Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/26/eabb5067/DC1

Supplementary Materials for

4D physiologically adaptable cardiac patch: A 4-month in vivo study for the treatment of myocardial infarction

Haitao Cui, Chengyu Liu, Timothy Esworthy, Yimin Huang, Zu-xi Yu, Xuan Zhou, Hong San, Se-jun Lee, Sung Yun Hann, Manfred Boehm, Muhammad Mohiuddin, John P. Fisher, Lijie Grace Zhang*

*Corresponding author. Email: lgzhang@gwu.edu

Published 24 June 2020, *Sci. Adv.* **6**, eabb5067 (2020)
DOI: 10.1126/sciadv.abb5067

The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/26/eabb5067/DC1)

Supplementary Materials:

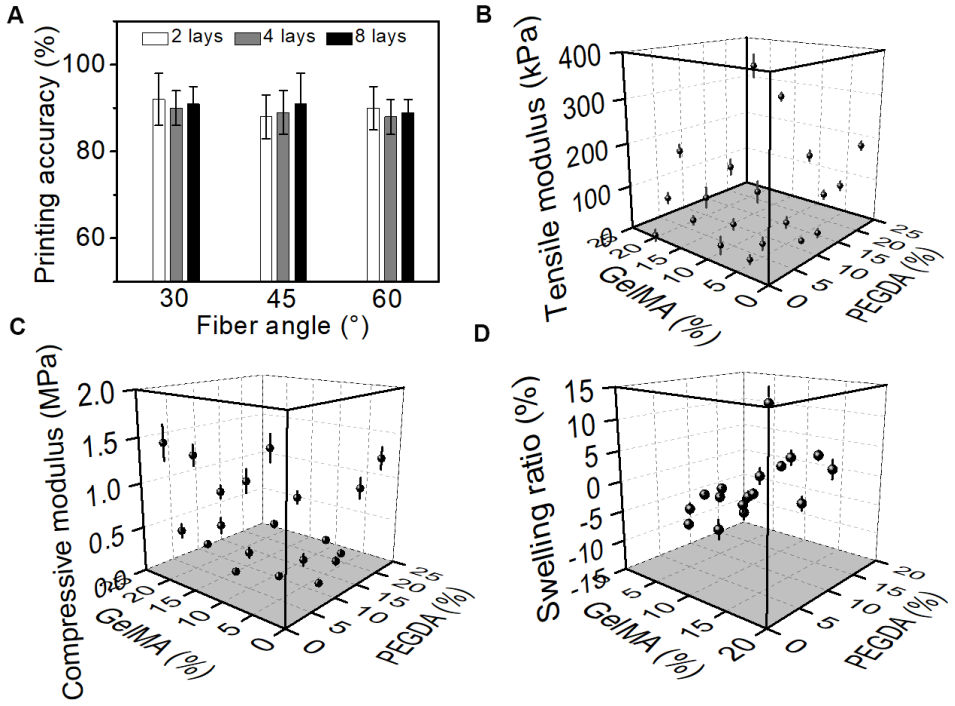

Fig. S1. Printing accuracy, mechanical modulus, and swelling behaviors of the printed cardiac patches. (**A**) Printing accuracy of the hydrogel patches as a function of fiber angle for different layer number; data are presented as the mean ± sd., n≥6. (**B**) Tensile moduli of the hydrogel patches with varying GelMA and PEGDA concentrations; data are presented as the mean ± sd., n≥6. (**C**) Compressive moduli of the hydrogel patches with varying GelMA and PEGDA concentrations; data are presented as the mean ± sd., n≥6. (**D**) Swelling ratio of the hydrogel patches with varying GelMA and PEGDA concentrations; data are presented as the mean ± sd., n≥6.

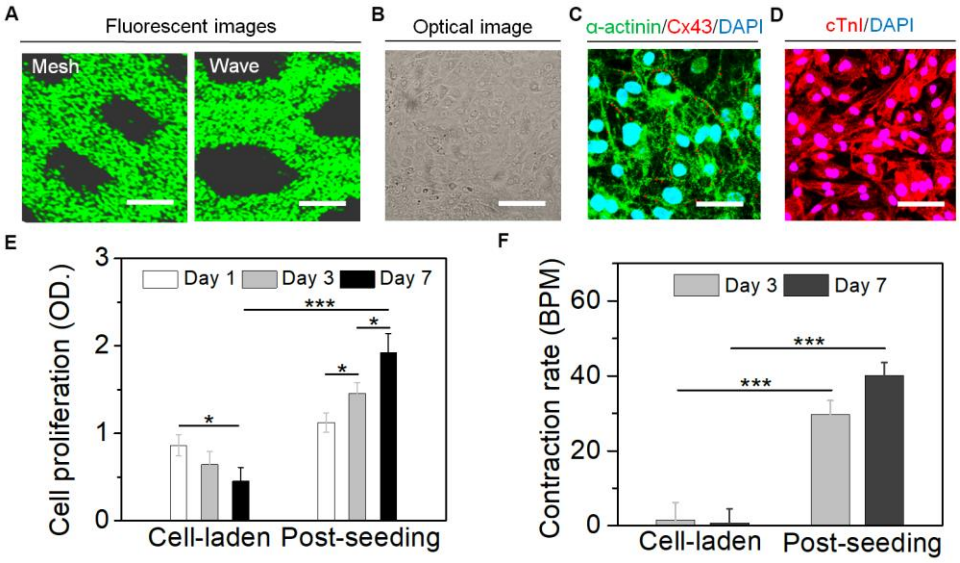

Fig. S2. Images, cell proliferation, and contraction rate of hiPSC-CMs on the well plate. (**A**) Fluorescent images of the mesh-patterned and wave-patterned hydrogel patches. The scale bar is 200 μm. (**B**) Optical images of monolayer hiPSC-CMs seeded on the well plate. The scale bar is 50 μm. Immunostaining of sarcomeric structure (α-actinin, green), gap junction (Cx43, red) (**C**), and contractile Protein (cTnI, red) (**D**) on the well plate. The scale bar is 30 μm. (**E**) Cell proliferation of hiPSC-CMs encapsulated in or seeded on the printed patches on day 1, day 3 and day 7; data are presented as the mean ± sd., n≥9, *p <0.05, ***p <0.001. (**F**) Beating rate of hiPSC-CMs on the printed patch and well plate on day 3 and day 7; data are presented as the mean ± sd., n≥6, ***p <0.001.

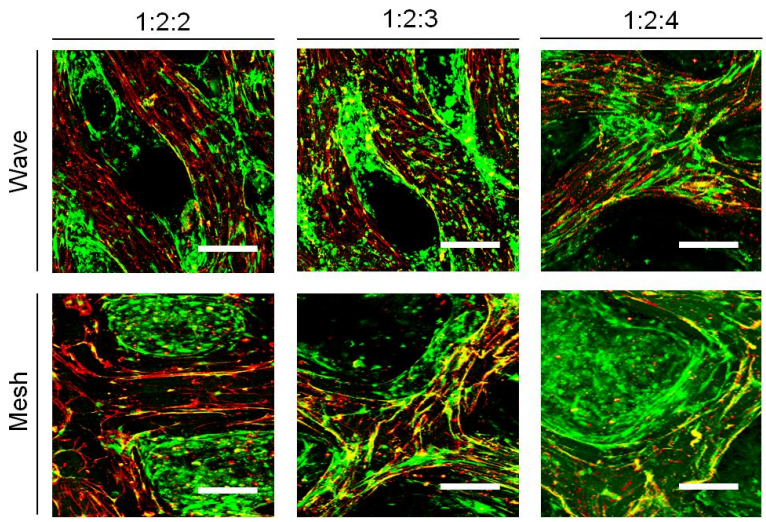

Fig. S3. Optimization of cell ratio for tri-cultured hiPSC-CMs, hECs, and hMSCs on the hydrogel patches. Optimization of cell ratio for tri-cultured hiPSC-CMs (green), hECs (red), and hMSCs (blue) on the hydrogel patches using cell tracker staining after 7 days of culture. The scale bar is 200 μm.

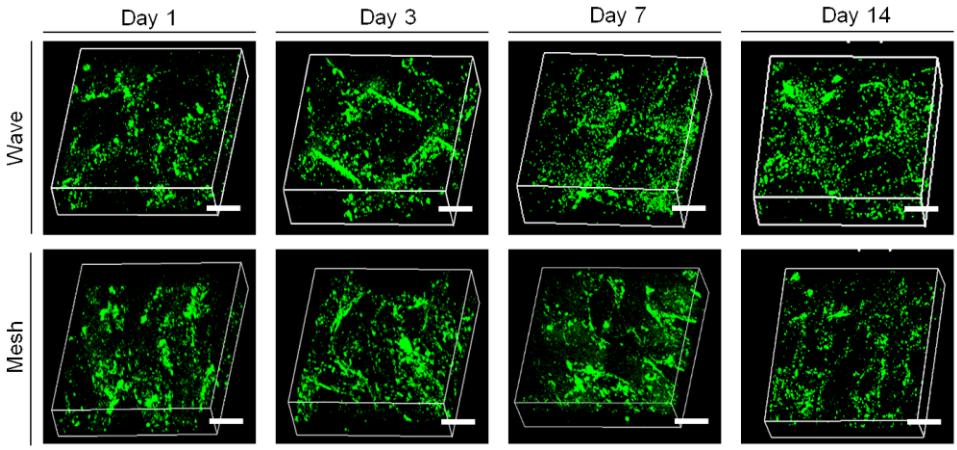

Fig. S4. Autofluorescence 3D images of GFP+ hiPSC-CMs on the patches for 14 days. Autofluorescence 3D images of GFP+ hiPSC-CMs on the mesh-patterned and wave-patterned patches on day 1, day 3, day 7, and day 14. The scale bar is 100 μm.

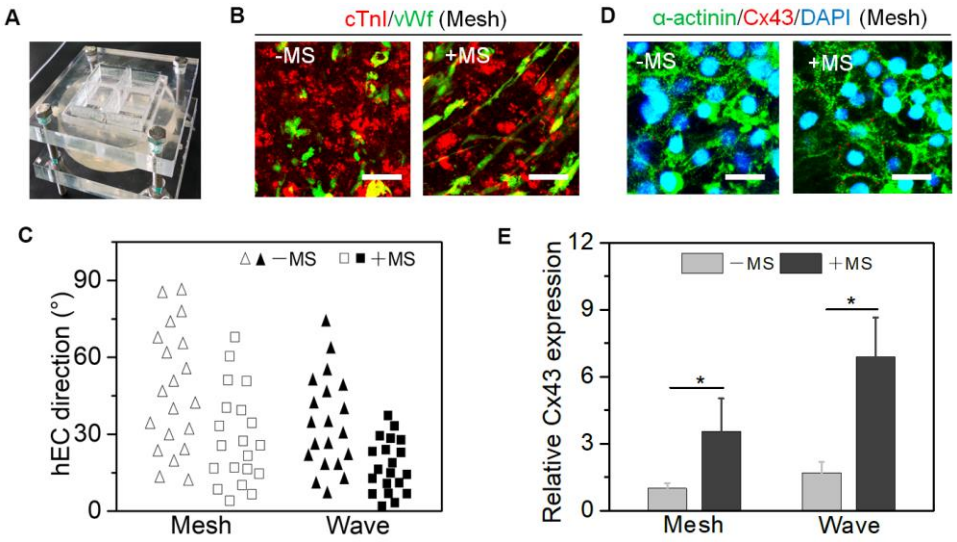

Fig. S5. **Immunostaining of hiPSC-CMs on the mesh-patterned patch, and directional distribution analysis of the hECs on the printed patches.** (**A**) Optical image of a custom-made bioreactor chamber to apply dual MS (mechanical loading and dynamic flow) for the maturation of engineered cardiac tissue on the hydrogel patches (Photo Credit: Haitao Cui, The George Washington University). (**B**) Immunostaining of contractile protein (cTnI, red) and vascular protein (vWf, green) on the mesh-patterned patch under MS condition (+MS) when compared to non-stimulated control (-MS). The scale bar is 50 μm. (**C**) Directional distribution analysis of the hECs on the printed patches under MS condition (+MS) when compared to non-stimulated control (-MS) on day 14; data are presented as the mean ± sd., n≥6 with 3 points for each image. (**D**) Immunostaining of the sarcomeric structure (α-actinin, green) and gap junction (Cx43, red) on the mesh-patterned patch under MS condition (+MS) when compared to non-stimulated control (-MS). The scale bar is 20 μm. (**E**) Relative Cx43 expression (fluorescence intensity) of hiPSC-CMs on the printed patches under MS condition (+MS) when compared to non-stimulated control (-MS) on day 14; data are presented as the mean ± sd., n≥6, *p <0.05.

Table S1. Primers of quantitative RT-PCR

Gene name	Protein name	Forward primer	Reverse primer
GAPDH	GAPDH	GGAGCGAGATCCCTCCAAA	GGCTCCCCCCTGCAAA
TNNI3	cTNI	CCTCACTGACCCTCCAAACG	GAGGTTCCCTAGCCGCATC
ACTN2	α-actinin 2	CTTCTACCACGCTTTTGCGG	CCATTCCAAAAGCTCACTCGC
RYR2	RYR2	TTGGAAGTGGACTCCAAGAAA	CGAAGACGAGATCCAGTTCC
vWf	vWf	CACCATTCAGCTAAGAGGAGG	GCCCTGGCAGTAGTGGATA
MYL2	MLC 2v	ACATCATCACCCACGGAGAAGAGA	ATTGGAACATGGCCTCTGGATGGA
PLN	Phospholamban	ACAGCTGCCAAGGCTACCTA	GCTTTTGACGTGCTTGTTGA
MYL7	MLC 2a	GGAGTTCAAAGAAGCCTTCAGC	AAAGAGCGTGAGGAAGACGG
MYH6	αMHC	CTCCGTGAAGGGATAACCAGG	TTCACAGTCACCGTCTTCCC
CD31	PECAM1	GAGTCCTGCTGACCCTTCTG	CACTCCTTCCACCAACACCT
ATP2A2	SERCA 2α	TCACCTGTGAGAATTGACTGG	AGAAAGAGTGTGCAGCGGAT
CASQ2	Calsequestrin 2	GTTGCCCGGGACAATACTGA	CTGTGACATTCACCACCCCA

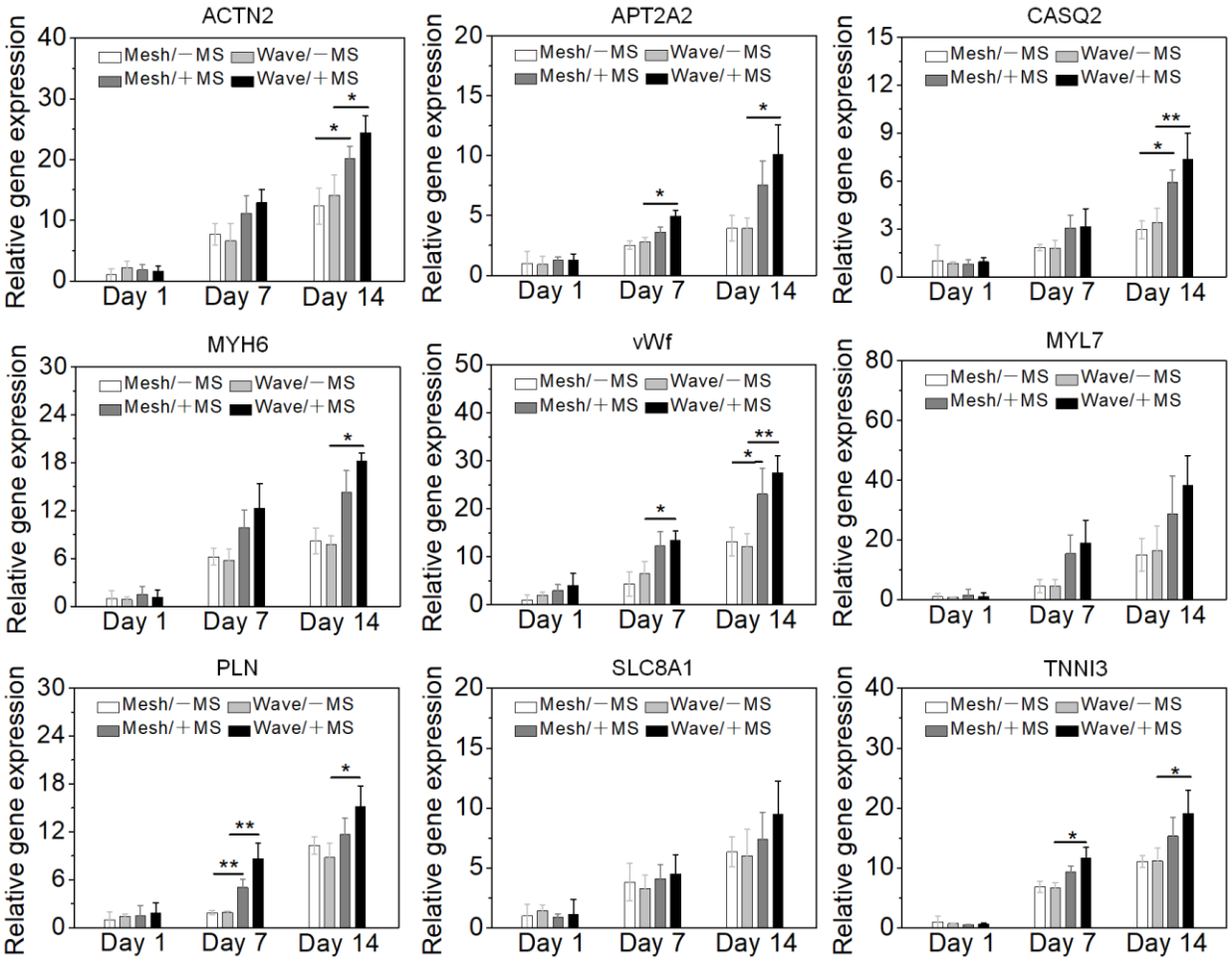

Fig. S6. Relative gene expression of myocardial structure, excitation-contraction, and angiogenesis on the patches. Relative gene expression of myocardial structure (ACTN2, TNNI3, MYH6, MYL7), excitation-contraction (RYR2, PLN, CASQ2, SLC8A1), and angiogenesis (vWf) on the patches under MS condition (+MS) when compared to non-stimulated control (-MS) on day 1, day 7, and day 14; data are presented as the mean ± sd., n≥9, *p <0.05, **p <0.01.

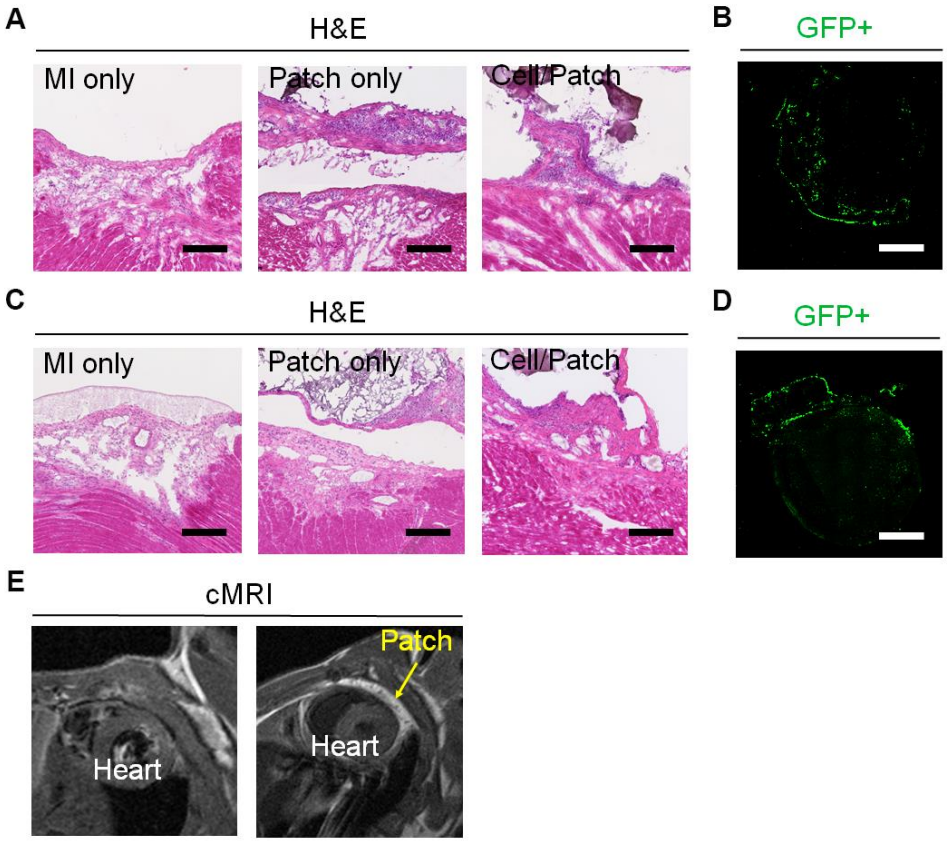

Fig. S7. H&E staining, GFP+, and cMRI images of printed patches. (**A**) Representative H&E staining images of mouse MI hearts without treatment (MI only), with the cell-free patch (Patch only), and with cellularized patch implantation (Cell/Patch) on the heart at week 10. The scale bar is 100 µm. (**B**) Representative fluorescent image of implanted (GFP+) iPSC-CMs on the patch at week 10. The scale bar is 500 µm. (**C**) Representative H&E staining images of mouse MI hearts without treatment (MI only), with the cell-free patch (Patch only), and with cellularized patch implantation (Cell/Patch) on the heart for 4 months. The scale bar is 100 µm. (**D**) Representative fluorescent image of implanted (GFP+) iPSC-CMs on the patch for 4 months. The scale bar is 500 µm. (**E**) Representative cMRI images of a mouse heart with the implanted patch. Left is MI only, and right is cellularized patch implantation into mice heart.

Movies S1. Spontaneous contractions of monolayer hiPSC-CMs on the well plate on day 7.
Movies S2. Spontaneous contractions of GFP+ hiPSC-CMs along the fiber direction of the printed patch on day 3.
Movies S3. Assembled hiPSC-CM fiber on the cardiac patch spontaneously contracted along with fibers after 2 weeks of MS.
Movies S4. Operation of patch implantation positioned over the infarcted site of the mice's heart.
Movies S5. cMRI video of the implanted patch on the heart area of mice showing contraction and relaxation with the heart beating at week 10.