Science Advances

AAAS

www.advances.sciencemag.org/cgi/content/full/1/7/e1500423/DC1

Supplementary Materials for

Platform technology for scalable assembly of instantaneously functional mosaic tissues

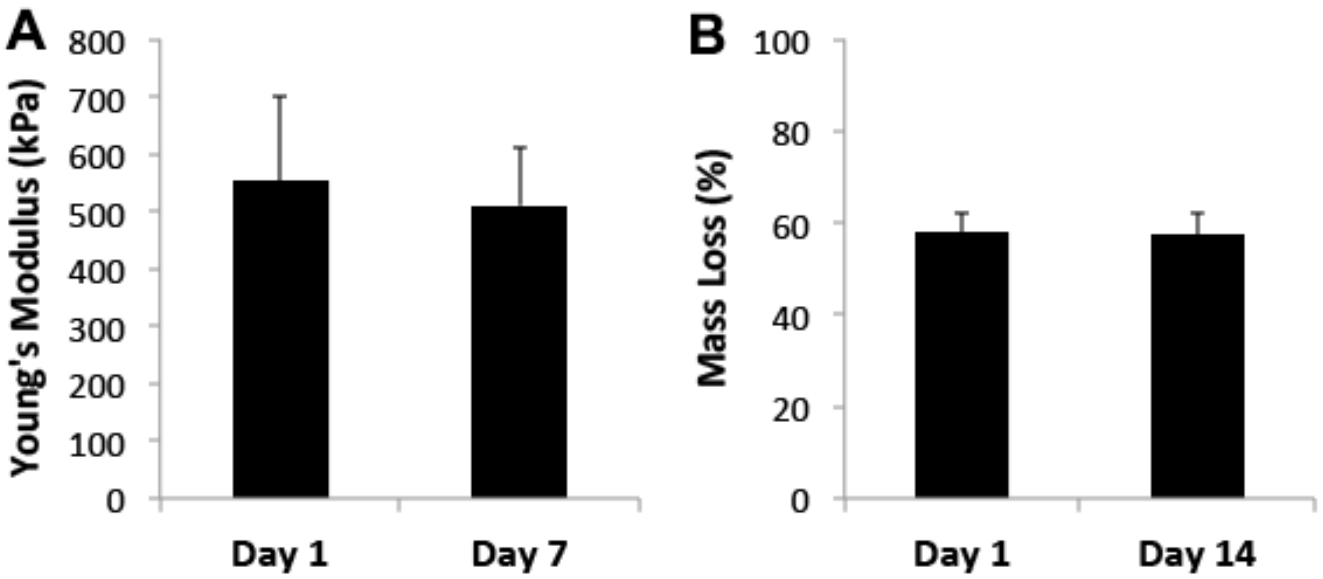
Boyang Zhang, Miles Montgomery, Locke Davenport-Huyer, Anastasia Korolj, Milica Radisic

Published 21 August 2015, *Sci. Adv.* **1**, e1500423 (2015)
DOI: 10.1126/sciadv.1500423

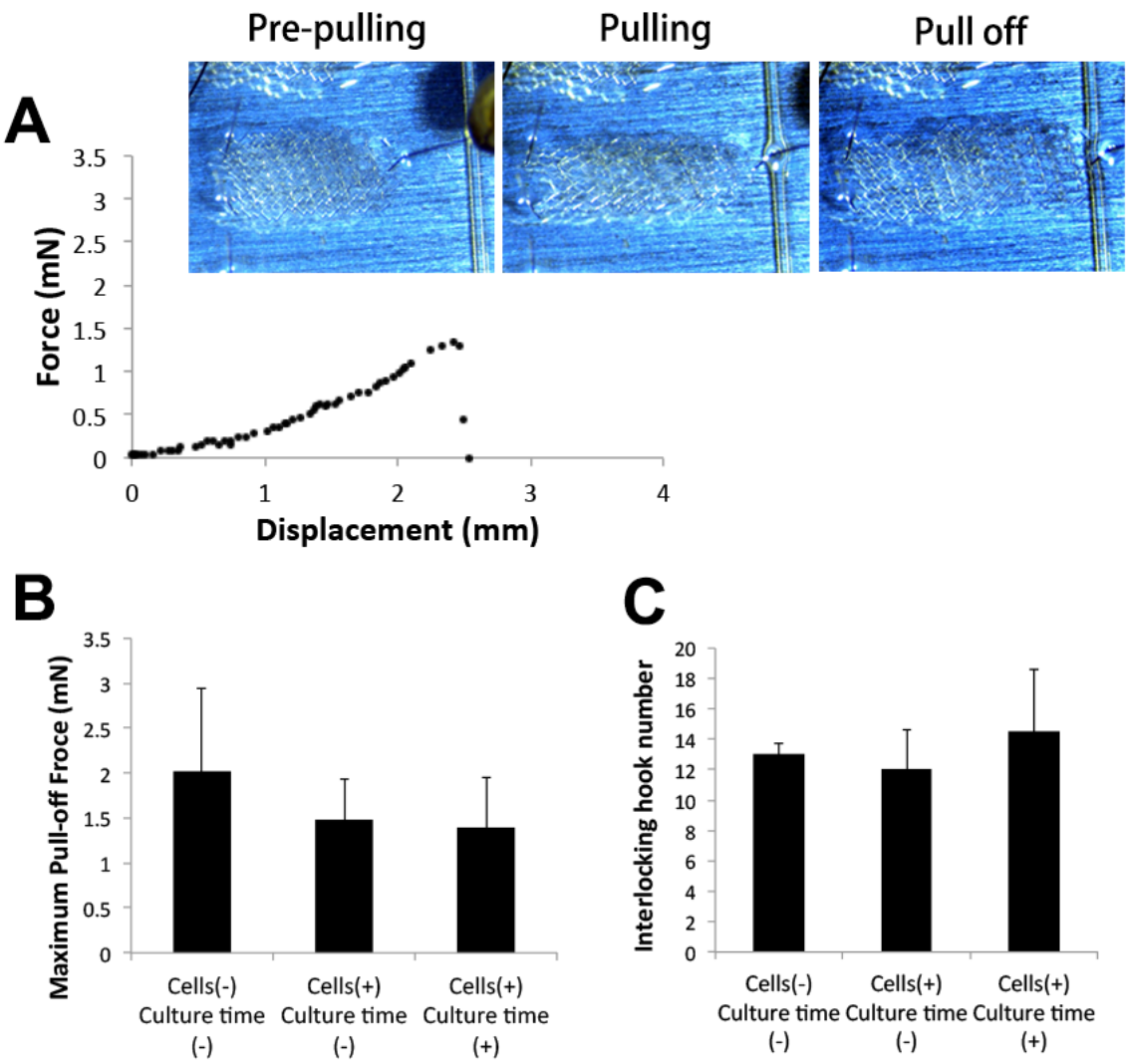
The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.advances.sciencemag.org/cgi/content/full/1/7/e1500259/DC1)

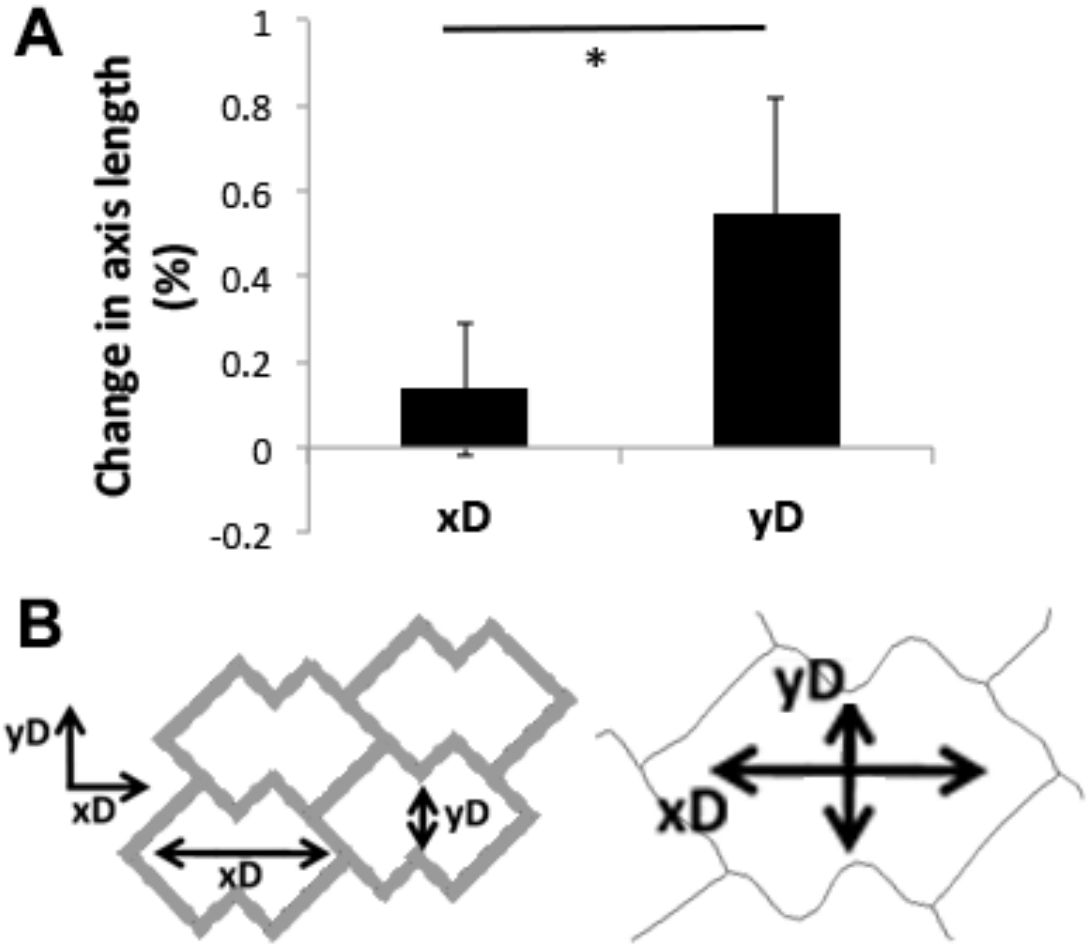
Movie S4 (.mov format). Time lapse of seeded CMs remodeling and compacting over a 3-day period on a single layer scaffold mesh (no hooks).
Movie S5 (.mov format). Contraction of cardiac tissue mesh after tissue remodeling, day 4.
Movie S6 (.mov format). Electrical field stimulation applied to a single scaffold mesh (no hooks) seeded with CMs, after 7 days in culture.
Movie S7 (.mov format). Autofluorescent scaffold contraction recorded and processed to measure fractional shortening.
Movie S8 (.mov format). Vertical scan of a three-layer Tissue-Velcro.
Movie S9 (.mov format). Electrically paced cardiac tissues contracting before assembly, after assembly, after disassembly, and 1 day after disassembly.
Movie S10 (.mov format). Spontaneous contraction of a two-layer cardiac tissue cultured for an additional 3 days after assembly.
Movie S11 (.mov format). Response of Tissue-Velcro (day 5) to epinephrine (300 nM) stimulation.



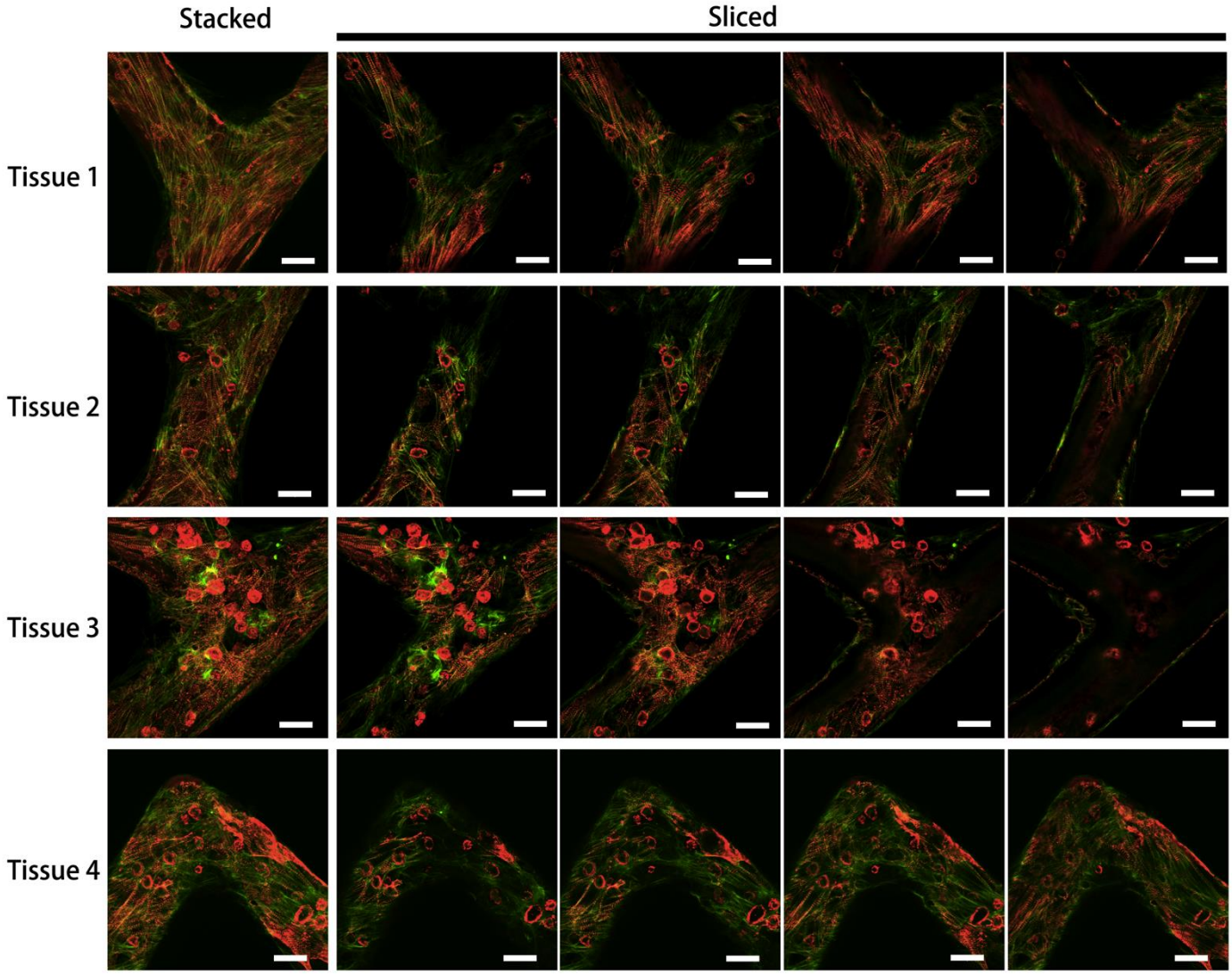
Supplementary Figure 1. Base material physical properties under cell culture conditions. (**A**) Young's (n=4). (**B**) Material mass (mean±s.d, day 1, n=6, day 14 n=5).



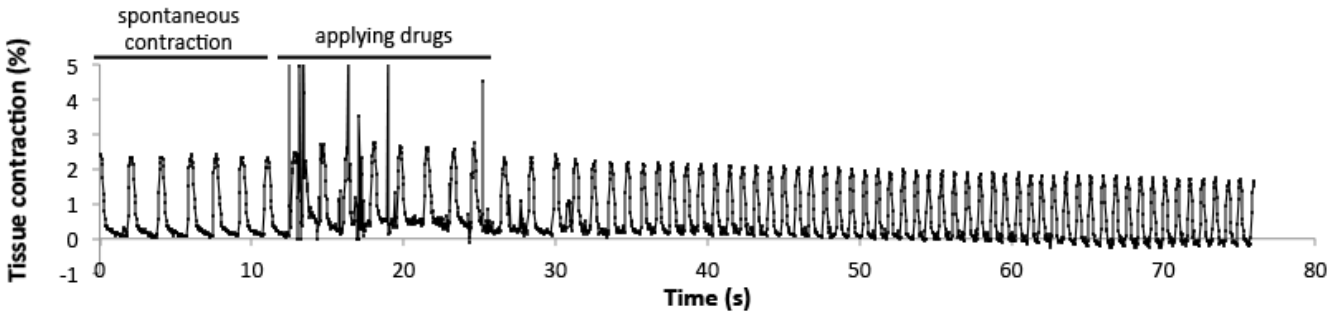
Supplementary Figure 2. Hook and loop interlocking mechanism is a dominant factor governing the mechanical stability of the assembled two-layer structures. (B) Representative pull-off force plot indicated a gradual rise followed by a sharp drop in force as the scaffold was pulled off. **(inset)** Set-up with two scaffolds or tissues for pull-off force measurement. Bottom scaffold was anchored down with two micro-pins on one side of the scaffold. The two scaffolds were off-set to leave room for the micro-pins. Upper scaffold was pulled from the opposite side with another micro-pin attached to the Myograph. **(B)** Quantification of maximum pull-off force (mean±s.d) generated under three different scenarios indicates the presence of cells or short culture time (3 days) does not affect mechanical stability of the assembled structures. Cells(-) Culture time (-) represents pull-off force between two cell-free scaffolds (n=5), Cells(+) Culture time (-) represents pull-off force between two tissues (day 7) assembled immediately before pull-off test (n=4), Cells(+) Culture time (+) represents pull-off force between two tissues (day 4) assembled and then cultured for additional 3 days before the pull-off test (n=4). **(C)** Number of interlocking hooks counted prior to pull-off test (mean±s.d).



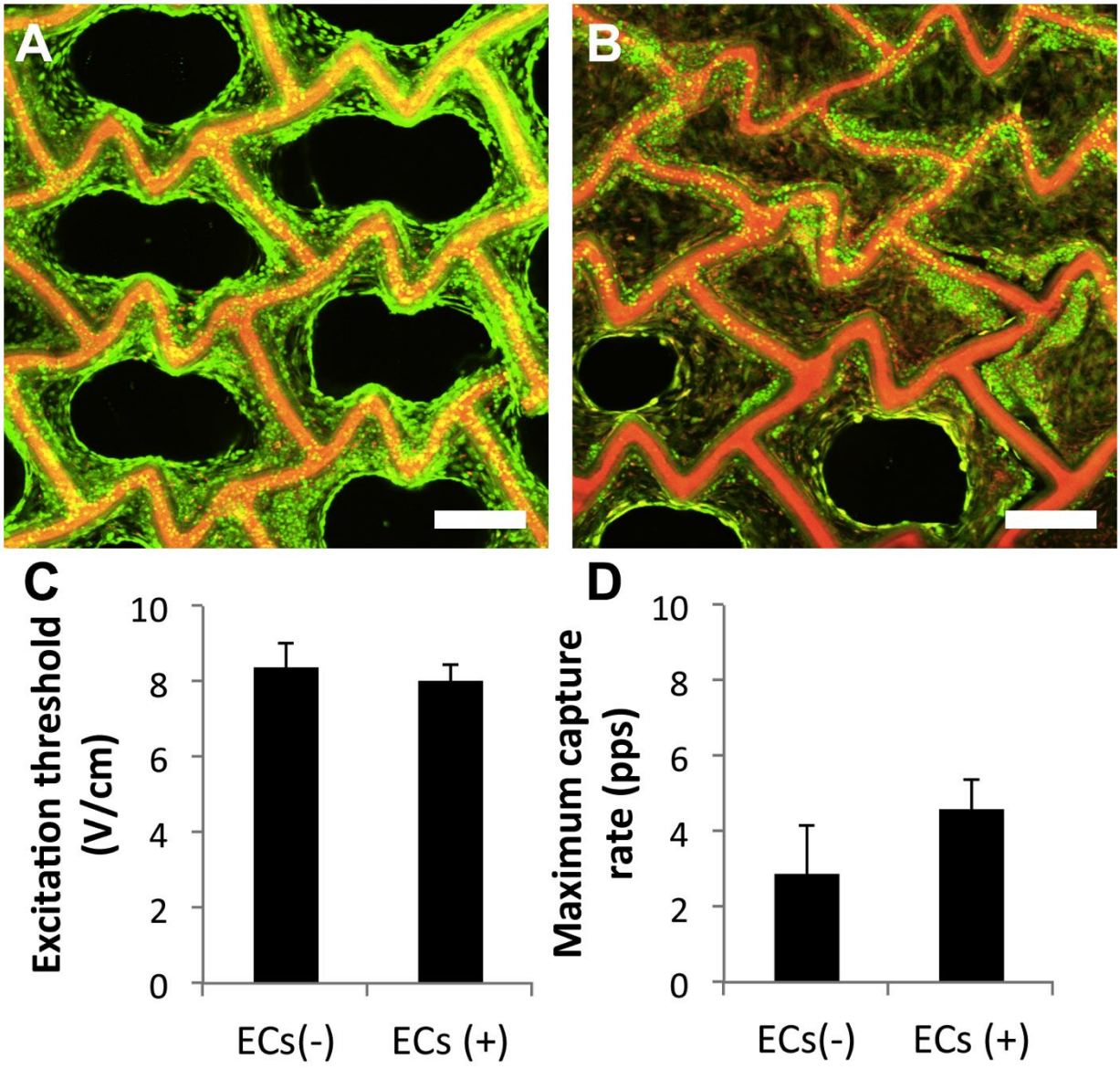
Supplementary Figure 3. Cardiac tissue contractility. (**A**) Quantification of axis shortening (%) during 1Hz paced contraction on day 8 of culture (mean±s.d, n =4). **(B)** Illustration and a representative skeletonized trace of the scaffold struts with labels indicating the two directions of compression (xD, long edge direction and yD short edge direction).



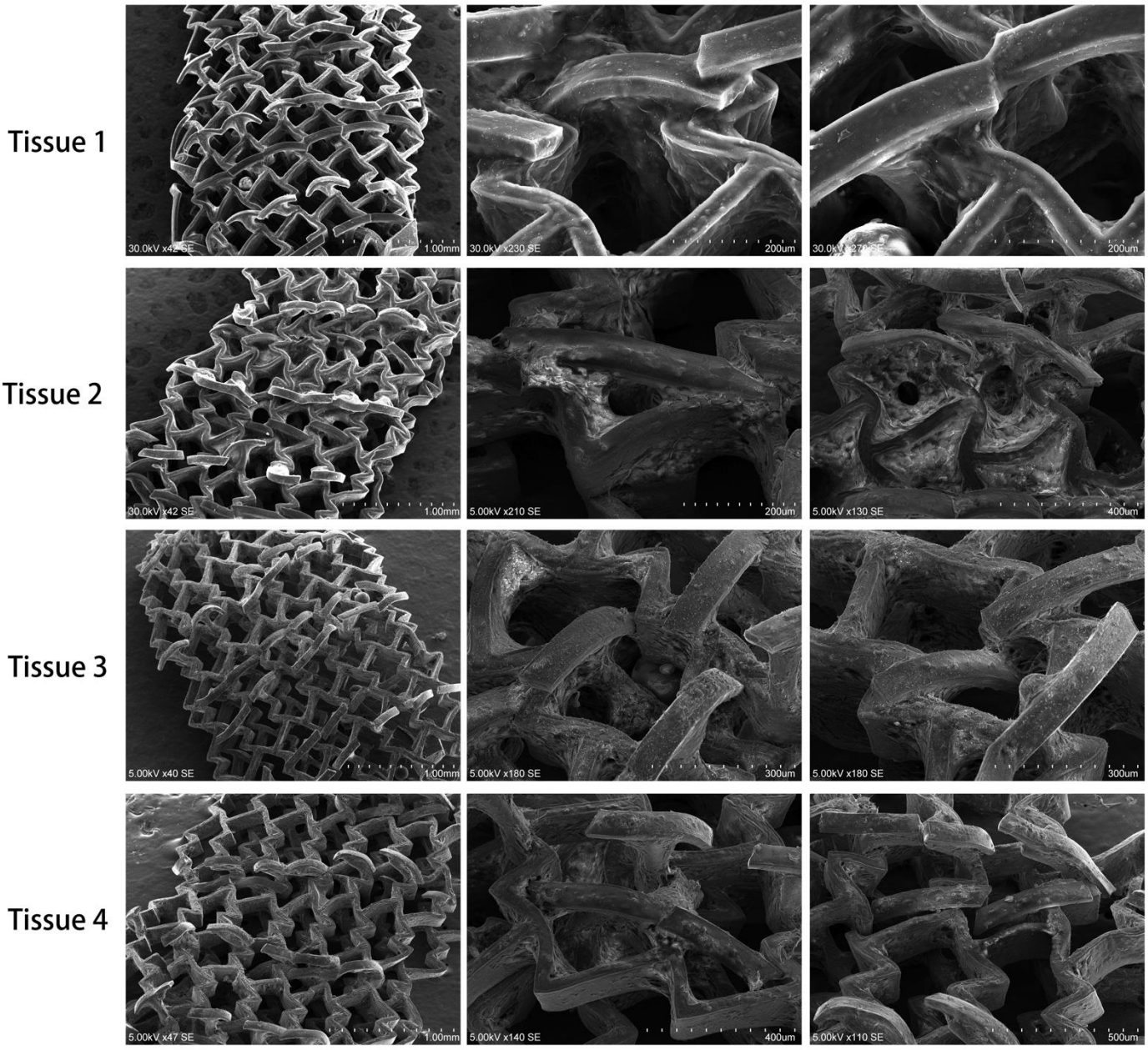
Supplementary Figure 4. Immunostaining of cardiac Tissue-Velcro on day 7 for sarcomeric α-actinin (red) and F-actin (green) at various locations of the tissues. Scale bar: 30 μm. Confocal sections were also shown individually to distinguish overlapping cells.



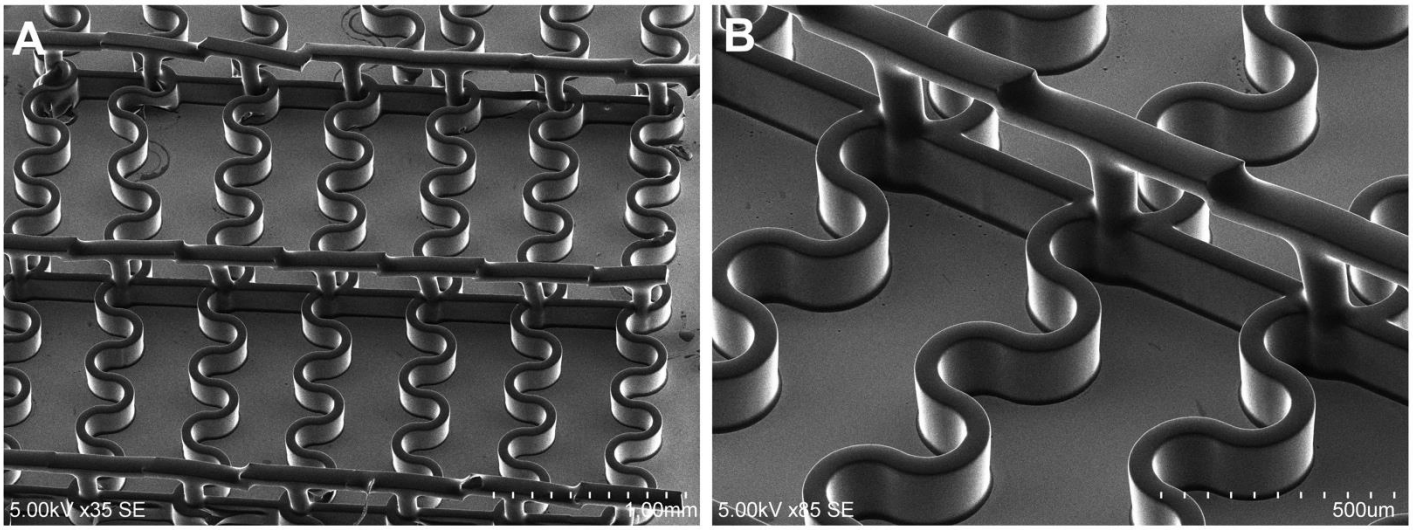
Supplementary Figure 5. Drug response. Spontaneously beating cardiac tissue (day 8) responding to stimulation with 300 nM epinephrine. EC50 for Epinephrine on rat cardiomyocytes were previously shown to range from 20 nM to 200 nM[57]. Increase in contraction rate is apparent.



Supplementary Figure 6. Coculture of cardiac and endothelial cells. (A, B) Fluorescent image of Tissue-Velcro stained with live and dead cell marker (CFDA, green and PI, red, scale bar: 200 μm). Scaffold struts exhibit autofluorescence in red. Tissues were first cultured in cardiomyocyte media for 4 days, then the tissues were coated (**A**) with or (**B**) without ECs and cultured for additional 4 days in EGM-2 media. Finally, the tissues were placed in 125 mL orbital shaker flasks at 160 RPM in 25 mL of EGM2 media for additional 3 days (n=4). Scale bar: 200 μm. (C, D) Quantification of the electrical excitability parameters at the end of the tissue culture (n=4).



Supplementary Figure 7. Scanning electron micrograph of the assembled two-layer cardiac tissue cultivated for 3 days. Hooks from the bottom Tissue-Velcro are locked onto the struts of the top Tissue-Velcro, forming a bridge for cell spreading and tissue integration. Scale bars shown on images.



Supplementary Figure 8. Scanning electron micrograph of an additional Tissue-Velcro design with spring-like structures that could potentially be used to enhance scaffold anisotropic mechanical properties and tissue anisotropic contraction. Scale bars shown on images.