Electronic Supplementary Information

Restoring Biophysical Properties of Decellularized Patches through Recellularization

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Seeding method analysis

Seeding methods were compared in terms of cell attachment and proliferation efficiency on the pcECM constructs. Sterile pcECM matrices (1cm diameter) were seeded with MSCs ($1x10^6$ cells/cm^2) using the following methods: Surface pipettation, needle injection, centrifugation and vacuum. For pipette surface seeding, 10 shots of 100 µl cell suspension were evenly pipetted over the endocardium surface of the pcECM. Needle injection seeding was performed by 10 injections of 100 µl cell suspension into the approximate mid-layer bulk of the pcECM, using a 25-gauge x1" hypodermic needle [Sterican®, B. Braun, Melsungen, Germany]. Centrifuge seeding was done by placing the ECM at the tip of 15mL centrifuge tube and submersed with 1mL of cell suspension. The tube was subsequently centrifuged at 500rpm for 2min at room temperature, with re-suspension of tube-bound cells after each centrifuge cycle for three times. For vacuum pump seeding, pcECM was placed on a Nalgene® PES filtering paper (Thermo Fisher Scientific, NY, U.S.A.) and subjected to 2min of 2bar vacuum suction with cell suspension pipetted on top over the time span.

Cell proliferation on the pcECM

AlamarBlue® Assay [Invitrogen, Carlsbad, CA] was used according to manufacturer's instructions and against a standard calibration curve of known cell concentrations performed in 6-well plates. Briefly, after pcECM samples were washed with PBS, 10% alamarBlue® in medium was added to each well and incubated for 4 hrs at 37^oC 5% CO_2. Following the incubation 100 µL of incubated solution in quadruplicate were transferred to a 96-well plate for fluorescence reading. Afterwards, scaffolds were washed gently and replenished with fresh media for further culture. Initial cell adherence

was evaluated 4h post seeding, and cell proliferation was evaluated 1, 3, 7 and 10 days post seeding using the AlamrBlue™ assay (Supplementary Fig. S1).

DiI-stained cell visualization

MSCs were pre-labeled with DiI (1, 1-diotadecyl-3, 3, 3, 3,-tetramethylindocarbocyanine perchlorate, Life-Technologies, CA, USA), and seeded on pcECM by surface pipettation. One day post seeding, samples were washed with PBS three times and fixated in 4% paraformaldehyde (PFA, Sigma-Aldrich, St-Louis, USA) for 4hr at 4˚C. Finally samples were washed 3 time in PBS and imaged using TCS SP5 confocal microscope (Leica, Wetzlar, Germany; Supplementary Fig. S2).

FTIR intensity raw data

Supplementary Fig. S3 displays the comparison of raw data intensities between the various peaks as measured for the different sample groups. Tukey's HSD with one way ANOVA revealed that the peak at 3000-3500nm is relatively stable and non-significantly different between the groups and was therefore used for normalization as appearing in the main manuscript Fig. 3D.

Biaxial tensile testing

To study the anisotropy of the samples, biaxial tensile experiments were performed in collaboration with Prof. Jacob Bortman's Heart Modeling team at Ben Gurion University (BGU). The custom-built heart tissue biaxial testing equipment was composed of four uniaxial motors, four load cells of 20N load capacity, an overhead camera and an integrated computer interface. Specimens were mounted in the orientation where their fiber-preferred direction was aligned with the Y-axis and the cross-fiber direction was

aligned with the X-axis of the biaxial testing equipment. Samples were equibiaxially preconditioned with 7 cycles of stretching-relaxation up to 10% elongation at 0.1 mm/s Following preconditioning, measurement was performed by equibiaxial stretching to 10% elongation at 0.15 mm/s. Loads were measured and computed into corresponding stress measurements. Deformations were measured by processing the video tracked images from the overhead camera and computed into corresponding strain measurements (Supplementary Fig. S5).

Supplementary Table S1: List of gene primers' accession (GenBank) and catalogue (Qiagen) numbers.

Gene - Symbol	GenBank accession number	Qiagen Catalog No.
ITGAV	NM_002210	#330001; PPH00628C-200
COL1A1	NM_000088	#330001; PPH01299F-200
NCAM1	NM_000615	#330001; PPH00639F-200
SPARC	NM_003118	#330001; PPH01175A-200
MMP2	NM_004530	#330001; PPH00151B-200
MMP10	NM_002425	#330001; PPH00896B-200
MMP11	NM_005940	#330001; PPH00236C-200
MMP14	NM_004995	#330001; PPH00198C-200
ACTB	NM_001101	#330001; PPH00073G-200
GAPDH	NM_002046	#330001; PPH00150F-200
HPRT1	NM_000194	#330001; PPH01018C-200
RPLP0	NM_001002	#330001; PPH21138F-200

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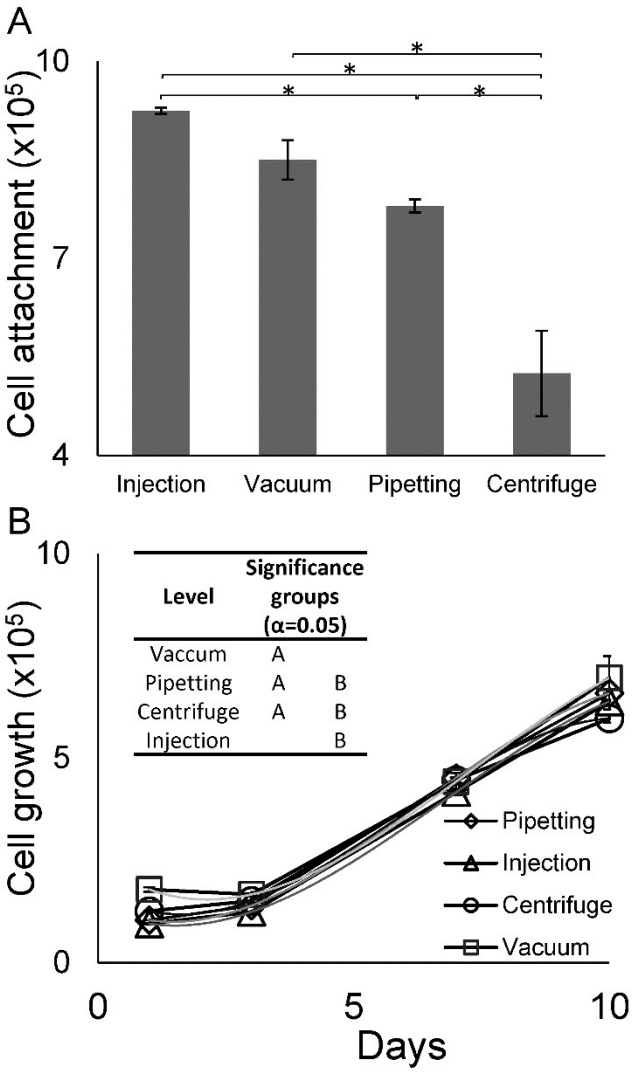
Supplementary Figures



Supplementary Fig. S1: Screening for optimal seeding method. Four seeding methods were evaluated (cell injection, vacuum distal to seeding site, cell suspension pipetting and centrifugation, as indicated) for their ability to deliver sustained cell quantities (**A,** 24 hours after seeding) and for their overall effect on the cell proliferation through time on the seeded pcECM constructs (**B**). Statistical significance (p<0.05) was calculated using one-way ANOVA with Tukey's HSD test for (**A**) and two-way ANOVA with Tukey's post hoc corrections for the effect of time and seeding groups in (**B**). Groups joined by the same letter in the statistical significance groups of panel (**B**) are insignificantly different.

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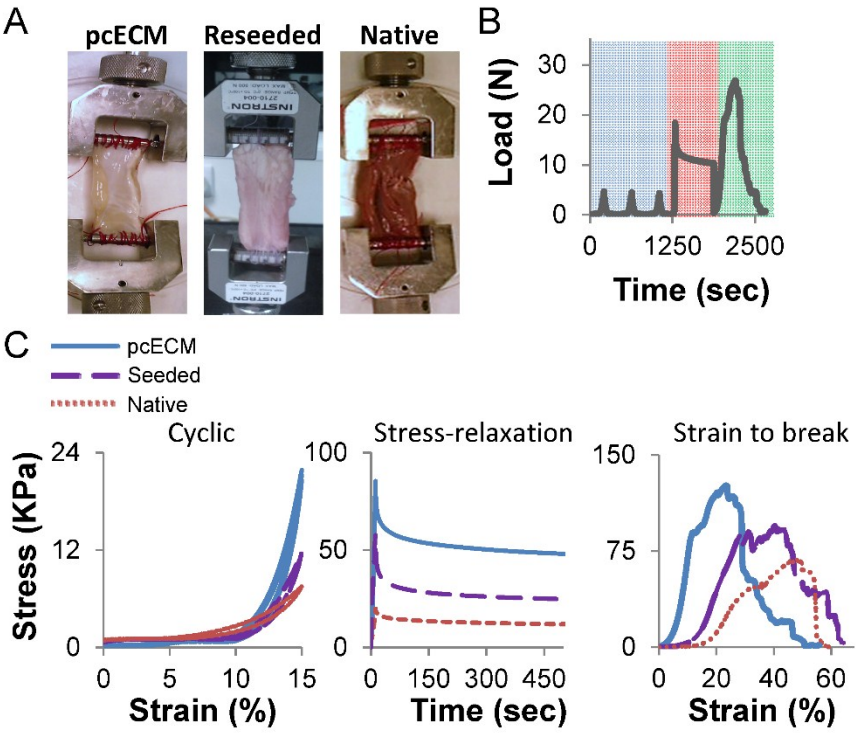

Supplementary Fig. S2: Representative Uniaxial mechanical testing outputs. Representative images of pcECM, reseeded and native tissue 30x70x15 mm slabs (**A,** as indicated). Typical testing scheme shows the load (N) as a function of time, in which three distinct testing assays are indicated by different background color—blue, indicating cyclic stress assay; red, indicating stress-relaxation assay; and green, indicating strain-to-break assay **(B**). Corresponding representative output curves from each assay are shown for all three sample types, as indicated in (**C**).

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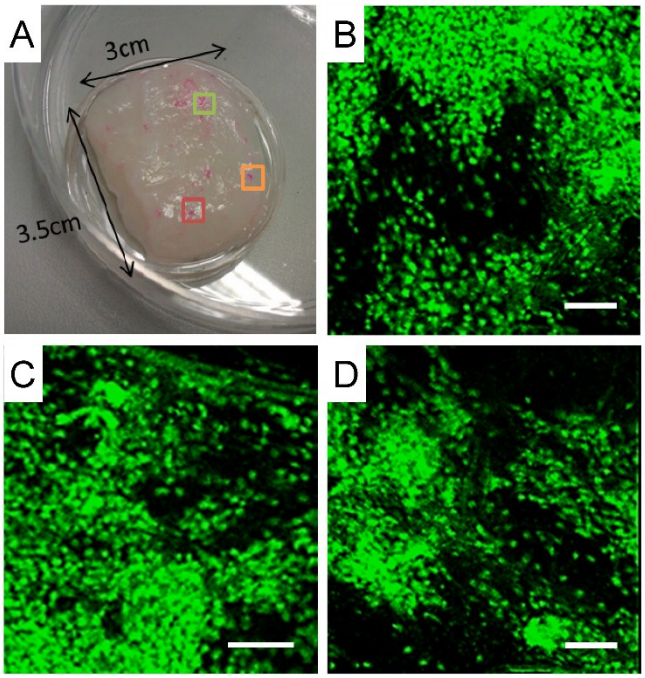

Supplementary Fig. S3: Confocal imaging of initial cell attachment to the pcECM. Pink staining of cell aggregates on pcECM two days after seeding (**A**). Confocal Z-stack superposition (maximum projection) of magnified ROIs from (**A**) visualizing the presence of cells on the seeding surface (**B-D**): Green-squared ROI (**B**); red-squared ROI (**C**); and orange-squared ROI (**D**). Scale bar: 100µm.

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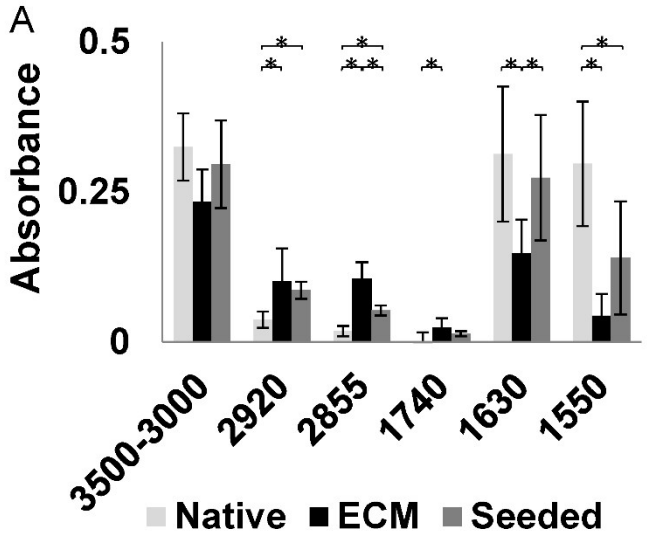

Supplementary Fig. S4: FTIR (Raw data) peak intensity comparison. (*) denotes statistical significance as assessed by one-way ANOVA with Tukey's HSD post hoc correction (p<0.05).

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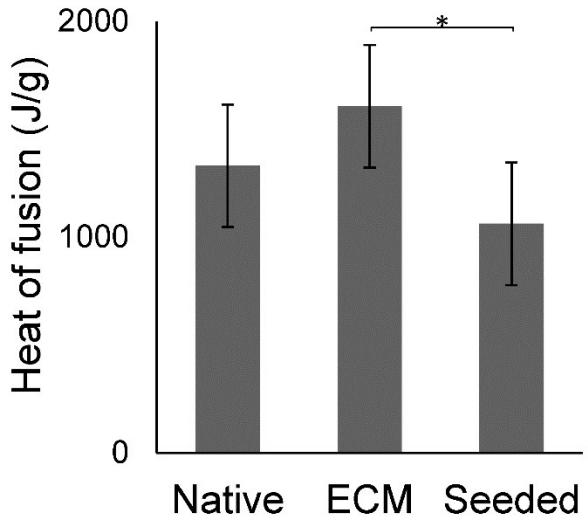

Supplementary Fig. S5: Heats of fusion for native tissue, decellularized pcECM and seeded pcECM as measured by differential scanning calorimetry (CM). (*) denotes statistical significance as assessed by one-way ANOVA with Tukey's HSD post hoc correction (p<0.05).

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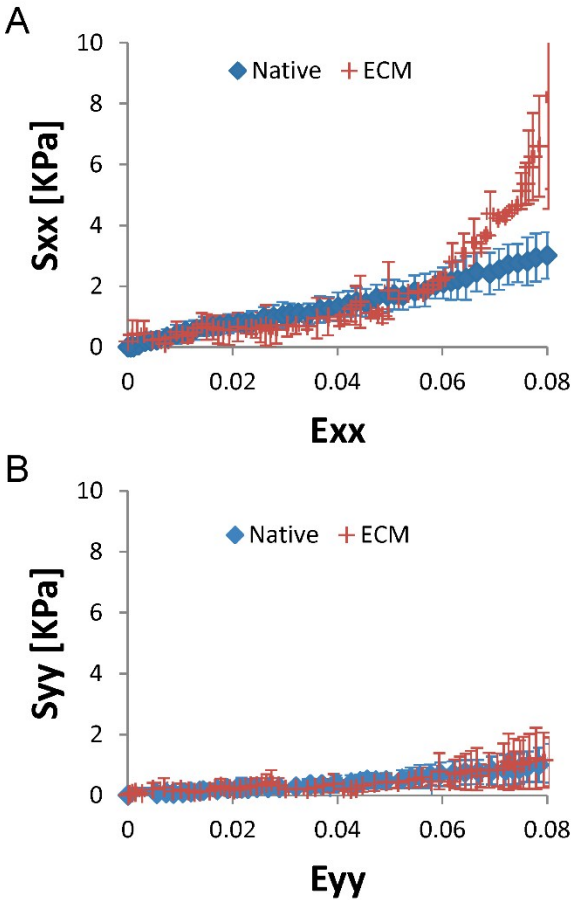

Supplementary Fig. S6: Biaxial mechanical testing of pcECM and native ventricular tissue. Stess (S) vs. strain (E) curves of pcECM and native myocardial tissue in the fiber preferred (XX, A) and cross fiber-preferred (YY, B) directions.