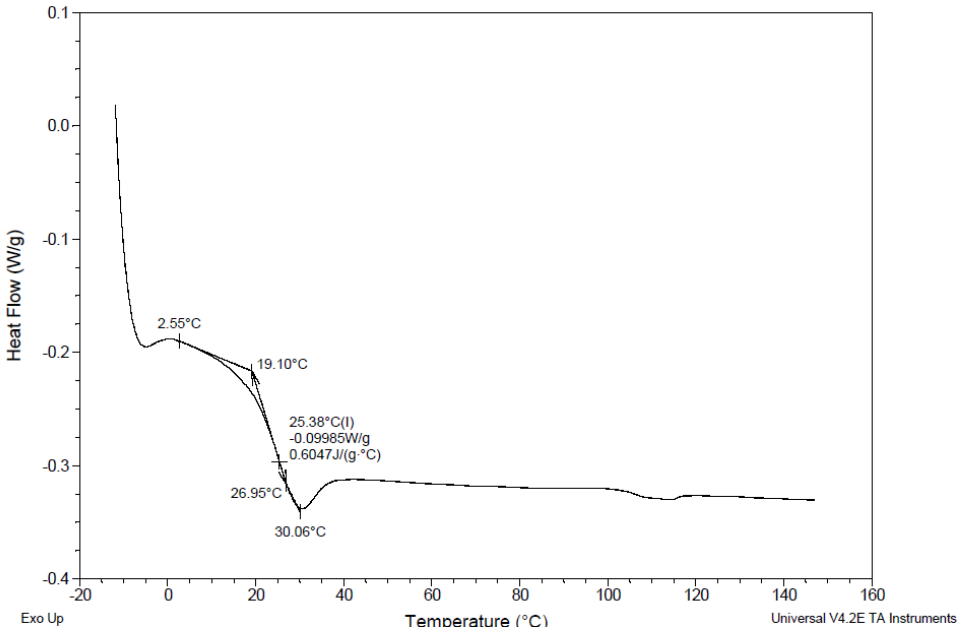

Fig. S10 A typical DSC thermogram indicating the glass transition temperature of polymer (**3**)

S5. Small angle X-ray scattering (SAXS)

SAXS data was collected on a Rigaku S-Max 3000 High Brilliance three pinhole SAXS system outfitted with a MicroMax-007HFM rotating anode (CuKR), Confocal Max-Flux Optic, Gabriel multiware area detector, and a Linkam thermal stage. Polymer samples were sandwiched between Kapton® discs and data was collected with an exposure of 7200 s.

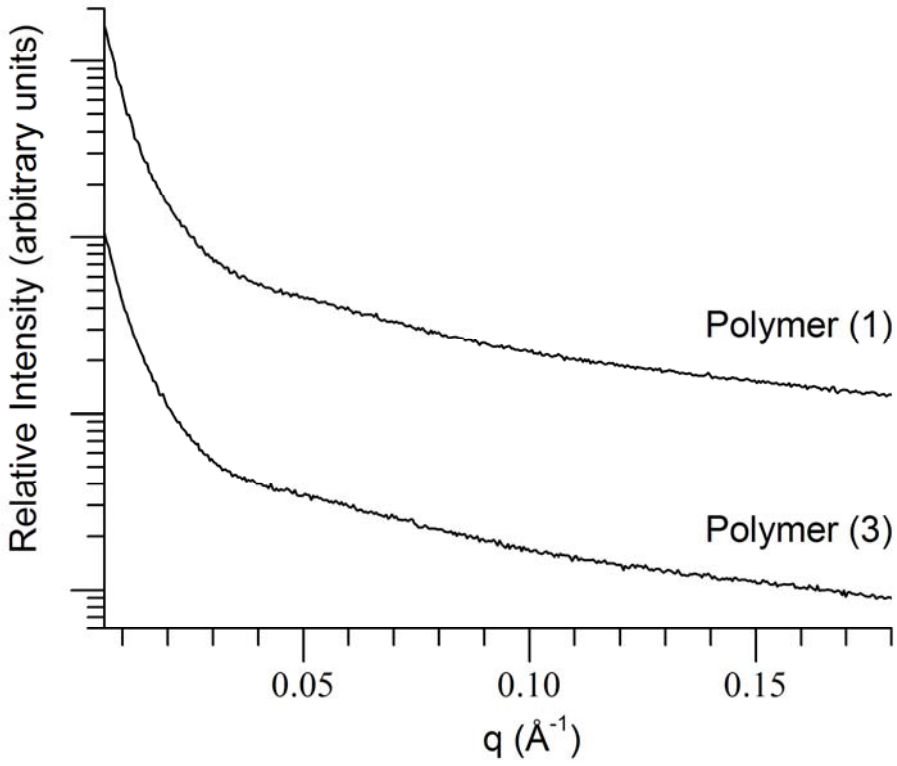

Fig. S11 SAXS intensity profile of polymers (**1**) and (**3**) at 25 °C

S6. Nitric oxide release profile

A Siever's chemiluminesence NO analyzer® (model 280i) was used for measuring the NO release from polymer (**4**). The instrument was calibrated before each analysis using nitrogen as the zero gas and a 45 ppm calibration NO gas. Samples were prepared by spin-coating a 100 mg/mL polymer solution in dry dichloromethane onto a glass slide using a SCS spin coater (model G3P-8) protected from light. The polymer coated slide was inserted into a clean dry NOA measurement cell, shielded from light. Once a stable baseline reading was obtained, 30 mL deoxygenated 10 mM phosphate buffered saline (PBS, 0.137 M NaCl, 2.7 mM KCl, pH 7.4) was introduced into the measuring cell and the system was maintained at 37 °C. Measurements were recorded in triplicate. Data was collected at a time interval of 5 sec and a flow rate of 200 mL/min with a cell pressure of 9.7 Torr and an oxygen pressure of 6 psig. In order to quantify the total amount of NO released from the sample, a solution of copper (II) chloride and glutathione was injected into the measurement cell at a net concentration of 0.5 mM copper (II) chloride ant glutathione. After the level of NO release reached 1 ppb, the experiment was terminated and the total amount of NO released from the material was determined by integrating the area of the curve. The average total NO released from the sample was 0.240±0.003 mmol per g of polymer (**4**).

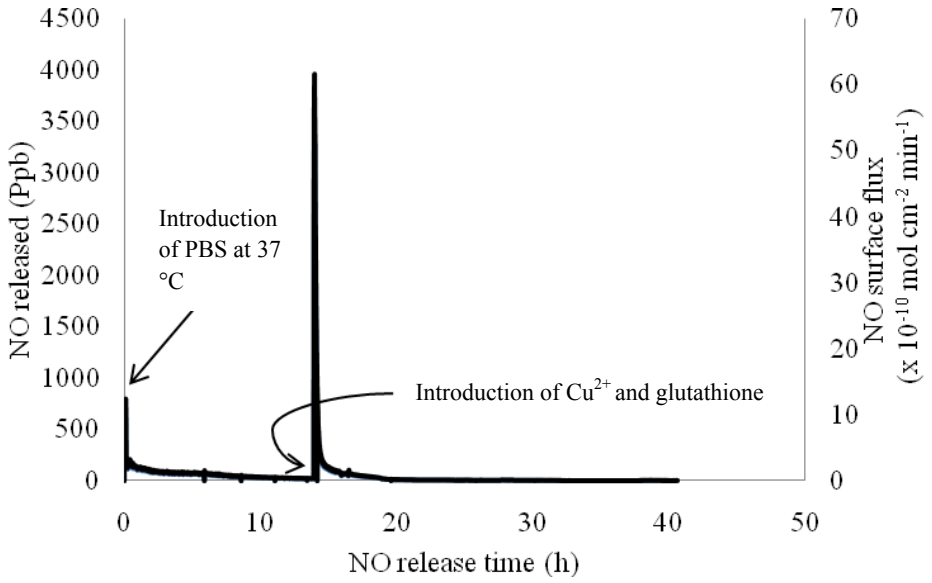

Fig. S12 Detailed NO release profile showing triggering of NO release after the introduction of $CuCl_2$ and GSH

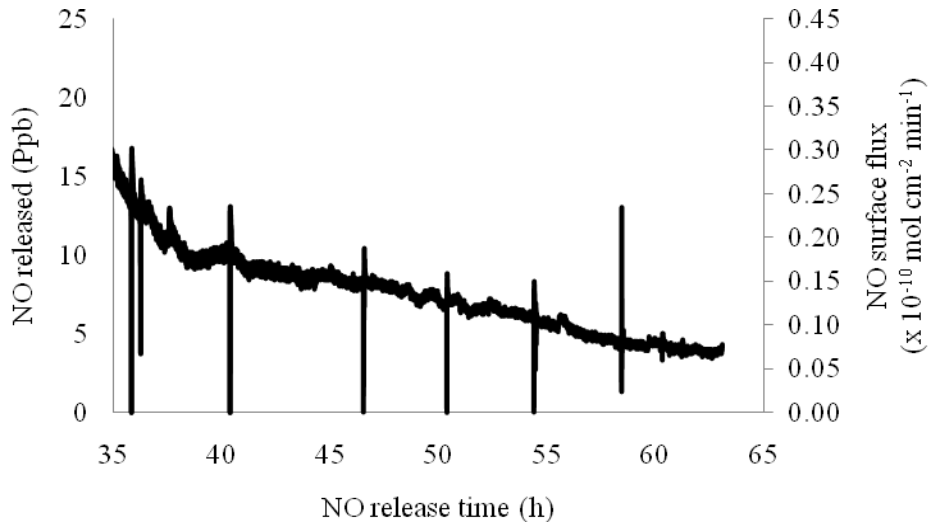

Fig. S13 Expanded NO release profile showing that the polymer releases NO above the baseline for prolonged periods

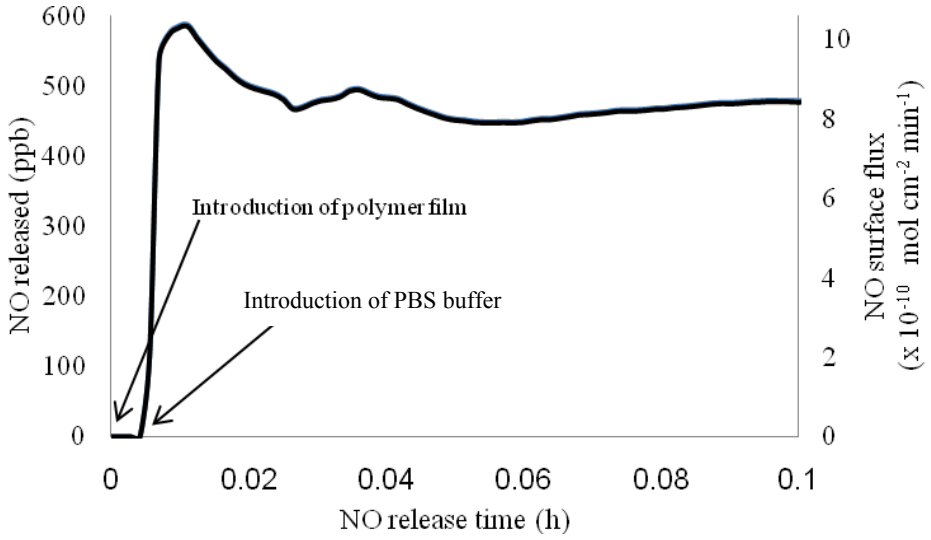

Fig. S14 An expanded version of figure S12 showing relative stability of S-nitrosated polymer protected from light

S7. Degradation measurements

Polymer degradation studies were performed by determining the weight loss as a function of time. Articles for this measurement were prepared by massing samples of polymer (**3**) (~150 mg each) to 1 mg. Each sample was then placed in 10 mL PBS buffer (0.01 M phosphate, 0.137 M NaCl, 2.7 mM KCl, pH 7.4) at 37 °C. At various

time points (t = 1, 2, 3, 4, 5, and 6 weeks), the samples were removed from the buffer, washed two times with 2 mL DI water and dried under vacuum until a constant mass was achieved. Buffer solutions were replaced weekly. Percentage weight loss at each time point was determined from the difference in mass of the initial and dried sample at each time point. Each time point represents as an average and standard deviation of three separate measurements.

S8. Cytotoxicity measurements

Cytotoxicity evaluations were performed according to protocols approved by the International Organization for Standardization (ISO-10993-5: Biological Evaluation of Medical Devices, Part 5: Tests for In Vitro Cytotoxicity) at NAMSA (Northwood, OH). In brief, a 60 cm^2 sample of polymer (**3**) as well as a positive (plasticized vinyl containing 10, 10'-oxybisphenoxarsine) and negative (high density polyethylene) control material were extracted in Minimal Essential Medium (MEM) at 37 °C for 24 h. After which time, mouse LDL fibroblast cells were dosed with the extract and allowed to incubate for 48 h. A reagent control of the MEM solution was used to validate the stability of the extract vehicle. Following incubation, the cells were examined microscopically for abnormal cell morphological changes and the degree of cell lysis. The cells incubated with polymer (**3**), the positive control, the negative control, and the reagent control were then assigned a reactivity score on a scale of 0 to 4 as described in the Table S2 (from NAMSA report November 4, 2010).

Table S2 Reactivity Score for Material Cytotoxicity

Score	Conditions of Cell Cultures
0	Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth.
1	No more than 20% of the cells are rount, loosely attached and without intracytoplasmic granules, or show changes in morphology; only sight growth inhibition observed.
2	Not more than 50% of the cells are round, void of intracytoplasmic granules; no extensive cell lysis; not more than 50% growth inhibition observed.
3	Not more than 70% of the cell layers are rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observed.
4	Nearly complete or complete destruction of the cell layers.