Supplementary Information

Materials

Unless otherwise stated, all chemicals for synthesis and physicochemical analysis were of ACS grade, purchased from Sigma-Aldrich and used as received without further purification. Phosphate buffered saline (PBS) was at pH 7.4. Reagents for cell culture were purchased from Invitrogen (Carlsbad, CA) unless otherwise noted. Human mesenchymal stem cells (HMSCs) from a 24 year old drug- and disease-free male were purchased from Lonza (Gaithersburg, MD).

Experimental methods

^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz NMR spectrometer, using residual solvent ^{1}H peaks as internal references for the ^{1}H NMR spectra. Mass spectra were recorded on an Agilent 6530 QTOF mass spectrometer in electrospray ionization mode. Infrared spectroscopy was carried out on a Thermo Scientific Nicolet 380 FT-IR Spectrometer (Thermo Fisher Scientific Inc., USA). Spectra were recorded in ATR mode at 21 °C, with a 1 cm^{-1} resolution and 64 scans (corrected for background and atmosphere using OMNIC software provided with the spectrometer). Gel permeation chromatography (GPC) was performed on a Viscotek GPCmax Solvent/Sample Module. Two fluorinated polystyrene columns (IMBHW-3078 and IMBLMW-3078) were used in series and maintained at 22 °C. DMF was used as the eluent at a flow rate of 1 mL min^{-1}. Detection was performed using a Viscotek VE 3580 refractive index detector. Molecular weight and dispersity data are reported relative to polystyrene standards in DMF with 0.01 M LiBr at 40 ^{o}C, and a commercially available sample of polycaprolactone (GPC: M_n = 40.1 kDa, M_w/M_n of 2.02) from Polysciences Inc., (Warrington, PA, USA) in DMF at 22 ^{o}C. Differential scanning calorimetry (DSC). DSC experiments were carried out with a DSC Q100 (TA Instruments, USA), using airtight aluminum pans. Material was weighed into aluminum pans (TA Instruments, USA), and analyses were carried out under a nitrogen atmosphere (flow rate of 50 mL min^{-1}). The samples were treated as follows: heated from room temperature to 125 °C (10 °C min^{-1}), cooled to 30 °C (10 °C min^{-1}), heated from 30 °C to 125 °C (5 °C min^{-1}) and finally cooled to 30 °C (5 °C min^{-1}). The first temperature ramp removed traces of volatile solvents and the T_m from the second ramp is reported.

Synthesis of alkyne-displaying aminopropylpyrrole derivative

Aminopropylpyrrole (3.6 g, 29 mmol), propiolic acid (1.4 g, 20 mmol), dicyclohexylcarbodiimide (DCC, 6.0 g, 29 mmol), hydroxybenzotriazole (HOBt, 3.9 g, 29 mmol) and triethylamine (3.1 g, 4.4 ml, 29 mmol) were stirred for 48 hours in dichloromethane (DCM, 100 mL), after which the mixture was filtered to remove the precipitated dicyclohexylurea. The filtrate was washed with aqueous solutions of $NaHSO_4$ (160 g L^{-1}), $NaHCO_3$ (saturated), $NaHSO_4$, $NaHCO_3$, water and finally brine. The solution was dried over MgSO4 and the volatiles removed with a rotary evaporator. The crude product was purified by silica column chromatography (eluting with a gradient of DCM 100%, to $CHCl_3$ 100%, to DCM:MeOH, 98:2) to give the product, a viscous oil, in a yield of (2.3 g, 13 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.65 (2H, C$H_{\beta\text{-pyrrole}}$), 6.16 (2H, C$H_{\alpha\text{-pyrrole}}$), 5.87 (1H, CON*H*), 3.95 (2H, CH_2), 3.29 (2H, CH_2), 2.75 (2H, CH_2). ESI-MS (m/z) calculated for $C_{10}H_{12}N_2O$ $[M + H]^+$ requires 177.09; found, 177.10. IR (ATR) ν_{max} cm^{-1} 3344 (CH alkyne), 3224 (NH, amide), 2105 (alkyne), 1636 (amide I), 1530 (amide II). See **Scheme S1**.

Synthesis of pyrrole-displaying polycaprolactone derivative

To a solution of 2-chlorocyclohexanone (25 g, 189 mmol) in DCM (250 mL) was added 3-chloroperbenzoic acid, (MCPBA, 50 g, 290 mmol) and the reaction mixture was stirred at room

temperature under an atmosphere of nitrogen for 96 hours. The reaction mixture was cooled to -20 °C, resulting in the precipitation of residual 3-chlorobenzoic acid. After filtration, the solution was washed three times with a saturated aqueous solution of $NaHSO_3$, three times with an aqueous solution of $NaHCO_3$, and once with water. The organic phase was dried over MgSO4, filtered, and the solvent was removed on a rotary evaporator. The residue was distilled under reduced pressure, yielding α-chloro-ε-caprolactone (22 g, boiling point 75-77 °C at 0.1 mm Hg). ε-caprolactone (4.0 g), α-chloro-ε-caprolactone (1.0 g), stannous stearate (0.212 g) and ethanol (1.8 μL) were dissolved in toluene (15 mL) and stirred under nitrogen for 24 hours at room temperature, after which the product was precipitated in cold hexanes, dissolved in DCM and reprecipitated in cold hexanes and dried under high vacuum for 48 hours, affording poly(ε-caprolactone-co-α-chloro-ε-caprolactone) as a white powder in a yield of 2.26 g. Poly(ε-caprolactone-co-α-chloro-ε-caprolactone) (2.26 g) and sodium azide (3.045 g) were dissolved in dimethylformamide (DMF, 15 mL) and stirred under nitrogen for 24 hours at room temperature, after which the product was precipitated in water. The polymer was redissolved in DMF and reprecipitated in water, isolated by filtration under vacuum, and dried under high vacuum for 48 hours, affording poly(ε-caprolactone-co-α-N_3-ε-caprolactone) as a white powder in a yield of 2.86 g. Poly(ε-caprolactone-co-α-N_3-ε-caprolactone) (1.14 g), alkyne-displaying aminopropylpyrrole derivative (0.05 g), copper(I) iodide (0.04 g) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.03g) were stirred under nitrogen for 24 hours at 35 °C. The polymer was precipitated in water and the solids were incubated in 50 mL of an aqueous solution of ethylenediaminetetraacetic acid (EDTA, 5g in 100 mL water, pH 8) for 24 hours at 4 °C, after which the EDTA solution was decanted and replaced with fresh EDTA solution and incubated for a further 24 hours at 4 °C, after which the EDTA solution was decanted and replaced with water (50 mL) in which it was incubated for 24 hours at 4 °C, after which the water was decanted and replaced with fresh water (50 mL) in which it was incubated for 24 hours at 4 °C, after which the water was decanted and the polymer dried under high vacuum for 48 hours. This process afforded the pyrrole-displaying polycaprolactone derivative in a yield of 1.1 g in the form of a light brown powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.52 (C*H* triazole), 7.09 (CON*H*), 6.53 ($CH_{\beta\text{-pyrrole}}$), 6.15 ($CH_{\alpha\text{-pyrrole}}$), 4.21, 4.04, 3.86, 3.65, 3.29, 2.32, 1.92, 1.64, 1.38, 1.10. IR (ATR) ν_{max} cm^{-1} 3326 (NH, amide), 2099 (residual azide), 1721 (ester), 1622 (amide I), 1571 (amide II), 1457 (triazole). GPC: M_n = 5.0 kDa (M_w/M_n of 1.95). DSC: T_m = 54.5 °C. See **Scheme S2**.

Preparation of films of pyrrole-displaying polycaprolactone derivative

Solutions of the pyrrole-displaying polycaprolactone derivative in chloroform (0.5 g in 20 mL) or hexalfuoroisopropanol (0.5 g in 20 mL) were cast on glass slides or in tissue-culture treated Corning® Costar® tissue culture plates, respectively. The solvent was allowed to evaporate for 24 hours and the films were subsequently dried under high vacuum for 48 hours.

Preparation of conductive films displaying amines

Aminopropylpyrrole (0.2 g) was dissolved in ethanol (2 mL), and added to a solution of ammonium persulfate (0.29 g), and ferric chloride (0.005g) in water (20 mL). 0.2 mL of this solution was coated on the surface of films of the pyrrole-displaying polycaprolactone derivative and allowed to polymerize for 24 hours, after which the brown-black films were washed extensively with water, followed by rinsing with aqueous ethanol (70%) and drying under high vacuum for 48 hours. See **Scheme S3**.

Preparation of conductive films displaying carboxylic acids

EDOT carboxylic acid (0.2 g), ammonium persulfate (0.29 g), and ferric chloride (0.005g) in water (20 mL). 0.2 mL of this solution was coated on the surface of films of the pyrrole-displaying polycaprolactone derivative and allowed to polymerize for 24 hours, after which the blue-grey films were washed extensively with water, followed by rinsing with aqueous ethanol (70%) and drying under high vacuum for 48 hours. See **Scheme S4**.

Preparation of conductive films displaying sulfonates

Hydroxymethyl EDOT (3 g, 17.8 mmol, Sarchem laboratories, Inc., Farmingdale, NJ, USA) and sodium hydride (0.47 g, 19.6 mmol, 1.1 eq.) and were stirred and heated at reflux in toluene (50 mL) for 2 hours. Butane sultone (2.42 g, 17.8 mmol, 1 eq.) was added and the mixture was heated at reflux for a further 2 hours. After cooling to room temperature, the product was precipitated in acetone, isolated by filtration and subsequently dried under high vacuum for 48 hours. The process yielded a brown powder, EDOT-S (3.71 g, 11.4 mmol). ^{1}H NMR (400 MHz, D_2O) δ_H 6.36, 4.70, 4.26, 4.12, 3.94, 3.59, 3.44, 2.78, 1.64, 1.55. EDOT-S (0.2 g), ammonium persulfate (0.29 g), and ferric chloride (0.005g) in water (20 mL). 0.2 mL of this solution was coated on the surface of films of the pyrrole-displaying polycaprolactone derivative and allowed to polymerize for 24 hours, after which the blue-black films were washed extensively with water, followed by rinsing with aqueous ethanol (70%) and drying under high vacuum for 48 hours. See **Scheme S5**.

Preparation of silica-coated conductive films

0.2 mL of phosphate buffer (pH 5.5) was added to conductive films displaying amines. Tetraethylorthosilicate (2.33 mL), water (3.85 mL), ethanol (3.85 mL) and 1N HCl (0.1 mL) were mixed and incubated for 10 minutes at room temperature, and aliquots of this solution (20 μL) were added to the phosphate buffer covered the amine-displaying conductive films. The reaction mixture was incubated for 1 hour after which it was removed and the films were washed thoroughly with Millipore water, rinsed with aqueous ethanol (70%) and dried under high vacuum for 48 hours.

Preparation of calcium phosphate-coated conductive films

Conductive films displaying carboxylic or sulfonic acids were incubated in an aqueous solution (1 mL) of calcium chloride (200 mM) for 20 minutes, after which the solution was removed and the samples were washed with water (3 x 1 mL). Thereafter, samples were incubated in an aqueous solution (1 mL) of sodium phosphate (120 mM) for 20 minutes, after which the solution was removed and the samples were washed with water (3 x 1 mL). The cycle of incubation with calcium chloride and sodium phosphate was repeated a further six times (i.e. a total of 7 cycles), after which the samples were washed with water, rinsed with aqueous ethanol (70%) and dried under high vacuum for 48 hours.

Preparation of calcium carbonate-coated conductive films

Conductive films displaying carboxylic or sulfonic acids were incubated in an aqueous solution (1 mL) of calcium chloride (10 mM). The films were placed in an airtight container with a beaker containing ammonium carbonate for 24 hours. The samples were subsequently washed with water until the pH was neutral, after which the samples were washed with water, rinsed with aqueous ethanol (70%) and dried under high vacuum for 48 hours.

Electrical properties

Resistance (R in Ω) was measured between the two silver electrodes using a digital multimeter (DM-8A, Sperry Instrument, Milwaulkee, WI). Sheet resistance (R_s) in Ω/square was calculated as follows:

$$R_S = RW/L$$

where W is the width of the electrode and L is the distance between the two silver electrodes. The electrodes were moved to different positions after each measurement, and the resistance *R* was recorded in at least ten different positions on the materials.

Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) spectroscopy

Samples were mounted on a Scanning Electron Microscopy (SEM) stub and sputter coated with carbon. All samples were imaged using a Hitachi S5500 SEM field emission scanning electron microscope equipped with an energy dispersive spectroscopy probe located at the Texas Materials Institute.

In vitro culture of human Mesenchymal stem cells without electrical stimulation

HMSCs were supplied by Lonza (Walkersville, MD). Samples were inserted in untreated polystyrene tissue culture plates and sterilized by incubation in 70% ethanol followed by exposure to UV for 60 min. After sterilization, the samples were incubated for 30 minutes in 24 well plates containing HMSC growth medium that was composed of: high glucose Dulbecco's Modified Eagle Medium (DMEM, 440 mL); fetal bovine serum (50 mL); antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), and 2 ng mL^{-1} basic fibroblast growth factor. Medium was aspirated and replaced prior to HMSC seeding. Cell viability before starting the experiment was determined by the Trypan Blue exclusion method, and the measured viability exceeded 95 % in all cases. HMSCs were seeded at 10,000 cells per cm^2, and incubated at 37 °C, 95 % humidity, and a CO_2 content of 5 %. After 3 days the medium was aspirated, the materials were washed gently with PBS and transferred to a fresh 24 well plate containing osteogenic medium that was composed of: high glucose Dulbecco's Modified Eagle Medium (DMEM, 425 mL); fetal bovine serum (50 mL); antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), dexamethasone (100 nM), β-glycerol phosphate (10 mM) and ascorbic acid (50 μM). Thereafter the osteogenic medium was aspirated and replaced every 2 days until the samples were analysed.

In vitro culture of human Mesenchymal stem cells with electrical stimulation

Electrical stimulation of Human Mesenchymal Stem cells was achieved employing a custom built setup. Non-conductive glass slides, polycarbonate wells (square polycarbonate blocks, thickness of 1 cm, sides of 2.5 cm, with square holes with sides of 0.9 cm cut out), Dow Corning® high vacuum grease, and medium binder clips (Staples®, Framingham, MA) were sterilized by autoclaving. Holes were drilled into the sides of 10 cm polystyrene Petri dishes using a Dremel saw (Lowes, Mooresfield, NC, USA), and the plates were sterilized by exposure to UV for 60 min. Adhesive-backed copper tape (5 mm width, Ted Pella, Inc.), waterproof Kapton® tape (1 cm width, Fisher Scientific, Waltham, MA, USA), wires and alligator clips were sterilized by exposure to UV for 60 min.

Electroactive PCL-based tissue scaffolds were placed on glass slides and secured in position with two thin strips of adhesive-backed copper tape that were attached to the scaffolds, parallel to one another and separated by a distance of ca. 4 cm. One face of the polycarbonate wells was coated with vacuum grease and placed on the electroactive tissue scaffolds, greased side down, in contact with the glass slide. A binder clip on either side of the well was used to secure this in position and render it water tight. A strip of copper tape was run between the parallel copper strips attached to the scaffolds and the ends of the slides as points of contact for the alligator clip-terminated wires attached to the multipotentiostat (CH Instruments, Austin, TX, USA). The counter and reference

electrodes were connected together and clipped to copper tape on one side of the slide, and the working electrode was clipped to copper tape on the other side of the slide.

The setup was sterilized by exposure to UV (30 minutes), the samples were incubated for 30 minutes in HMSC growth medium that was composed of: high glucose Dulbecco's Modified Eagle Medium (DMEM, 440 mL); fetal bovine serum (50 mL); antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), and 2 ng mL^{-1} basic fibroblast growth factor. Medium was aspirated and replaced prior to HMSC seeding. Cell viability before starting the experiment was determined by the Trypan Blue exclusion method, and the measured viability exceeded 95 % in all cases. HMSCs were seeded at 10,000 cells per scaffold, and incubated at 37 °C, 95 % humidity, and a CO_2 content of 5 %. After 2 days the medium was aspirated, the materials were washed gently with PBS and transferred to a fresh 24 well plate containing osteogenic medium that was composed of: high glucose Dulbecco's Modified Eagle Medium (DMEM, 425 mL); fetal bovine serum (50 mL); antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), dexamethasone (100 nM), β-glycerol phosphate (10 mM) and ascorbic acid (50 µM).

The tips of the wires attached to the samples were wound around alligator clip-terminated wires attached to the multipotentiostat (CH Instruments, Austin, TX, USA). The counter and reference electrodes were connected together and clipped to the wire protruding from one end of the sample, and the working electrode was clipped to the wire protruding from the other side of the sample. Wires and alligator clips were secured in position with adhesive copper tape (Ted Pella, Inc., Reading, CA, USA) and wrapped in Parafilm® to render them electrically insulating and waterproof (i.e. suitable for use inside an incubator). The electrical stimulation paradigm was as follows: a potential step of 10 mV mm^{-1} was placed across the samples for the duration of 8 hours followed by 40 hours without stimulation, repeated three times (32 hours of stimulation in total), after which the wires were disconnected and the substrates cultured as normal. Throughout the electrical stimulation experiments the osteogenic medium was aspirated and replaced every 2 days. Thereafter the osteogenic medium was aspirated and replaced every 2 days until the samples were analysed.

Biochemical assays

The DNA content and Alkaline Phosphatase (ALP) activity of samples that were broken up in a buffer of 0.2% Triton X-100 were quantified concurrently, using the PicoGreen® assay (Life Technologies, Thermo Fisher Scientific Inc., USA) for DNA quantitation in accordance with the manufacturer's protocol, a SensoLyte® pNPP Alkaline Phosphatase Assay Kit (AnaSpec, Inc., Freemont, CA, USA) for ALP quantitation in accordance with the manufacturer's protocol, and a Synergy HT Multi-Mode Microplate Reader (Bio-tek US, Winooski, VT).

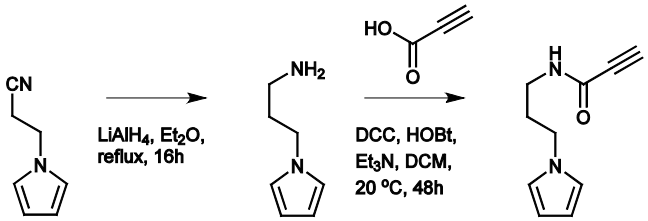
Fluorescence staining and imaging of cells

Cells fixed with paraformaldehyde were permeabilized with 0.1% Triton X-100 (Fluka) and 2% bovine serum albumin (BSA) in PBS buffer for 5 min, followed by blocking with 2% BSA in PBS buffer for 30 min at room temperature. Actin filaments and cell nuclei within cells were stained with Alexa Fluor 488® Phalloidin (Life Technologies, USA) for 30 min and 4',6-diamidino-2-phenylindole (DAPI, Invitrogen, USA) for 5 min, respectively. The cells were thereafter washed three times with PBS and stored at 4 °C until images were acquired. Fluorescence images of cells were obtained using an Olympus IX70 inverted microscope equipped with an Olympus DP80 dual color and monochrome digital camera (a 1.4 megapixel Bayer mosaic color CCD camera) that was attached to the

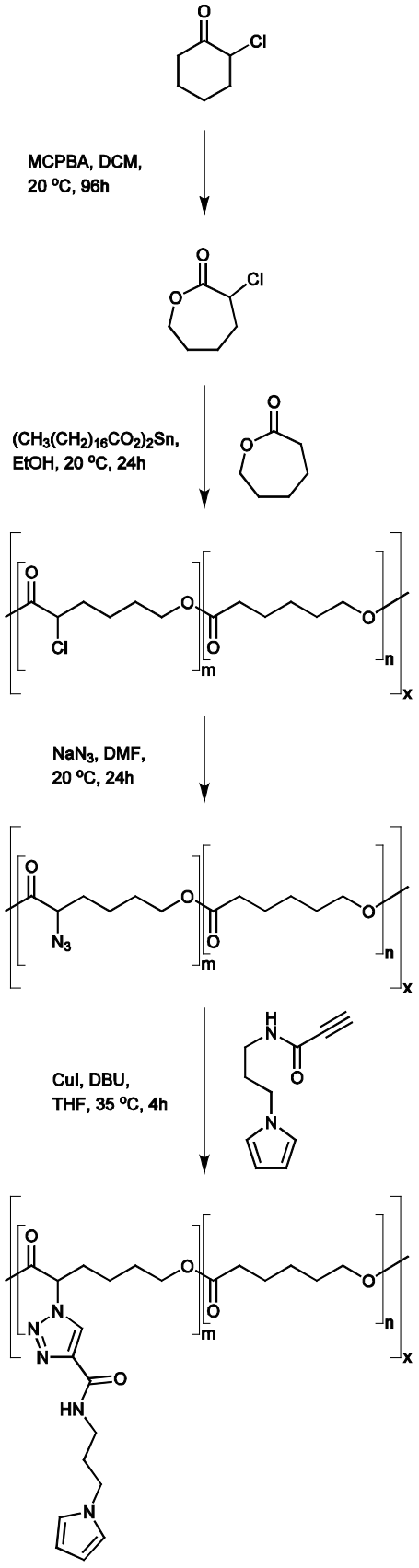
microscope with a 0.63 B-mount. Image Analysis was done using Olympus cellSens® imaging software, Version 1.11.

Supplementary Schemes and Figures

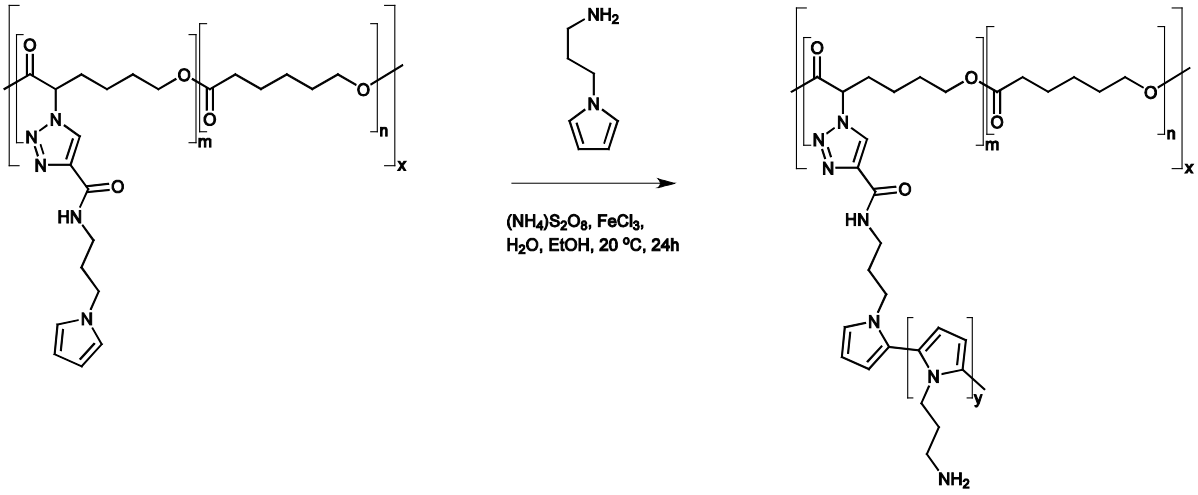
Scheme S1. Synthesis of alkyne-displaying aminopropylpyrrole derivative



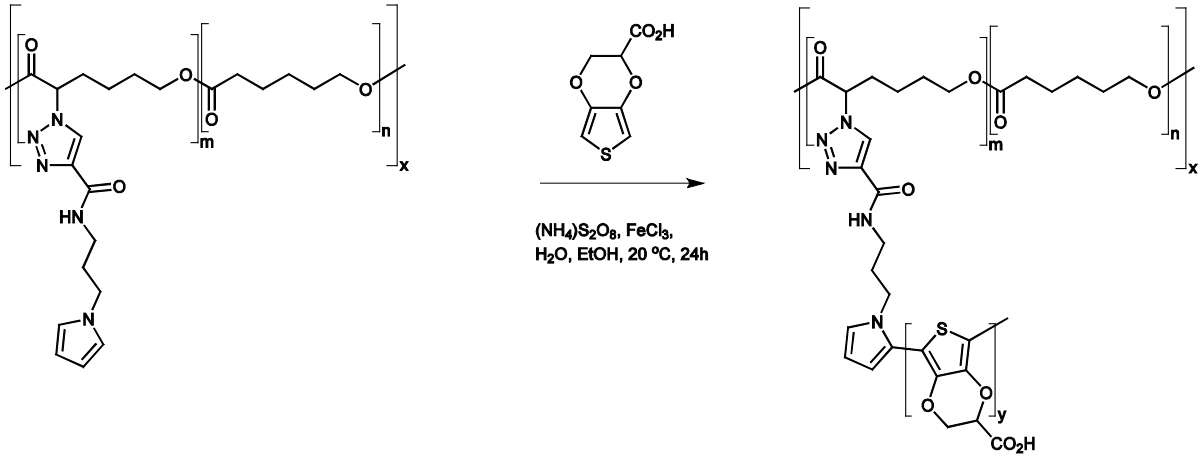
Scheme S2. Synthesis of pyrrole-displaying polycaprolactone derivative



Scheme S3. Synthesis of amine-displaying polycaprolactone derivative



Scheme S4. Synthesis of carboxylic acid-displaying polycaprolactone derivative



Scheme S5. Synthesis of sulfonate-displaying polycaprolactone derivative

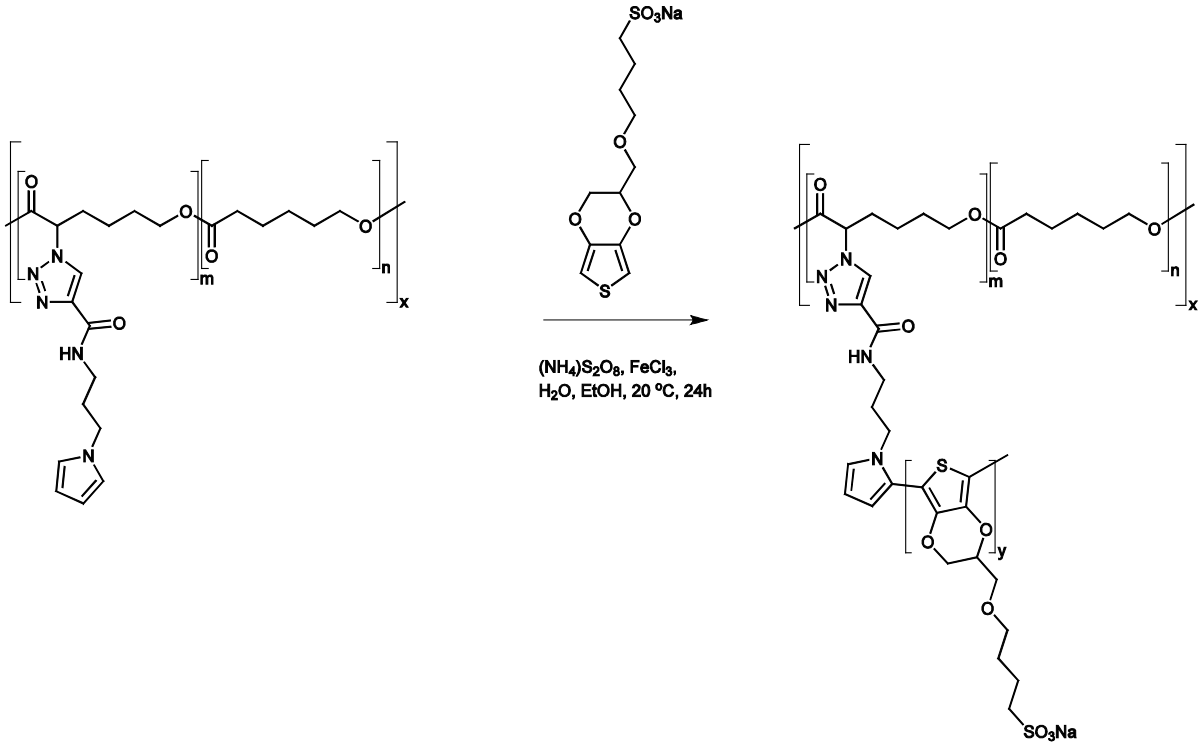


Figure S1. Gel permeation chromatograms of pyrrole-displaying polycaprolactone derivative (2) and a commercially available sample of polycaprolactone in DMF at 22 ^{o}C. Grey line) commercially available sample of polycaprolactone (GPC: M_n = 40.1 kDa (M_w/M_n of 2.02) from Polysciences Inc., (Warrington, PA, USA). Black line) pyrrole-displaying polycaprolactone derivative (2), GPC: M_n = 5.0 kDa (M_w/M_n of 1.95).

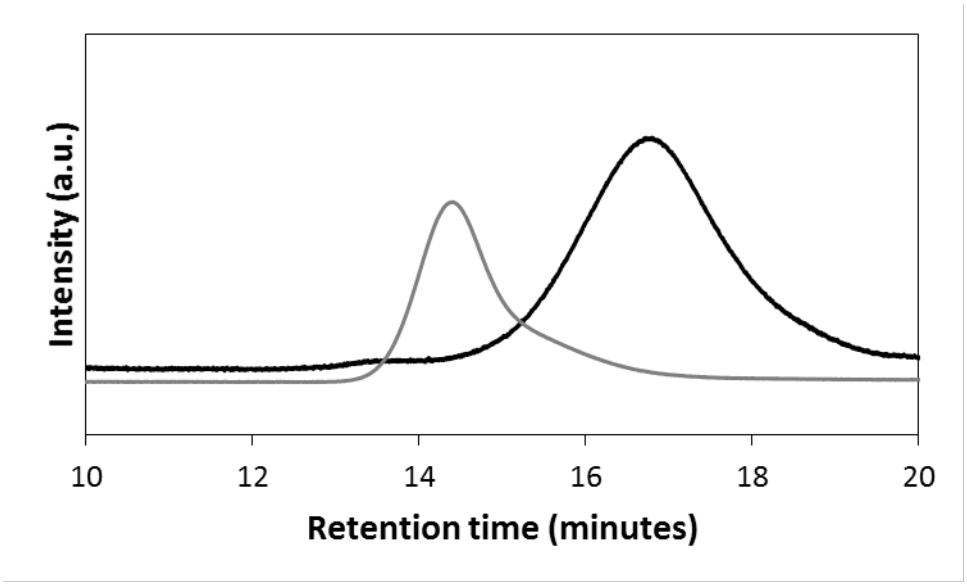


Figure S2. EDX spectrum of films of interpenetrating networks of PCL and PEDOT-CO_2H biomineralized with calcium-carbonate. Peaks in the EDX spectra at 0.277 and 0.525 keV are the characteristic Kα emissions of carbon and oxygen, respectively, and the very weak emission at 0.392 keV is the Kα emission of nitrogen. The peaks in the spectra of the films after the polymerization reactions at 2.621 and 6.398 keV are characteristic Kα emission lines of chlorine and iron, the peak at 0.705 keV is the Lα emission line of iron, and the peak at 2.307 keV is the Kα emission line of sulphur present in the backbone of the PEDOT-CO_2H. The successful biomineralization of the PEDOT-CO_2H films with calcium carbonate is clear from the appearance of the peak at 3.690 keV that is characteristic of the Kα emission of calcium.

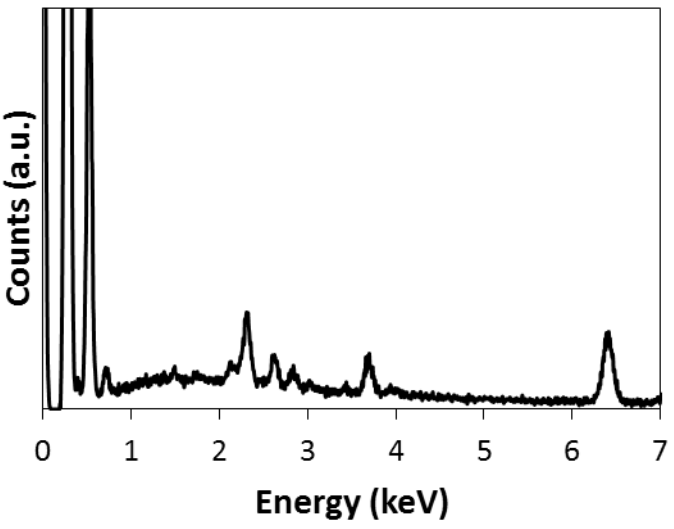


Figure S3. Films of interpenetrating networks of PCL and PPy-PSS films (prepared as reported by Hardy and coworkers in *Bioengineering*, 2015, **2**, 15) could be biomineralized with calcium carbonate as shown in the optical micrograph (scale bar represents 200 μm).