Supporting Information

Binding of Anti-Cell Adhesive Oxime-Crosslinked PEG Hydrogels to Cardiac Tissues

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Experimental Section

All solvents and reagents were obtained from Sigma, Fischer, or Acros and used as received unless otherwise stated. Star, hexaglycerol core, eight-arm poly(ethylene glycol) (10,000 g/mole) was purchased from Creative PEG works and used as received. NMR spectra were acquired on Bruker 500 and 600 MHz spectrometers, and processed using Topspin 2.1. Rheological measurements were performed with a TA Instruments ARG2 Rheometer using a parallel-plate geometry (20 mm diameter) at 37 °C with 0.3 N normal force, for runs longer than ten minutes samples were surrounded with mineral oil.

Synthesis of 8-arm aldehyde-PEG: Star PEG (1.0 g, 0.1 mmol) was dissolved in anhydrous dichloromethane (20 mL), followed by addition of 4-carboxybenzaldehyde (360.3 mg, 2.4 mmol). The reaction flask was placed into an ice bath followed by addition of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (460.1 mg, 2.4 mmol) and 4-(dimethylamino)pyridine (13.0 mg, 0.1 mmol). After 48 h methanol (0.5 mL) was added and stirred for 3 h. The crude reaction product was then dialyzed (molecular weight cut off 3,500 g/mole) against methanol to afford the 8-arm aldehyde-PEG (ald-PEG) in 94% yield and 91% functionalization. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.13 (bs, 1H, HCO), 8.28-8.24 (m, 2H, C6H4), 7.97-7.93 (m, 2H, C6H4), 4.55-4.52 (m, 2H, CH2OC=O), 3.88-3.85 (m, 2H, CH2CH2OC=O), 3.8-3.4 (m, PEG protons).

Synthesis of 8-arm N-hydroxyphthalimide-PEG: Star PEG (1.0 g, 0.1 mmol) was dissolved in anhydrous dichloromethane (20 mL) and tetrahydrofuran (2 mL), followed by

addition of *N*-hydroxyphthalimide (407.3 mg, 2.5 mmol) and triphenylphosphine (655.7 mg, 2.5 mmol), and the reaction flask was placed into an ice bath. Diisopropyl azodicarboxylate (427.5 μL, 2.4 mmol) was added dropwise over 30 minutes. After 48 h methanol (0.5 mL) was added and stirred for 3 h. The crude reaction product was then dialyzed (molecular weight cut off 3,500 g/mole) against methanol to afford the 8-arm *N*-hydroxyphthalimide-PEG in 95% yield and 89% functionalization. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.88-7.85 (m, 2H, C6H4), 7.78-7.75 (m, 2H, C6H4), 4.41-4.35 (m, 2H, CH2ON), 3.8-3.4 (m, PEG protons).

Synthesis of 8-arm aminooxy-PEG: N-hydroxyphthalimide-PEG (331.0 mg, 0.033 mmol) was dissolved in acetonitrile (5.296 mL). Hydrazine monohydrate (121.8 μL, 2.5 mmol) was slowly added over 15 minutes. The reaction was stirred for 2 hr followed by addition of dichloromethane (46 mL) and filtered over a plug of Celite® 545 to afford the 8-arm aminooxy-PEG (AO-PEG) in 91% yield with 86% functionalization. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.88-3.85 (m, 2H, CH2ONH2), 3.8-3.4 (m, PEG protons).

Characterization of Oxime-Crosslinked PEG-Hydrogel: AO-PEG and ald-PEG were separately dissolved in deionized water at 100 mg/mL. For lower concentrations samples were diluted with additional deionized water. AO-PEG (250 μL) and ald-PEG (250 μL) were added simultaneously to 4 mL glass vial and the timer started. Gelation was determined at the inability of the solution to flow (n=3 for each concentration). For swelling ration and water content, PEG hydrogels (100 μL, 100 mg/mL) were prepared as described above. The gels were incubated at 37 °C for 24 h. The gels were then placed into PBS pH 7.4 (1 mL) (n=3) for 24 hrs after which the gels were lyophilized. The percent swelling was calculated as follows: Volume(swollen)/Volume(initial)*100. The water content of the swollen hydrogels was calculated as follows: (Mass(swollen)-Mass(dehydrated))/Mass(swollen)*100%.

Cell adhesion experiments: Oxime-crosslinked PEG hydrogel (100 μL, 100 mg/mL) were formed in a 96 well plate using ratios of 1:1, 1:3, and 3:1 ald-PEG:AO-PEG. The gels

were swollen in Dulbecco's PBS for 48 h at 37 °C. The membranes of mouse 3T3 fibroblasts and RAW 264.7 macrophages were labeled with PKH26 following manufacture protocol. The PBS on the swollen gels was removed and either labeled macrophages (100 μL, 100,000 cells/cm^2) or fibroblasts (100 μL, 50,000 cells/cm^2) were seeded on top of the gels (n=3 for each formulation for each cell type); tissue culture plastic was the positive control. After 24 h at 37 °C fluorescent images were taken of both cell types on tissue culture plastic, 1:1 gel, 1:3 gel, and 3:1 gel. Five pictures were taken per well with three wells per group. The fluorescence per area was quantified using ImageJ and analyzed with one-way ANOVA with a Tukey post-test.

Agar diffusions assay: Fibroblasts (L929) were labeled with CellTrace (cytosol stain) according to the manufacturer's protocol. An agar diffusion based test was performed according to International Organization for Standardization (ISO) 10993-5 guidelines. Labeled L929s were cultured for 48 hours in 25 mm dish followed by formation of a 2 wt. % serum free agar gel over the cells. After serum starving for 24 hours 1:1 (n=3), 1:3 (n=3), 3:1 (n=3) aldehyde:aminooxy gels were placed on top of the agar gel. Latex samples (n=3) as well as filter paper samples (n=3) were also placed on agar gels. Cell morphology and density was then evaluated directly underneath the substrates and at the border zones. Five pictures were taken of cells beneath the substrate in each well (5 pictures per well, 15 pictures per group). Cells were scored based upon morphology from 1 to 3. A score of 1 was a rounded cell where the long and short axes were of equal length. A score of 3 was a spread-out cell with multiple protrusions where the long axis was >2 times longer than the short axis. A score of 2 was a cell morphology that was not scored 1 or 3.

Doped media assay: Fibroblasts (3T3) were cultured until confluent in a 96 well plate. Following monolayer formation, cells were serum starved for 24 hours and then cultured in 5% elution extract (n = 3), 25% elution extract (n=3), 50% elution extract (n=3) and serum free

media (n=3) for 24 hours at 37°C. Positive controls were media doped with the same volume of PBS. Metabolic activity of cells was quantified using the AlamarBlue assay following the manufacture's protocol.

Material retention of oxime-crosslinked PEG-hydrogels on cardiac tissues. AO-PEG (300 mg) was dissolved in 0.1 M PBS (10 mL, pH 9.0) and Alexa Fluor® 594 Succinimidyl Ester (330 μL of 1 mg/mL in dimethyl sulfoxide) was added. After 1 h the crude product was dialyzed (MWCO 3,500 g/mole) in ethanol resulting in fluorescent-AO-PEG (AO-PEG*) with 0.7 % functionalization by ^{1}H NMR. Ald-PEG (330 mg) was dissolved in 0.1 M PBS (10 mL, pH 4.0) and Alexa Fluor® 594 Hydrazide (330 μL of 1 mg/mL in dimethyl sulfoxide) was added. After 1 h the crude product was dialyzed (MWCO 3,500 g/mole) in ethanol resulting in fluorescent-ald-PEG (ald-PEG*) with 0.6 % functionalization by ^{1}H NMR. Porcine heart was obtained within hours of euthanasia and the coating of tissue performed following previously published procedure with slight modification.[1] Biopsies of adipose, ventricle, atrium, and aorta/pulmonary artery were taken with 5 mm punches. The aorta and superior vena cava were combined due to amount of available tissue; biopsies from the aorta and superior vena cava were randomly mixed into the separate groups. Each biopsy was dipped into PBS pH 7.4 to damped the tissue to mimic the fluid present during a surgical procedure. The materials were then applied using two pipettes (each containing 5 μL) resulting in 10 μL of 100 mg/mL PEG-hydrogel on the surface of the tissue. Three different formulations of PEG-hydrogel were investigated per tissue 1:1, 1:3, 3:1 volumes of ald-PEG:AO-PEG (n=3 for each formulation for each tissue). After five minutes the PEG-covered biopsies were aggressively dunked in PBS 3-5 times and then placed into a 48 well plate with PBS (400 μL) containing 0.625% vol/vol pen strep (life technologies) to completely cover the gel coated tissue. Buffer was removed, fluorescence was measured on a BioTek plate reader (excite 589 nm, emission 615 nm,

sensitivity 100), and fresh buffer was added. The PBS was replaced after 1, 4, 8 h, and then every 24 h for two weeks.