Supporting Information

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SI Materials and Methods

Cytotoxicity Evaluation. Cytotoxicity of the formed gels was evaluated by exposure to human mesenchymal stem cells (6,000 cells cm^{-2}) (Lonza) in a Transwell (Corning) format ($n = 3$ per group). Hydrogel disks were sterilized by exposure to germicidal UV light for 1 h and placed into a Transwell insert into a well 24 h after seeding. The AlamarBlue fluorescence assay (10% in medium) was used to quantify viability at days 1 and 3 post-exposure to the hydrogels.

Uniaxial Tensile Testing. Samples ($20 \times 5 \times 2$ mm, $n = 4$–8 per group from four different hearts) were removed from the midwall of the left ventricle of explanted (not infarcted) tissue in the longitudinal direction. Uniaxial testing was completed using an Instron 5848 Microtester with a 50-N load cell and equipped with custom grips and a PBS reservoir. A 0.05-N preload was applied for 60 s. Samples were preconditioned with 15 cycles of 0.005% of gauge length at 0.1% sec^{-1} followed by a ramp to failure at 0.1% strain s^{-1}. The modulus was determined as the slope between 10% and 15% strain (the linear region) of the resulting stress-versus-strain curve using a custom Matlab program.

Echocardiographic Analysis. Briefly, transapical epicardial real-time 3D echocardiography was performed through the left thoracotomy using a Philips IE 33 platform with a 7-MHz ultrasound probe (Philips Medical Systems). Full-volume 3D datasets were acquired. These datasets were exported to a dedicated workstation for image manipulation and analysis using QLAB 3D Advanced Quantification software (Philips Medical Systems). The 3D image acquired was manipulated to display two orthogonally related long-axis views, bisecting each other on the central long axis of the left ventricle. Ventricular volumes were obtained according to the software manufacturer's recommended method: In both end-diastole (defined as the frame before closure of the mitral valve) and in end-systole (defined as the frame before closure of the aortic valve), the basal and apical limits of the left ventricle are defined by manually placing reference points on the image in the two orthogonally related long-axis views. The software then defines the interface between the endocardium and LV cavity, and thus the LV envelope, by inserting splines to connect the manually inserted reference points for each of these frames. The 3D image for each of these two time points then may be rotated about its long axis, and thus the line defining the endocardial envelope of the left ventricle may be fine-tuned manually to correct for interpolation error. After these two frames have been traced in this manner, the remaining frames are traced in sequence by means of automated contour detection. The resulting 4D LV model then is divided automatically into the 17-segment model of the American Society of Echocardiography, and the global and segmental volume–time curves are exported to Microsoft Excel. At each time point, global end-diastolic and end-systolic volumes were defined as the maximum and minimum LV cavity volumes, respectively. Global ejection fraction was defined as (end-diastolic volume − end-systolic volume)/end-diastolic volume. The length of the anterior apical wall motion abnormality (i.e., infarct length) was measured in the 2D apical two-chamber view. A pulmonary artery catheter was used to measure cardiac output via the thermodilution method.

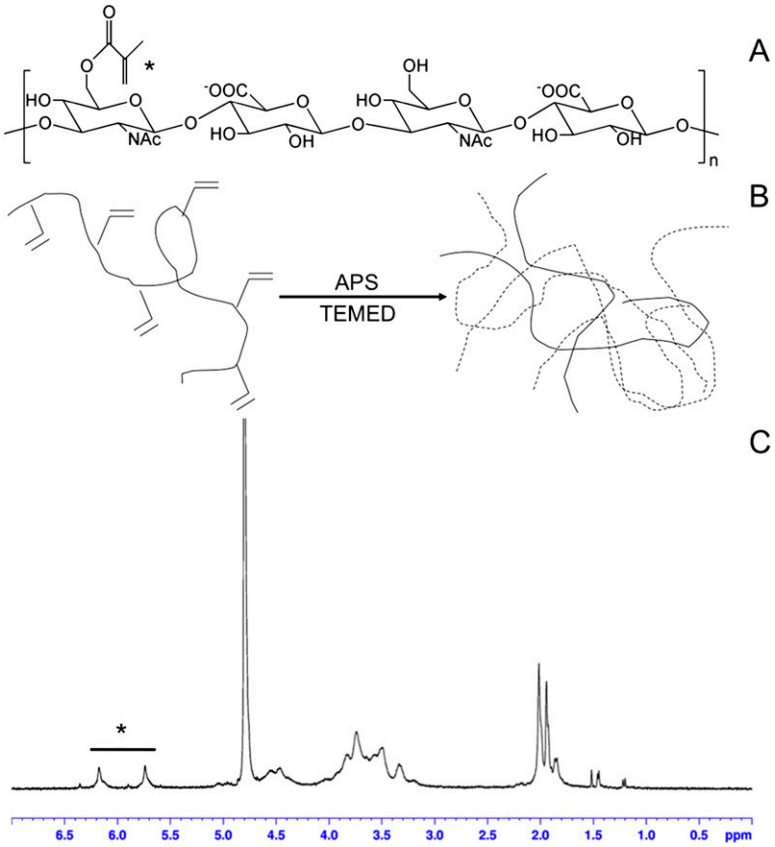

Fig. S1. (*A*) Methacrylated hyaluronic acid (MeHA) chemical structure. (*B*) Schematic of hydrogel formation. (*C*) Representative ^{1}H NMR spectra of MeHA. *Methacrylate group.

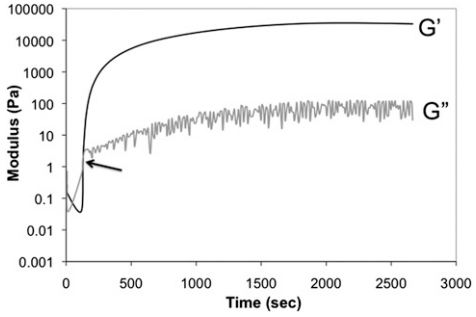

Fig. S2. Representative MeHA High [5.0 mM ammonium persulfate (APS)/5.0 mM *N,N,N′,N′*-tetramethylethylenediamine (TEMED)] time sweep depicting gelation onset, as indicated by the arrow.

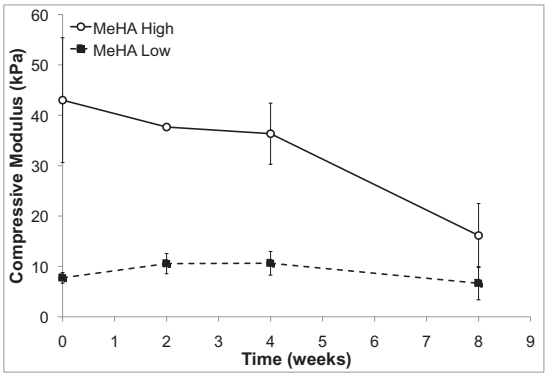

Fig. S3. Compressive modulus of MeHA High (circle, solid line) and MeHA Low (square, dashed line) with in vitro degradation over 8 wk.

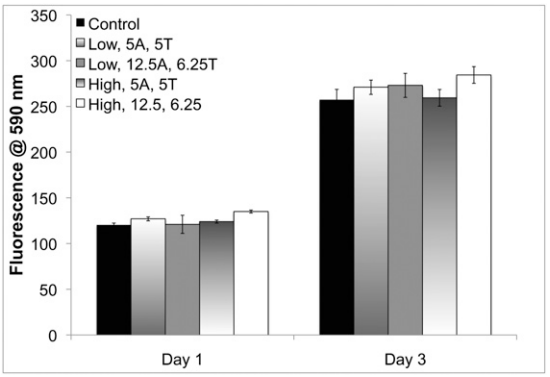

Fig. S4. In vitro cytotoxicity. Human mesenchymal stem cells were cultured in the presence of the different hydrogels in a Transwell format, and viability was assessed via the AlamarBlue assay. Data are plotted as mean ± SD. There were no statistically significant differences between any groups at each time point (n = 3 per group at each time point).

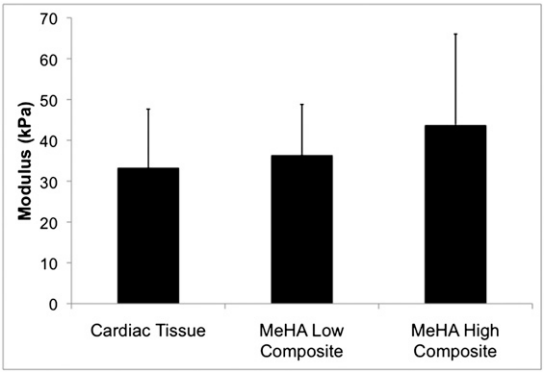

Fig. S5. Moduli of cardiac tissue and hydrogel tissue composites as determined using uniaxial tensile testing. Samples (20 × 5 × 2 mm) were prepared from the midwall of the left ventricle in the indicated direction followed by injection of 0.3 mL of macromer/initiator solution (if necessary). n = 4–8 per group. Data are presented as mean ± SD.

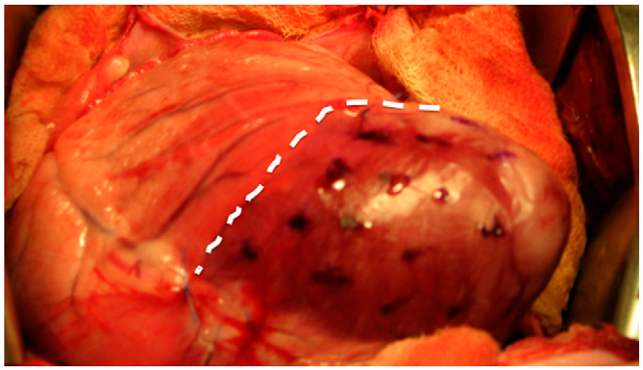

Fig. S6. Sheep heart (as viewed from left thoracotomy) depicting the infarct area (discolored region to the right of the dashed line) and the injection sites (dots).

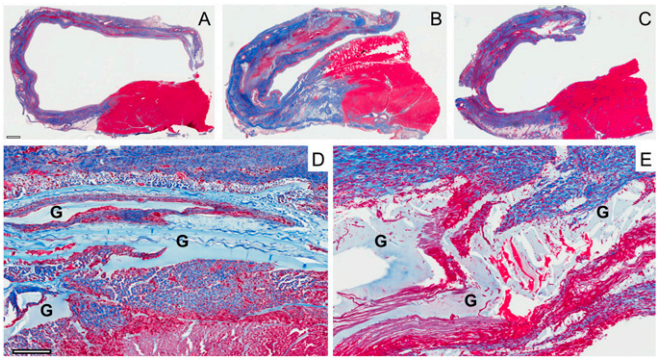

Fig. S7. Histological images 8 wk after myocardial infarction (MI) and injection. Representative trichrome-stained samples of (*A*) control infarct, (*B* and *D*), MeHA High, and (*C* and *E*) MeHA Low treatment in which the gel (labeled "G") stains light blue. (Scale bar, 1 mm in *A*–*C* and 200 μm in *D* and *E*.)

Table S1. In vivo effects of MeHA treatment on infarct size, geometry, and function

Metric	Control infarct	MeHA high	MeHA low
Number	9	6	5
Weight (kg)	40.6 ± 0.7	40.8 ± 0.7	39.4 ± 1.3
Infarct area	28.64 ± 1.0	23.87 ± 0.93*	26.42 ± 1.56
Infarct length			
Post-MI	7.41 ± 0.23	7.37 ± 0.16	7.34 ± 0.10
2 wk	8.28 ± 0.39	8.04 ± 0.12	8.26 ± 0.14
8 wk	8.91 ± 0.55	8.47 ± 0.16	8.98 ± 0.15
Normalized end-diastolic volume			
Post-MI	1.30 ± 0.08	1.32 ± 0.08	1.50 ± 0.13
2 wk	1.76 ± 0.22	1.63 ± 0.12	1.80 ± 0.23
8 wk	2.06 ± 0.20	1.70 ± 0.13	2.08 ± 0.25
DoB 2.5	1.41 ± 0.15	1.22 ± 0.07	1.58 ± 0.29
DoB 5.0	1.19 ± 0.13	0.90 ± 0.07	1.20 ± 0.28
Normalized end-systolic volume			
Post-MI	1.38 ± 0.08	1.45 ± 0.08	1.66 ± 0.18
2 wk	2.10 ± 0.30	1.90 ± 0.12	2.22 ± 0.40
8 wk	2.43 ± 0.29	2.00 ± 0.16	2.52 ± 0.38
DoB 2.5	1.64 ± 0.21	1.32 ± 0.10	1.88 ± 0.44
DoB 5.0	1.38 ± 0.18	0.95 ± 0.10	1.50 ± 0.43
End-diastolic volume			
Baseline	51.64 ± 2.27	56.97 ± 2.92	51.40 ± 2.37
Post-MI	65.94 ± 2.30	71.73 ± 3.81	72.26 ± 2.88
2 wk	88.08 ± 7.99†	91.23 ± 5.22†	91.60 ± 9.39†
8 wk	103.44 ± 8.10†	95.78 ± 6.50†	106.08 ± 8.84†
DoB 2.5	71.53 ± 7.63	69.75 ± 5.64	79.5 ± 11.68
DoB 5.0	60.46 ± 6.29	51.48 ± 3.73	60.26 ± 10.97
End-systolic volume			
Baseline	31.69 ± 1.90	34.17 ± 1.58	30.20 ± 1.36
Post-MI	42.67 ± 2.12	47.42 ± 2.64	46.50 ± 2.65
2 wk	63.38 ± 6.67†	64.22 ± 3.77†	65.94 ± 9.98†
8 wk	74.03 ± 6.38†	67.18 ± 4.64†	75.32 ± 8.72†
DoB 2.5	50.87 ± 6.12	44.98 ± 3.80	55.82 ± 11.00
DoB 5.0	42.07 ± 5.20	33.13 ± 3.17	43.64 ± 11.24
Cardiac output			
Baseline	4.27 ± 0.24	3.82 ± 0.31	4.32 ± 0.26
2 wk	3.06 ± 0.13†	3.18 ± 0.26	3.30 ± 0.33
8 wk	3.04 ± 0.35†	3.28 ± 0.18	4.18 ± 0.75
DoB 2.5	4.51 ± 0.45	4.38 ± 0.35	5.02 ± 1.08
DoB 5.0	4.92 ± 0.61	5.15 ± 0.42	5.14 ± 0.62
Ejection fraction			
Baseline	38.89 ± 1.81	40.00 ± 1.16	41.22 ± 0.94
Post-MI	35.47 ± 1.62	33.80 ± 1.69	35.76 ± 1.67
2 wk	28.39 ± 1.41†	29.40 ± 0.95†	29.18 ± 3.37†
8 wk	28.65 ± 0.99†	29.67 ± 1.21†	29.60 ± 2.67†
DoB 2.5	29.54 ± 1.09	35.33 ± 1.97	31.20 ± 2.76
DoB 5.0	31.23 ± 1.29	35.97 ± 2.00	30.26 ± 4.31

Data are plotted as the mean ± SEM. DoB, dobutamine.
*$P < 0.01$ compared with control infarct.
†$P < 0.05$ compared with respective baseline value.