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Supplementary Materials for

An off-the-shelf artificial cardiac patch improves cardiac repair after myocardial infarction in rats and pigs

Ke Huang, Emily W. Ozpinar, Teng Su, Junnan Tang, Deliang Shen, Li Qiao, Shiqi Hu, Zhenhua Li, Hongxia Liang, Kyle Mathews, Valery Scharf, Donald O. Freytes, Ke Cheng*

*Corresponding author. Email: ke_cheng@ncsu.edu, ke_cheng@unc.edu

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MATERIALS AND METHODS

Derivation of human cardiac stromal cells (CSCs)

Human cardiosphere-derived CSCs were obtained from human hearts as previously described *(10)*. Briefly, myocardial samples harvested from human hearts were cut into fragments of 2 mm^3, washed with phosphate-buffered saline, and partially digested with collagenase (Sigma-Aldrich). The tissue fragments were cultured as cardiac explants on a 0.5 mg/ml fibronectin (Corning) solution coated surface in Iscove's modified Dulbecco's medium (Invitrogen) containing 20% fetal bovine serum (Corning). A layer of stromal-like cells emerged from the cardiac explant with phase-bright cells over them. The explant-derived cells were harvested using TryPEL Select (Gibco). Harvested cells were seeded at a density of 2×10^4 cells/ml in Ultra Low Attachment flasks (Corning) for cardiosphere formation. In about one week, explant-derived cells spontaneously aggregated into cardiospheres. The cardiospheres were collected and plated onto fibronectin-coated surfaces to generate cardiosphere-derived CSCs. All cultures were incubated in 5% CO2 at 37°C. Our lab has banked >50 human cardiosphere-derived CSC lines. These cells were ready for use in the lab so there was no need for recruitment of new human subjects.

Enzyme-mediated degradation of artCPs

A round-shaped myoECM or artCP (diameter: 5 mm, thickness: 1 mm) was placed in deionized water for 24 h and then placed in HEPES-buffered saline (HBS) solution with 0.2 mg/mL sodium azide (Sigma-Aldrich) and 2 mg/mL collagenase (Sigma-Aldrich). As control, a round-shaped artCP (diameter: 5 mm, thickness: 1 mm) was placed in HBS solution with 0.2

mg/mL sodium azide. Samples were weighed and incubated at 37 °C for five continuous days. Three samples from each group were weighed and imaged on each day.

Cardiac functional assessment of rats

To measure rat cardiac dimensions and function, echocardiography was performed at 4h and 21 days after MI surgical operation following induction of anesthesia with a 1.5% isofluorane-oxygen mixture. Echocardiography was performed with a Philips Cx-30 Ultrasound System with a L15-7io high frequency probe. Two-dimensional long axis images were record from the left caudal (apical) view. Two-dimensional guided M-mode images at chordae tendineae level were evaluated. M-mode measurements of left ventricle end-diastolic and end-systolic dimensions (LVEDD and LVESD, respectively) were performed by using the leading-edge method of the American Society of Echocardiography *(69)*. For estimation of each parameter, the average of three measurements from three different cycles in an image was obtained. Left ventricular end-diastolic and systolic volumes (LVEDV and LVESV, respectively) were calculated by the biplane method of disks (modified Simpson's rule). Ejection fraction (EF) was determined by using (LVEDV-LVESV/LVEDV) × 100%, and fractional shortening (FS) was calculated from the M-mode echocardiography images as (LVEDD-LVESD/LVEDD) × 100%.

Heart morphometry studies

After all animals were euthanized, hearts from rats or cubic pieces (1×1×1 cm) of infarcted pig hearts were collected and frozen in optimum cutting temperature (OCT) compound (Tissue-Tek) after equilibration with increasing sucrose solutions up to 30% overnight. Hearts were then snap-frozen in liquid nitrogen, and cryosectioned with a thickness of 5 μm from the apex to the ligation level with 100 μm intervals for Masson's Trichrome staining. Masson's trichrome staining was performed as described in previous studies *(40,41)*. Images were acquired with a PathScan Enabler IV slide scanner (Advanced Imaging Concepts). From the Masson's trichrome images, morphometric parameters including viable myocardium and infarct thickness were measured in each section with ImageJ software. The cryo-sectioned with a thickness of 10 μm for Haematoxylin and eosin staining (H&E). Slides were fixed in Hematoxylin (Sigma-Aldrich) for 5 min at room temperature, and then rinsed in running water for 2 minutes; after decolorizing in acid alcohol for 2 seconds, rinsed again in sodium bicarbonate for 5 dips; rinsed out container with Dehydrant after 95% iso for 30 seconds, and then fixed in Eosin (Sigma-Aldrich) for 2 minutes, and then washed in dehydrant 100% (Richard-Allan Scientific) and Xylnene (VWR) for 3 times. The slides were photographed using an AZ100 multi-purpose zoom microscope system.

Triphenyltetrazolium chloride (TTC) assay

TTC assay was performed to differentiate the viable cardiac tissue and the infarcted cardiac tissue. Sterilized solution of 2,3,5-Triphenyltetrazolium Chloride (TTC) was made by dissolving TTC (2g; MP Biomedicals) into 200 ml of sterilized PBS and then pre-warmed at 37°C incubator for 30 min. The heart was collected and washed with sterilized PBS and then placed in freezer until the heart became stiff. Five sections (1cm in thickness) were cut from Apex to left ventricle ligation level and incubated in pre-warmed TTC solution at 37 °C for 30 mins. Afterwards, the sections were fixed in 10% formaldehyde solution for 2 hours.

Cardiac functional assessment of pigs

To measure pig cardiac dimensions and function, echocardiography was performed 24 h and 7 days after MI surgical operation. Following induction of sedation with TKX cocktail (1 mL/13-30 kg IM) and anesthesia with a mixture of isoflurane (0-5% in 100% oxygen), echocardiography was performed using a Philips Cx-30 Ultrasound System with an S4-2 broadband sector array transducer. Two-dimensional long axis images were recorded from the left caudal (apical) view. Two-dimensional guided M-mode images at chordae tendineae level were evaluated. M-mode measurements of left ventricle end-diastolic and end-systolic dimensions (LVEDD and LVESD, respectively) were performed by using the leading-edge method of the American Society of Echocardiography *(69)*. For estimation of each parameter, the average of three measurements from three different cycles in an image was obtained. Left ventricular end-diastolic and systolic volumes (LVEDV and LVESV, respectively) were calculated by the biplane method of disks (modified Simpson's rule). Ejection fraction (EF) was determined by using (LVEDV-LVESV/LVEDV) × 100%, and fractional shortening (FS) was calculated from the M-mode echocardiography images as (LVEDD-LVESD/LVEDD) × 100%.

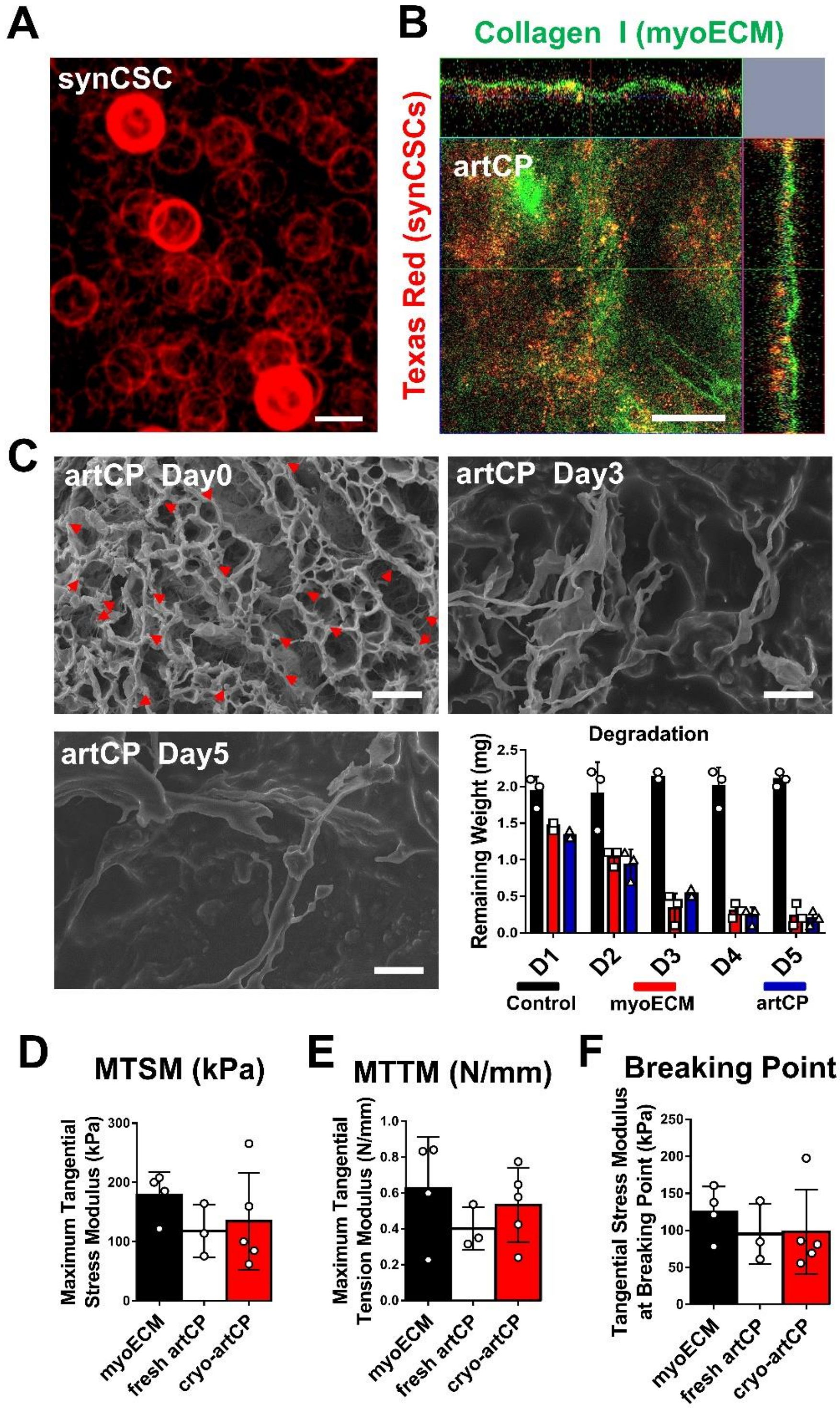

Fig. S1. Characterization of artCP. (**A**) Representative fluorescent micrographs showing DiD labelled synCSCs. Scale bar = 10 μm. (**B**) Representative 3D confocal images showing the distribution of synCSCs (red; DiD-labeled) in myoECM (green; stained with anti-collagen I antibodies). Scale bar = 25 μm. (**C**) Enzyme-mediated degradation of myoECM and artCPs. The control group was artCP in HBS solution with 0.2 mg/mL sodium azide. *n* = 3 in each group. Scale bars = 50 μm. Maximum tangential stress modulus (**D**), maximum tangential tension modulus (**E**), and tangential stress modulus at breaking point (**F**) were measured by uniaxial mechanical testing. *n* = 4 in myoECM group, *n* = 3 in fresh artCP group, *n* = 5 in cryo-artCP group. All data are means ± SD. Comparisons among groups were performed using one-way ANOVA followed by post hoc Bonferroni test.

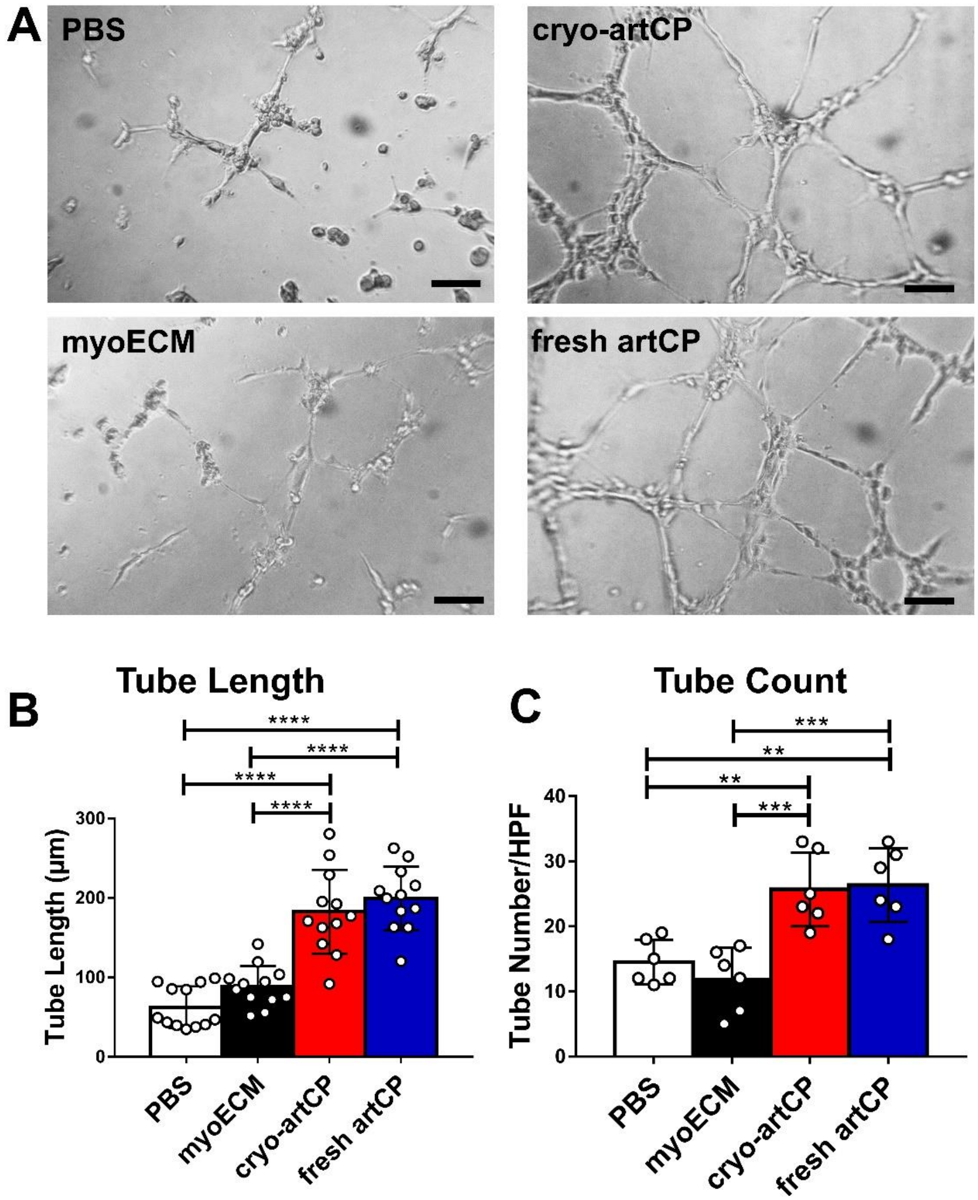

Fig. S2. Effects of artCP on endothelial cells in vitro. (**A**) Representative micrographs showing tube formation of HUVECs in PBS, myoECM, cryo-artCP and fresh artCP groups.

Scale bars = 100 μm. The tube lengths (**B**) (*n* =12) and tube numbers (**C**) (*n* = 6) were quantified by ImageJ. All data are means ± SD. Comparisons among groups were performed using one-way ANOVA followed by post hoc Bonferroni test. The comparisons between samples are indicated by lines, and the statistical significance is indicated by asterisks above the lines. **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

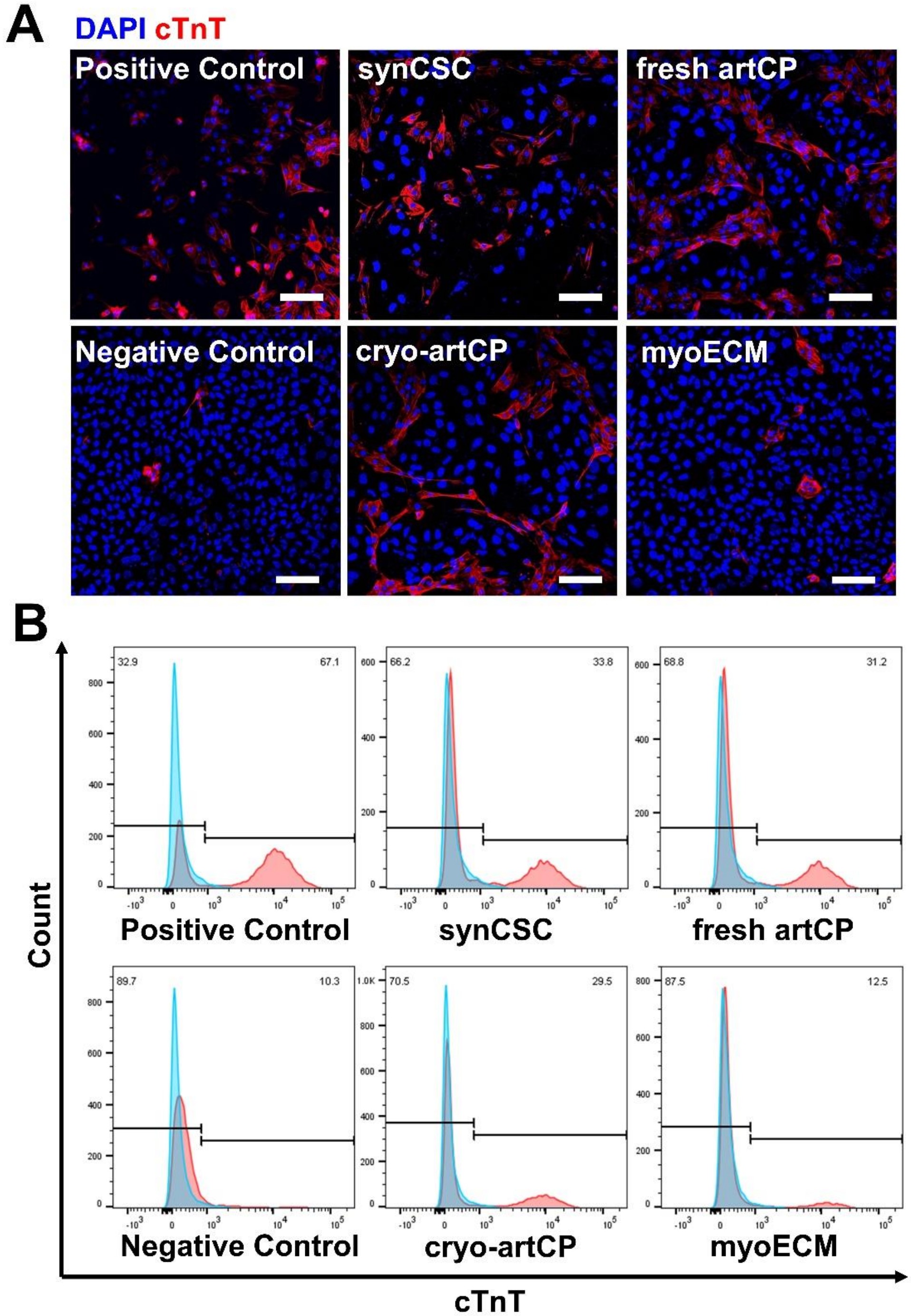

Fig. S3. Effects of artCP on CPC differentiation in vitro. (**A**) Representative micrographs showing differentiation of CPCs in positive control, negative control, PBS, myoECM, cryo-artCP and fresh artCP groups. Scale bars = 100 μm. (**B**) cTnT $^{+}$ cardiomyocytes were quantified through flow cytometry and analyzed with FlowJo software.

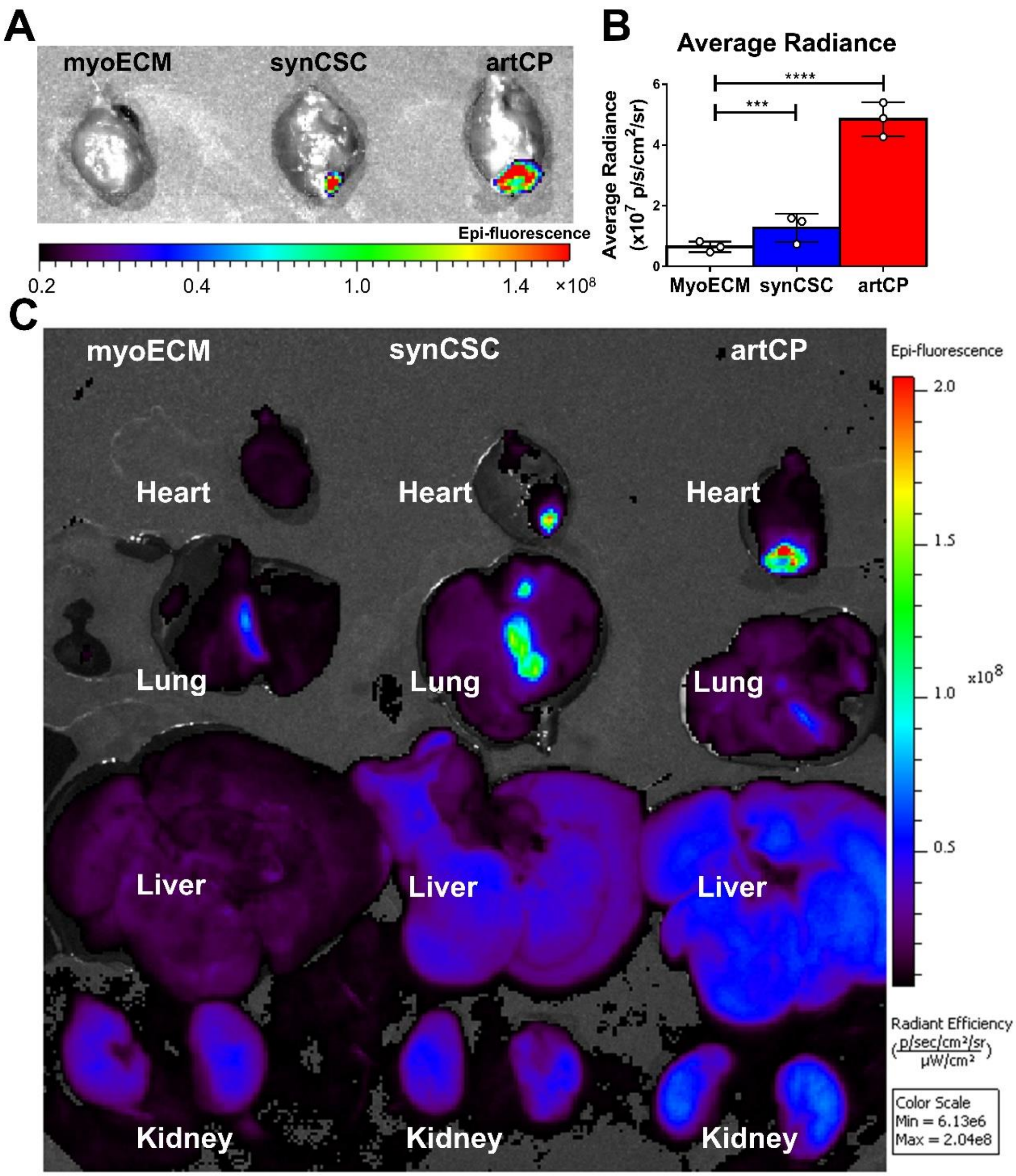

Fig. S4. synCSC retention and biodistribution in rats. To track the synCSC in vivo, we transplanted a DiR labelled artCP onto the heart of a rat with MI. Other rats received intramyocardial injection of DiR labelled synCSC or transplantation of empty myoECM transplantation as controls. (**A**) The hearts were collected and placed in the Xenogen IVIS imaging system (Caliper Life Sciences) to detect DiR fluorescence as an indicator of synCSC retention in the heart. (**B**) The average radiance in each group was measured by the Xenogen IVIS software. $n = 3$ in each group. (**C**) All major organs were collected and placed in the IVIS system for biodistribution studies. All data are means ± SD. Comparisons among groups were performed using one-way ANOVA followed by post hoc Bonferroni test. The comparisons between samples are indicated by lines, and the statistical significance is indicated by asterisks above the lines. ***$P < 0.001$ and ****$P < 0.0001$.

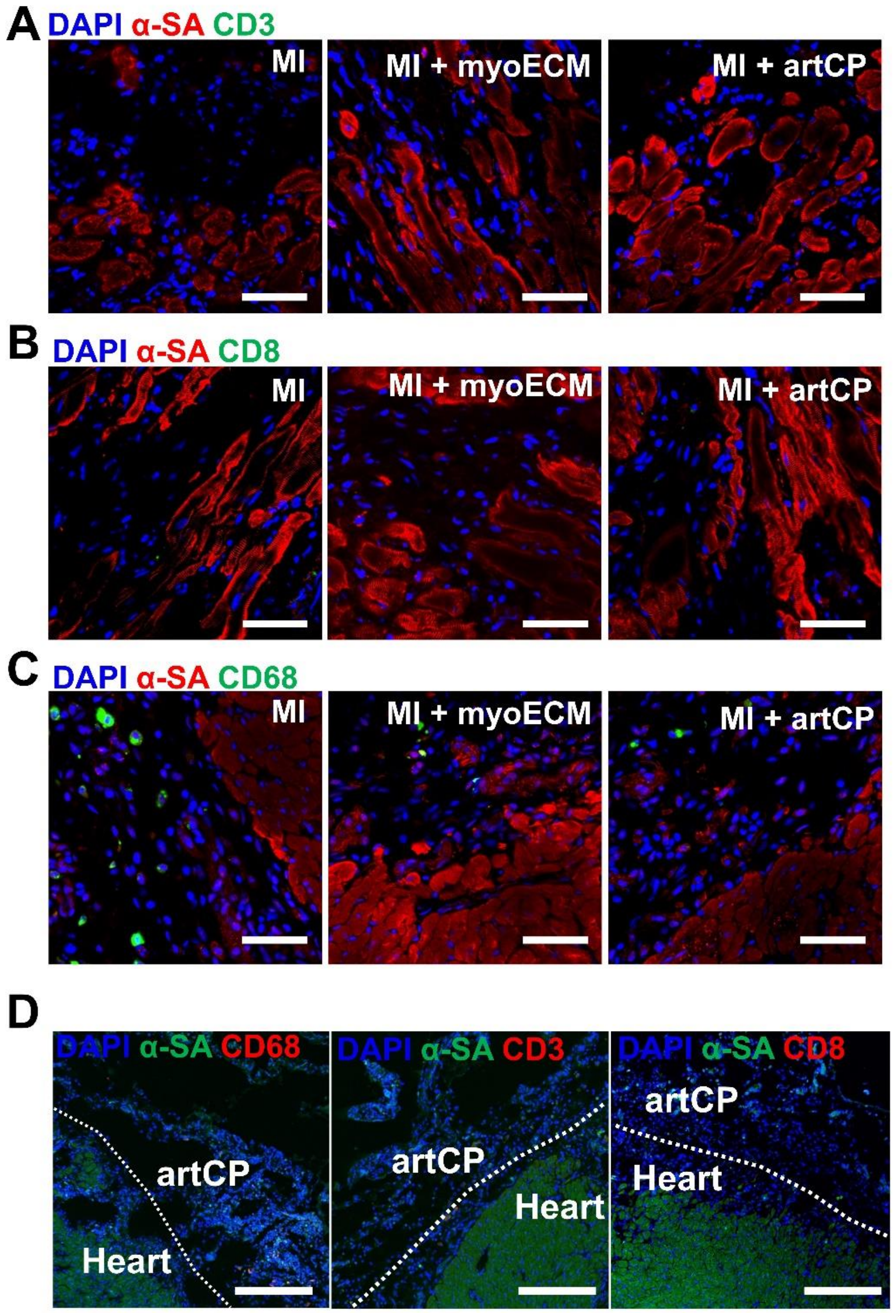

Fig. S5. **Immunogenicity of artCP in the post-MI rat heart.** 7 days after artCP or myoECM transplantation we analyzed the infiltration of T-lymphocytes, detected by anti-CD3 (**A**) (green) and anti-CD8 (green) (**B**) antibodies. Nuclei were counterstained with DAPI (blue). Scale Bars = 50 μm. (**C**) Macrophages were labelled with anti-CD68 (green) antibodies. Scale Bars = 50 μm. (**D**) The same IHC staining was performed 21 days after transplantation. Cardiomyocytes were counterstained with anti-alpha sarcomeric actinin (α-SA) and FITC-conjugated secondary antibodies. Scale Bars = 200 μm.

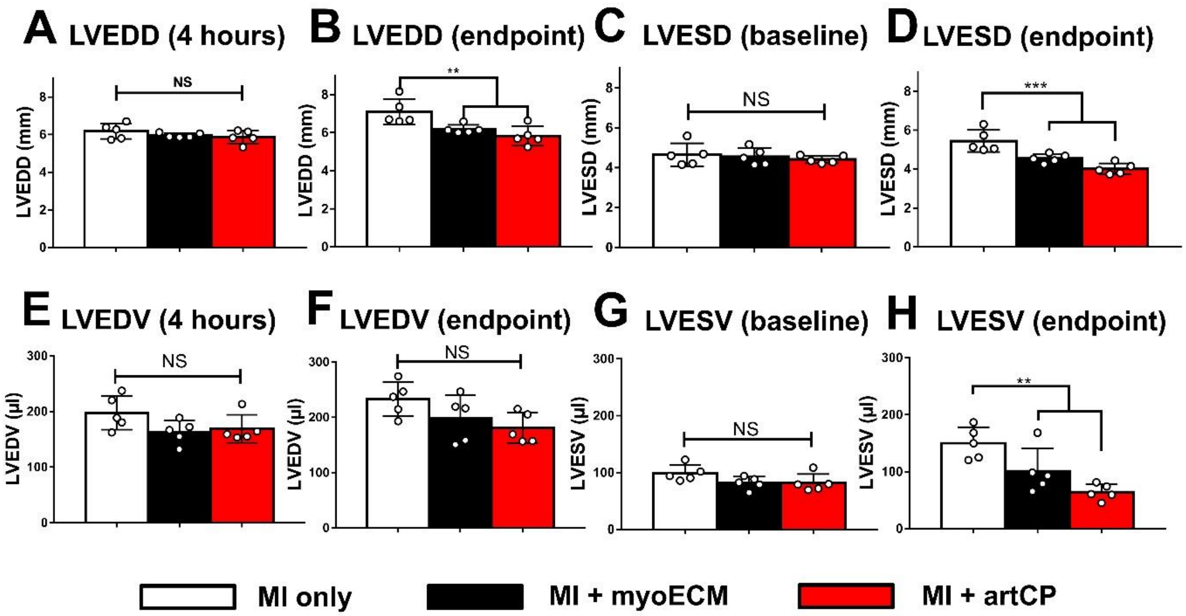

Fig. S6. Rat heart dimensions measured by echocardiography. From the M-mode images at chordae tendineae level, left ventricle end-diastolic diameter (LVEDD) at 4 hours (baseline) (**A**) and 21 days (end point) (**B**) were measured. Also, the left ventricle end-systolic diameter (LVESD) at 4 hours (baseline) (**C**) and 21 days (end point) (**D**) were measured. From B-mode at long axis, left ventricle end-diastolic volume (LVEDV) at 4 hours (baseline) (**E**) and 21 days (end point) (**F**) were measured. Also, left ventricle end-systolic volume (LVESV) were also measured at 4 hours (baseline) (**G**) and 21 days (end point) (**H**). $n = 5$ in each group. All data are means ± SD. Comparisons among groups were performed using one-way ANOVA followed by post hoc Bonferroni test. The comparisons between samples are indicated by lines, and the statistical significance is indicated by asterisks above the lines. NS indicates $P > 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

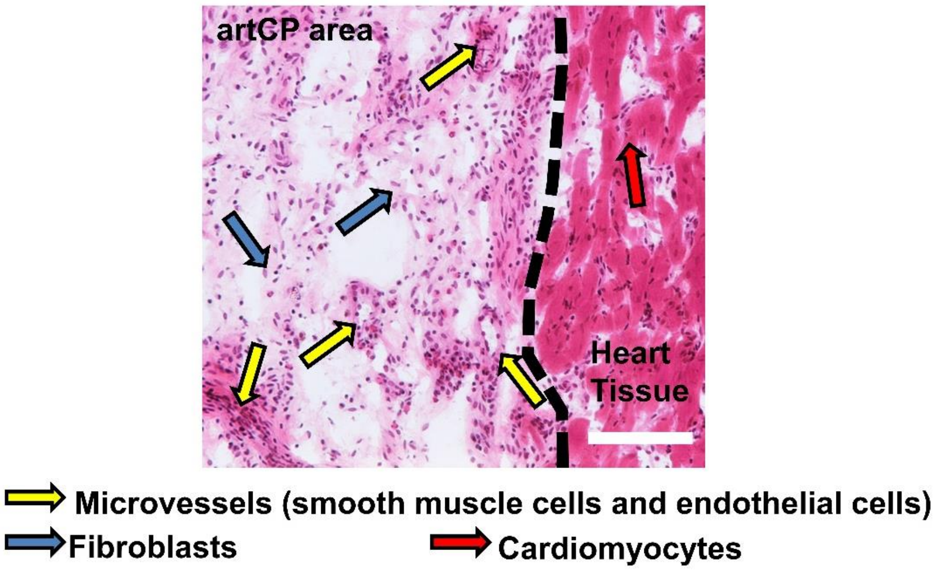

Fig. S7. H&E staining of artCP and adjacent myocardium 21 days after transplantation in a rat. Representative H&E staining image showing the artCP integration to heart tissue. Fibroblasts, smooth muscle cells, and endothelial cells are indicated by arrows. Scale Bars = 100 μm.

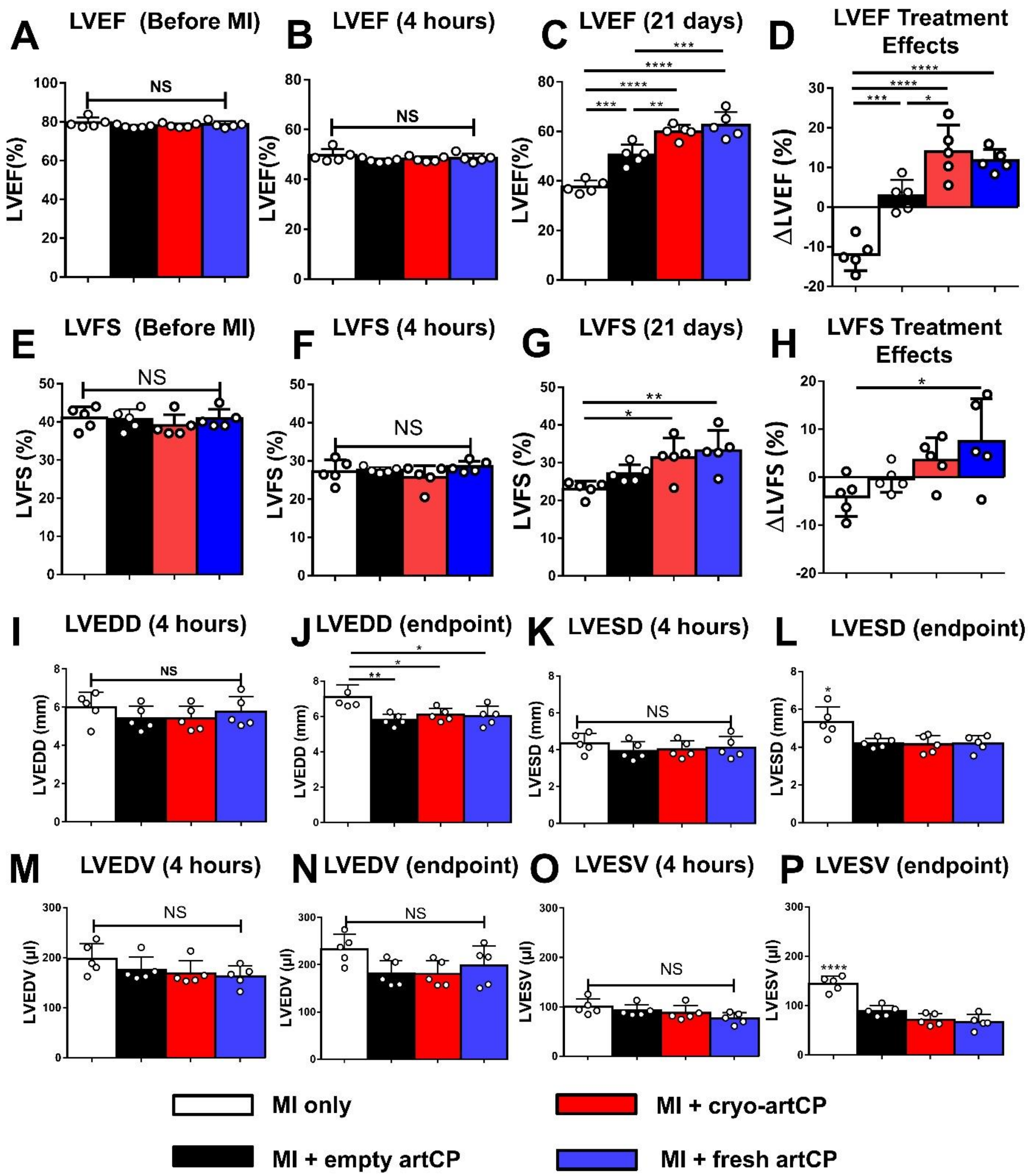

Fig. S8. Treatment effects from empty, cryopreserved, or fresh artCP transplantation in rats. LVEFs were analyzed before MI (**A**), 4 hours after MI (**B**) and 21 days after MI (**C**).

Treatment effects were determined as the changes in LVEF from 4 hours after MI to 21 days after MI (**D**). The LVFSs were also analyzed before MI (**E**), 4 hours after MI (**F**) and 21 days after MI (**G**). Treatment effects were determined as the changes in LVFS from 4 hours after MI to 21 days after MI (**H**). $n = 5$ in each group. From M-mode images at chordae tendineae level, LVEDDs at 4 hours (baseline) (**I**) and 21 days (end point) (**J**) were measured. Likewise, LVESDs at 4 hours (baseline) (**K**) and 21 days (end point) (**L**) were measured. From B-mode at long axis, LVEDVs at 4 hours (baseline) (**M**) and 21 days (end point) (**N**) were measured. LVESVs were also measured at 4 hours (baseline) (**O**) and 21 days (end point) (**P**). $n = 5$ in each group. All data are means ± SD. Comparisons among groups are performed using one-way ANOVA followed by post hoc Bonferroni test. The comparisons between samples are indicated by lines, and the statistical significance is indicated by asterisks above the lines. NS indicates $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.

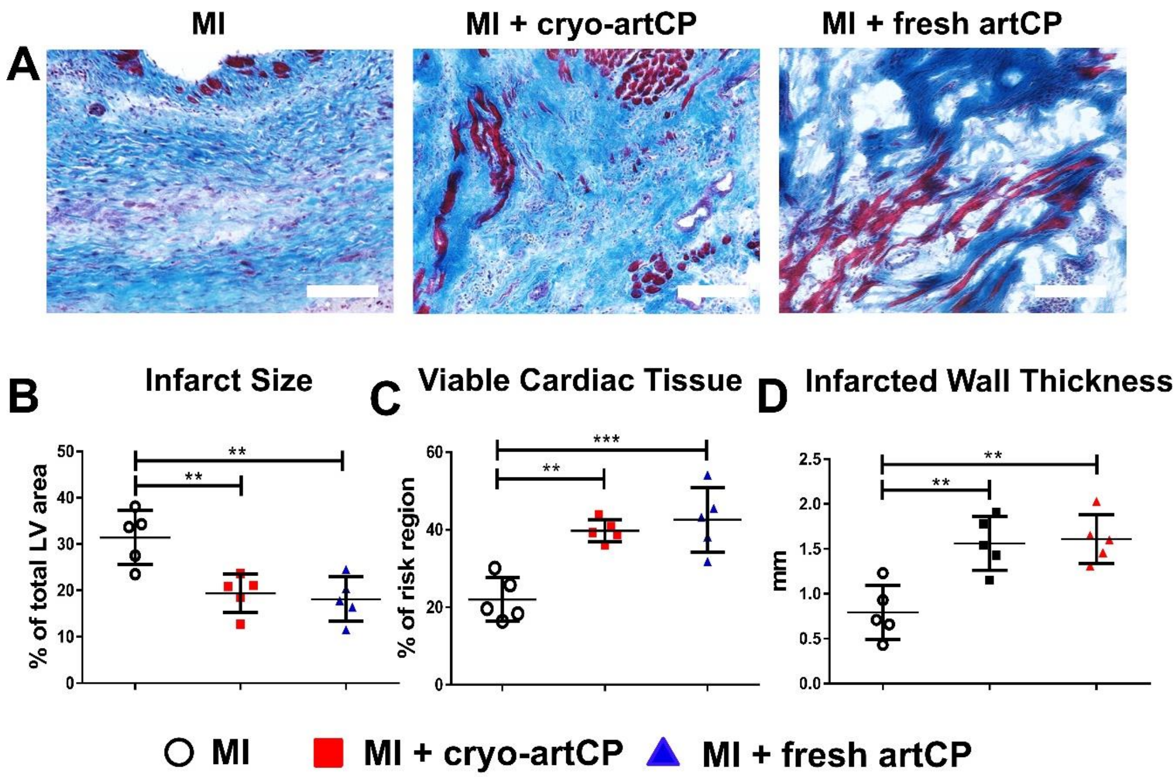

Fig. S9. Heart morphometric analysis after patch transplantation in rats. (**A**) Masson's Trichrome staining was performed 21 days after MI on 5 μm cryo-sections. Scale Bars = 100 µm. Morphometric parameters including infarct size (**B**), the percentage of viable myocardium at risk area (**C**), and infarct wall thickness (**D**) were measured from the Masson's Trichrome stained images using the NIH ImageJ software. n = 5 in each group. All data are means ± SD. Comparisons among groups were performed using one-way ANOVA followed by post hoc Bonferroni test. The comparisons between samples are indicated by lines, and the statistical significance is indicated by asterisks above the lines. **$P < 0.01$ and ***$P < 0.001$.

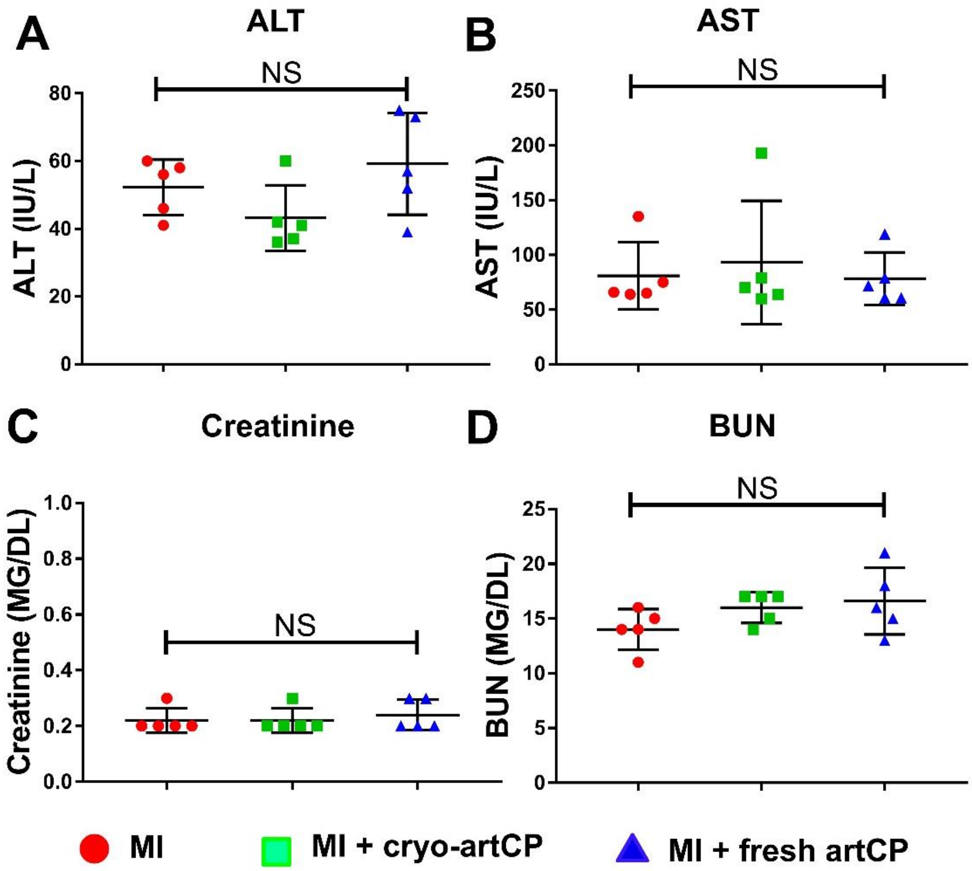

Fig. S10. Liver and kidney functions 21 days after artCP transplantation in rats. (**A**) Alanine aminotransferase (ALT) and (**B**) aspartate aminotransferase (AST) concentrations in sera. (**C-D**) Blood creatinine (**C**) and urea nitrogen (**D**) concentrations to evaluate the potential kidney toxicity of artCP transplantation. *n* = 5 in each group. All data are means ± SD. Comparisons among groups were performed using one-way ANOVA followed by post hoc Bonferroni test. NS indicates $P > 0.05$.

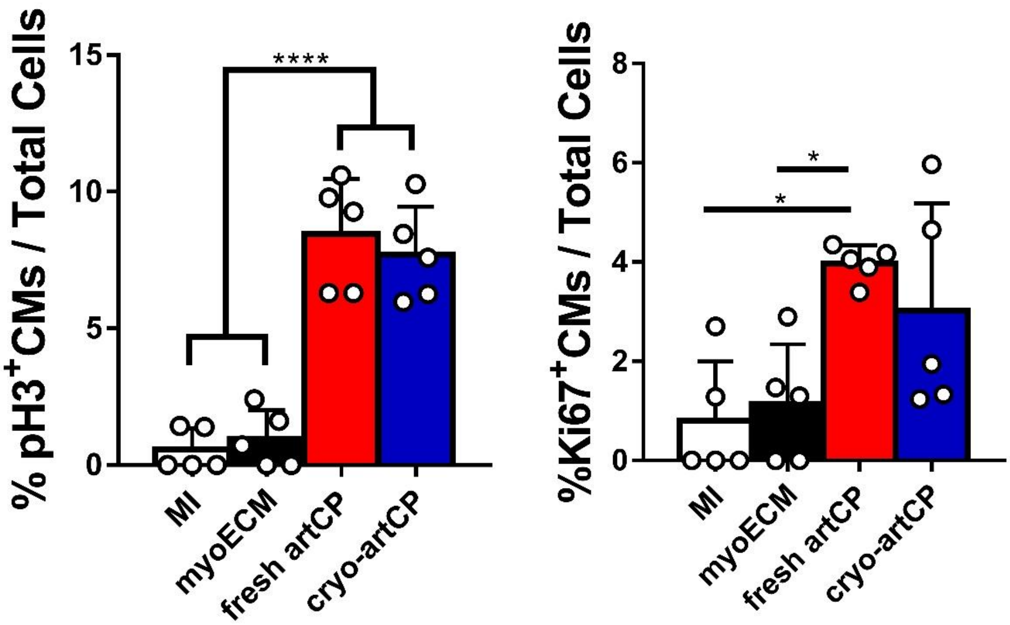

Fig. S11. Percentage of pH3$^+$ or Ki67$^+$ cardiomyocytes in total cells 21 days after patch transplantation in rats. $n = 5$ in each group. All data are means ± SD. Comparisons among groups were performed using one-way ANOVA followed by post hoc Bonferroni test. *$P < 0.05$ and ****$P < 0.0001$.

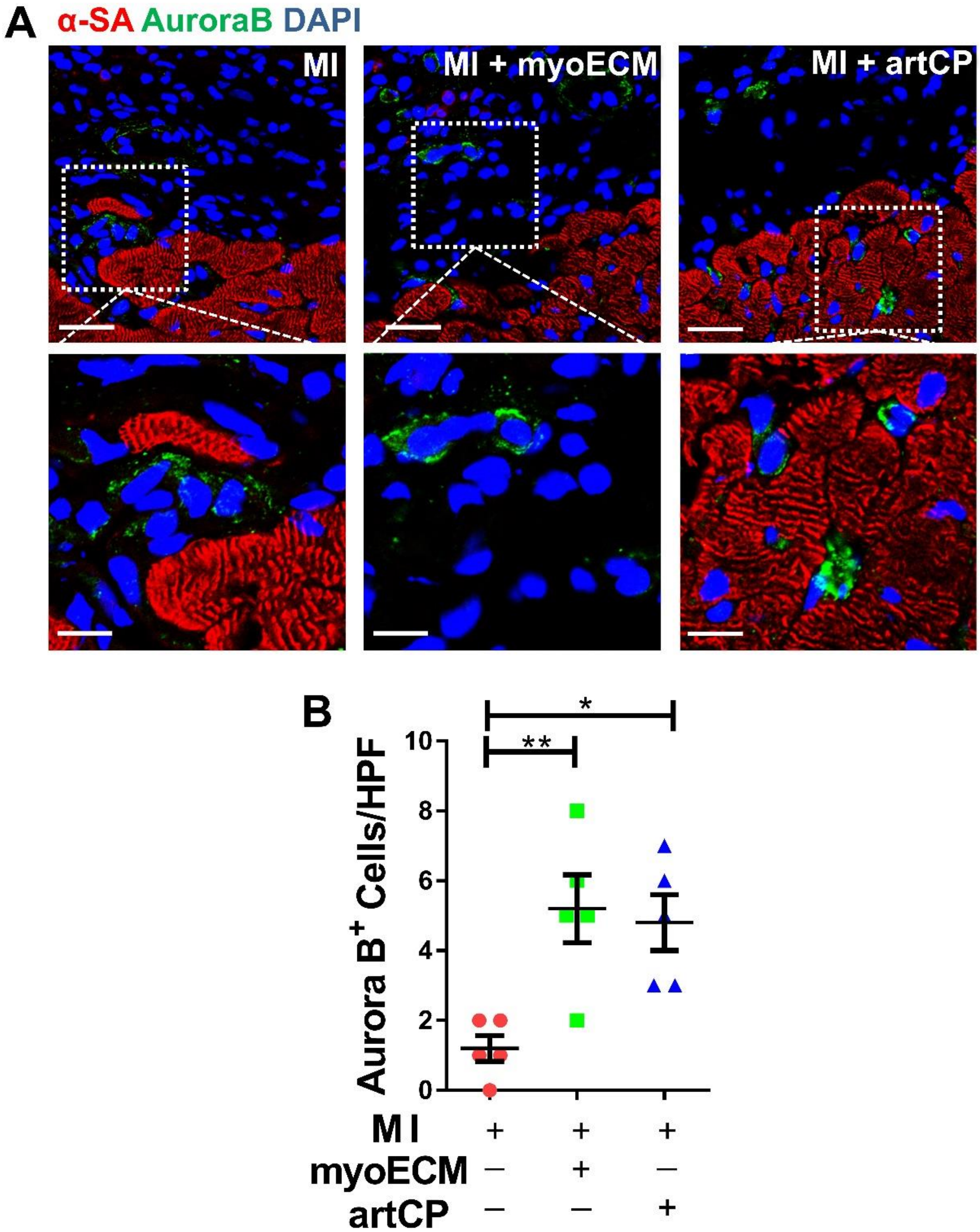

Fig. S12. **Staining of proliferation marker Aurora B after artCP or myoECM transplantation in rats.** (**A**) Aurora B (green) was detected in the infarcted area from the hearts of MI only group, artCP, and myoECM transplanted group. Scale bars = 25 μm. Scale bars in snapshot = 10 μm. Regions outlined by dashed box shown at higher magnification below. (**B**) Aurora B^+ cells were quantified. $n = 5$ per group. All data were means ± SD. Comparisons among groups were performed using one-way ANOVA followed by post hoc Bonferroni test. The comparisons between samples are indicated by lines, and the statistical significance is indicated by asterisks above the lines. *$P < 0.05$ and **$P < 0.01$.

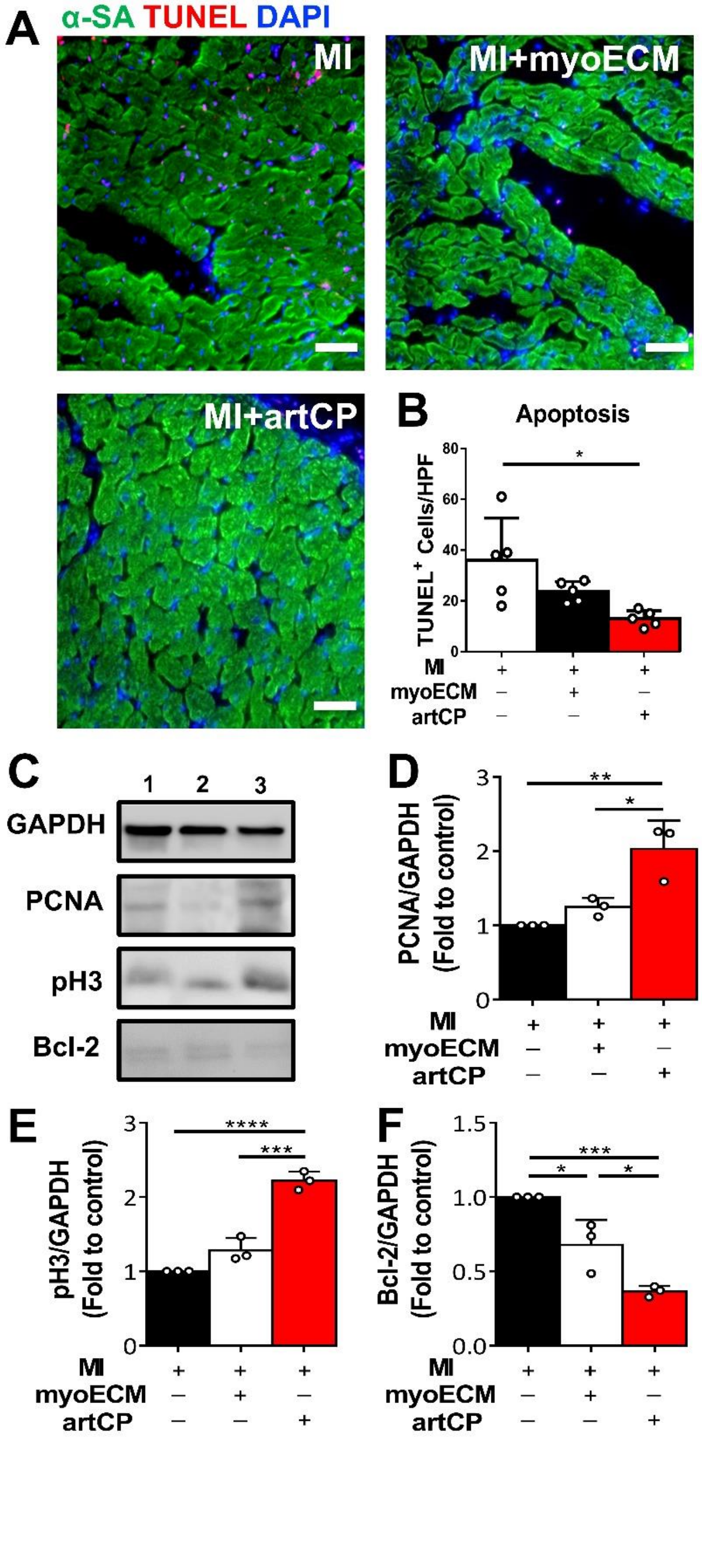

Fig. S13. Analyses of myocardial proliferation and apoptosis 21 days after patch transplantation in rats. (**A**) Representative fluorescent micrographs showing TUNEL^{+}

apoptotic cells in the myocardium. All images were taken with an epi-fluorescent microscope (Olympus IX81). Scale bars = 25μm. (**B**) TUNEL^{+} cells were counted using the NIH ImageJ software. *n* = 5 in each group. (**C**) Western blot analyses of pCNA, pH3, and Bcl-2 expressions in the myocardium treated by: 1) MI only group; 2) myoECM group; or 3) artCP group. Quantification of pCNA (**D**), pH3 (**E**), and Bcl-2 (**F**) normalized to MI control. *n* = 3 in each group. All data are means ± SD. Comparisons among groups were performed using one-way ANOVA followed by post hoc Bonferroni test. The comparisons between samples are indicated by lines, and the statistical significance is indicated by asterisks above the lines. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

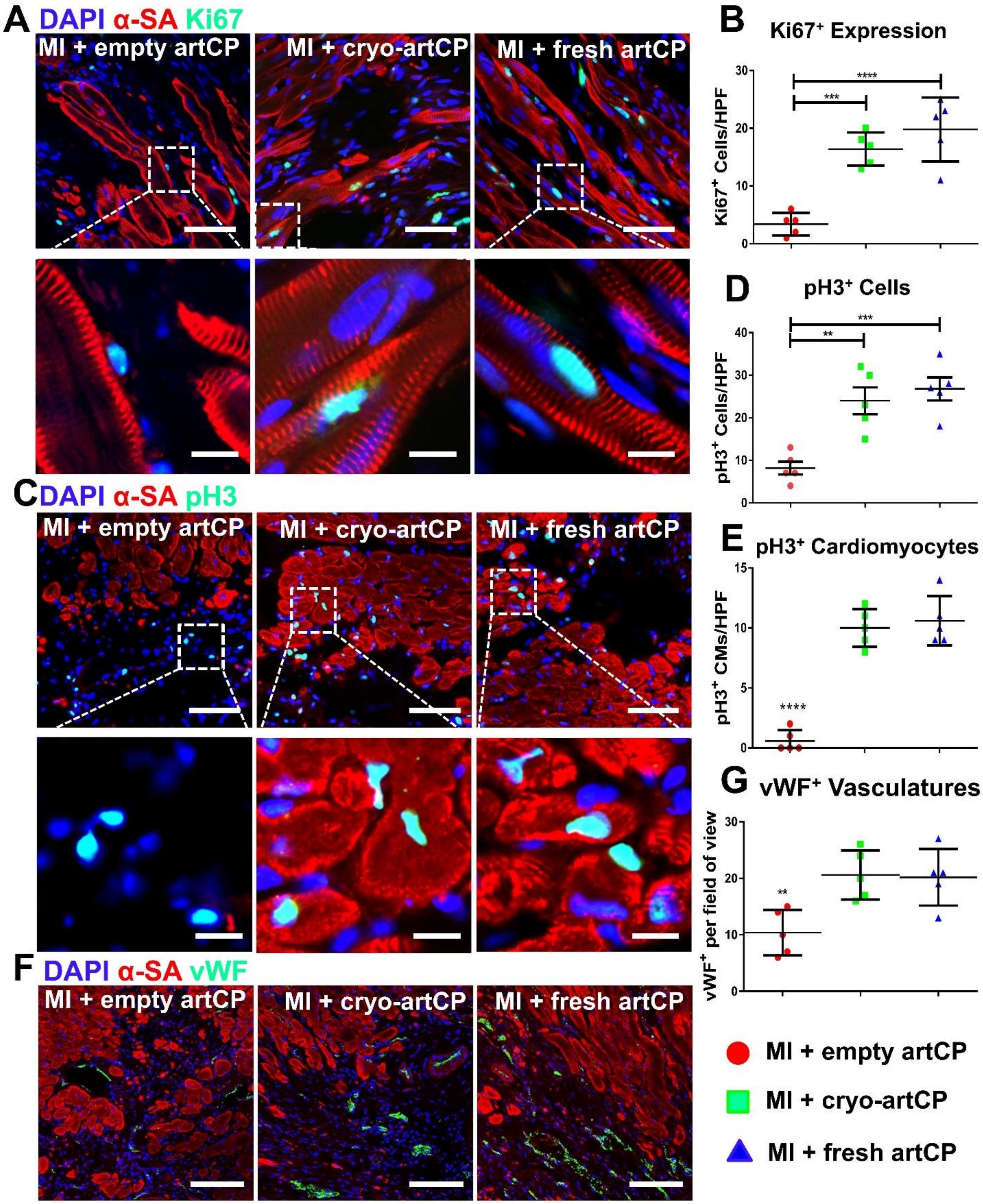

Fig. S14. **Effects of artCP therapy on angiomyogenesis in rats.** (**A**) Ki67^{+} cells (green) were detected and quantified in the peri-infarct area 21 days after MI. Scale bars = 50 μm. Regions outlined by dashed box shown at higher magnification below. Scale bars in snapshot =10 μm. (**B**) Ki67$^+$ cells were counted per HPF via the NIH ImageJ software. n = 5 in each group. (**C**) pH3$^+$ (green) were detected in the peri-infarct area. Scale bars = 50 μm. Regions outlined by dashed box shown at higher magnification below. Scale bars in snapshot =10 μm. (**D**) pH3$^+$ cells and (**E**) pH3$^+$ cardiomyocytes (nuclei that overlaid with α-SA$^+$ cells also overlaid with pH3$^+$ signals) were counted per HPF via the NIH ImageJ software. n = 5 in each group. (**F**) vWF$^+$ (green) was detected in the peri-infarct area. Scale bars = 100 μm. (**G**) Pooled data of vWF$^+$ signal quantification via the NIH ImageJ software. n = 5 in each group. All data are means ± SD. Comparisons among groups were performed using one-way ANOVA followed by *post hoc* Bonferroni test. The comparisons between samples are indicated by lines, and the statistical significance is indicated by asterisks above the lines. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

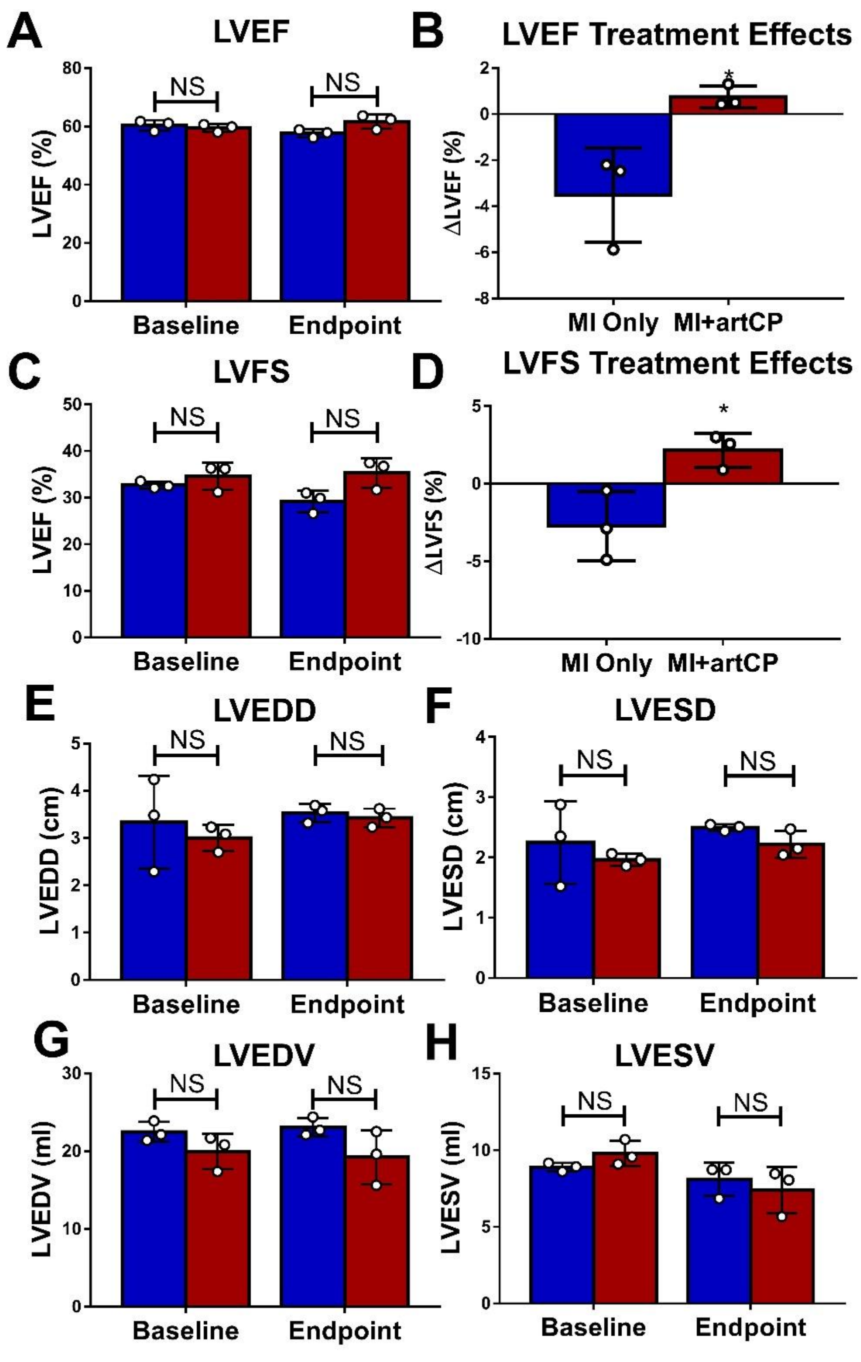

Fig. S15. **Cardiac functional assessment of artCP therapy in a porcine MI model.** (**A**) LVEF was measured 4 hours (baseline) and 7 days (end point) post-MI. (**B**) Treatment effects were determined as the changes in LVEF from 4 hours after MI to 7 days after MI. (**C**) LVFS was measured at 4 hours (baseline) and 7 days (end point) after MI. (**D**) Treatment effects were determined as the changes in LVFS from 4 hours after MI to 7 days after MI. $n = 3$ in each group. From M-mode images at chordae tendineae level, LVEDDs at 4 hours (baseline) and 7 days (end point) after MI (**E**) were measured. Likewise, LVESDs at 4 hours (baseline) and 7 days (endpoint) after MI (**F**) were measured. From B-mode at long axis, LVEDVs at 4 hours (baseline) and 7 days (end point) after MI (**G**) were measured. LVESVs were also measured at 4 hours (baseline) and 7 days (end point) after MI (**H**). $n = 3$ in each group. All data are means ± SD. Comparisons among groups were performed using one-way ANOVA followed by post hoc Bonferroni test. The comparisons between samples are indicated by lines, and the statistical significance indicates by asterisks above the lines. NS indicates $P > 0.05$ and $*P < 0.05$.

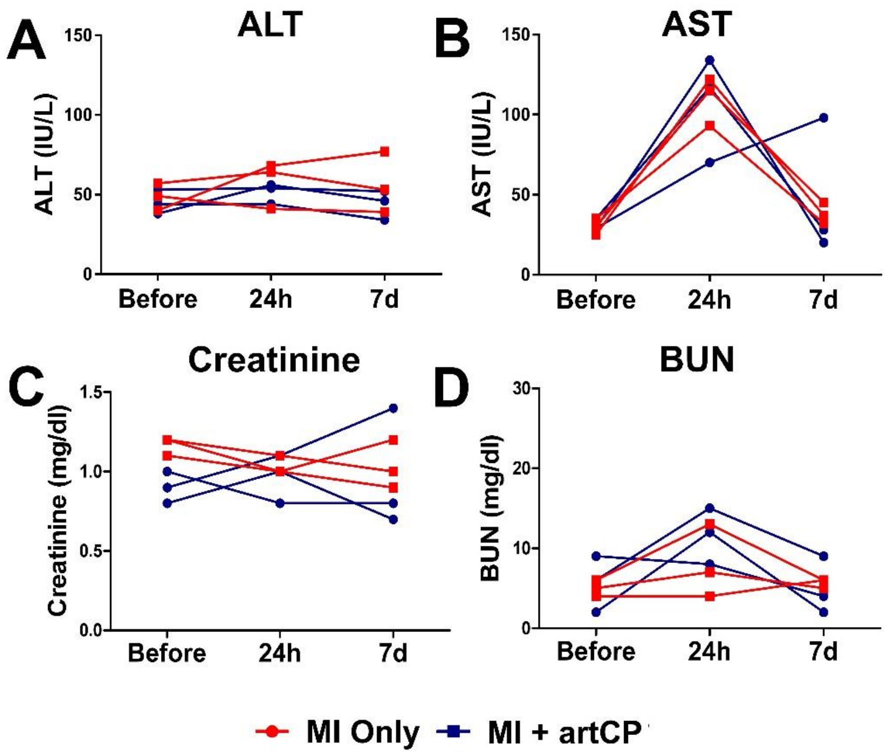

Fig. S16. **Effects of artCP transplantation on liver and kidney functions in the porcine MI model.** (**A**) Alanine aminotransferase (ALT) and (**B**) aspartate aminotransferase (AST) concentrations in sera. (**C-D**) Blood creatinine (**C**) and urea nitrogen (**D**) concentrations were determined to evaluate the potential kidney toxicity of artCP transplantation. $n = 3$ in each group. All data are means ± SD. Comparisons among groups were performed using one-way ANOVA followed by post hoc Bonferroni test. NS indicates $P > 0.05$.

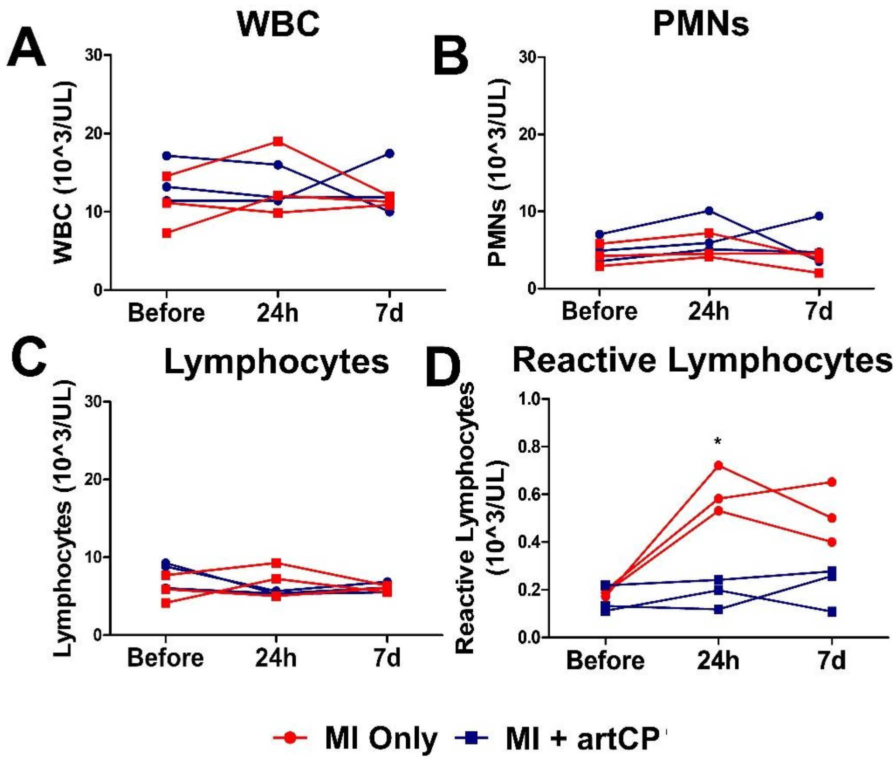

Fig. S17. Immunogenicity analysis of artCP transplantation in the porcine MI model. (**A**) WBCs, (**B**) PMNs, (**C**) lymphocytes, and (**D**) reactive lymphocytes were quantified before MI, 24 hours after MI, and 7 days after MI. $n = 3$ in each group. All data are means ± SD. Comparisons among groups were performed using one-way ANOVA followed by post hoc Bonferroni test. NS indicates $P > 0.05$ and $^*P < 0.05$.

Table S1. myoECM average tension/stress versus strain.

myoECM Average Tension/Stress vs Strain				
Strain Zeroed (mm/mm)	Tension (N/mm)	SE	Stress (kPa)	SE
0.00000000	0.00288102	0.00038703	1.95243197	0.39317894
0.00124474	0.00306895	0.00041314	2.07050067	0.39934343
0.00238667	0.00317183	0.00044181	2.13666173	0.41310129
0.00349637	0.00346492	0.00049945	2.35680913	0.50886676
0.00461028	0.00364234	0.00051122	2.46469504	0.50489859
0.00572445	0.00369881	0.00046926	2.49494112	0.47088724
0.00682416	0.00385991	0.00047319	2.61602773	0.49982681
0.00794144	0.00395734	0.00051085	2.67939515	0.52069605
0.00905432	0.00416820	0.00052079	2.82248808	0.54440265
0.01016352	0.00424432	0.00053331	2.87079025	0.55200993
0.01126351	0.00440505	0.00055259	2.98289021	0.57722940
0.01236969	0.00463599	0.00059492	3.15584427	0.64511677
0.01347662	0.00475212	0.00054812	3.21857535	0.59109741
0.01458798	0.00485358	0.00063350	3.28351217	0.63712024
0.01569717	0.00505358	0.00063256	3.42063167	0.65633983
0.01681055	0.00514050	0.00062307	3.47924725	0.65523822
0.01792339	0.00531192	0.00062387	3.61338551	0.70026833
0.01903150	0.00548505	0.00067218	3.73209168	0.73805565
0.02014149	0.00568209	0.00071522	3.86397645	0.77474803
0.02125885	0.00581858	0.00068740	3.94218221	0.73655351
0.02236266	0.00599164	0.00072691	4.08014501	0.80525318
0.02347033	0.00615511	0.00073608	4.17670966	0.79760263
0.02457915	0.00620740	0.00073448	4.21149716	0.79620053
0.02568964	0.00646784	0.00081821	4.39840675	0.88222194
0.02679791	0.00661654	0.00079696	4.49935818	0.88285285
0.02790818	0.00679985	0.00080896	4.61832977	0.88263739
0.02902148	0.00692698	0.00082330	4.71397567	0.91863803
0.03013260	0.00709861	0.00084703	4.83644604	0.95818710
0.03123786	0.00717316	0.00085639	4.89067206	0.97261789
0.03234320	0.00739023	0.00088782	5.03428836	0.99641053
0.03346329	0.00761854	0.00091332	5.19121567	1.02651169
0.03456264	0.00781147	0.00092112	5.31820372	1.03862589
0.03567038	0.00788299	0.00093597	5.36509707	1.05099600
0.03678145	0.00817781	0.00098557	5.57571146	1.11012057
0.03789325	0.00821916	0.00099389	5.59834383	1.11307776
0.03900272	0.00845224	0.00099887	5.76069007	1.13858794
0.04011996	0.00868415	0.00108576	5.93620166	1.22963491

0.04122374	0.00887310	0.00110847	6.04573889	1.21794175
0.04233498	0.00897294	0.00110089	6.11865394	1.23476062
0.04344062	0.00914748	0.00110623	6.23653181	1.24990666
0.04455846	0.00933168	0.00114935	6.38148683	1.31870779
0.04566584	0.00959019	0.00118343	6.54931201	1.33731399
0.04676878	0.00979515	0.00121394	6.68288384	1.36075891
0.04787715	0.00999079	0.00128348	6.82540824	1.42901934
0.04899029	0.01008074	0.00124834	6.88674567	1.41525735
0.05009881	0.01032902	0.00126362	7.05789720	1.44431709
0.05120906	0.01070771	0.00128271	7.31352016	1.47577093
0.05231396	0.01072966	0.00131832	7.33114360	1.50000748
0.05342782	0.01086709	0.00134725	7.42590310	1.52877579
0.05453969	0.01115003	0.00138993	7.62696158	1.58267979
0.05564404	0.01125450	0.00144077	7.70356055	1.62638282
0.05676350	0.01150231	0.00144128	7.86501707	1.63345963
0.05786318	0.01169747	0.00151503	8.01558146	1.71995054
0.05897550	0.01193215	0.00149656	8.16419104	1.70508457
0.06008339	0.01211919	0.00151295	8.29813422	1.73760589
0.06120547	0.01228040	0.00155735	8.41095447	1.77533026
0.06230465	0.01253533	0.00161473	8.59351312	1.84075261
0.06341288	0.01276735	0.00161482	8.74436745	1.85032874
0.06452563	0.01299114	0.00165103	8.90394616	1.89461857
0.06564412	0.01309296	0.00167868	8.98490995	1.93412971
0.06675126	0.01328785	0.00174839	9.12750191	2.00470084
0.06784670	0.01355737	0.00178199	9.29940952	2.02230072
0.06895426	0.01393627	0.00176973	9.54384187	2.01843979
0.07007653	0.01402733	0.00180600	9.62117917	2.07487328
0.07117999	0.01411514	0.00185752	9.70323287	2.13905734
0.07229141	0.01442371	0.00190172	9.90385587	2.17655004
0.07339762	0.01460354	0.00197253	10.04131483	2.24469482
0.07451210	0.01476094	0.00198402	10.14643818	2.25995317
0.07562048	0.01503376	0.00200931	10.33380585	2.29487829
0.07673372	0.01520365	0.00202918	10.44925968	2.32235122
0.07784081	0.01545890	0.00207388	10.62717557	2.36844035
0.07895736	0.01561198	0.00214541	10.74697158	2.43988929
0.08005455	0.01569216	0.00221522	10.82018460	2.51551653
0.08117341	0.01604454	0.00223178	11.05143232	2.53582830
0.08228031	0.01628120	0.00222019	11.20673510	2.53671509
0.08338966	0.01648706	0.00229449	11.36849515	2.62329862
0.08449134	0.01671856	0.00231048	11.52471915	2.64551098
0.08559985	0.01686557	0.00236507	11.63039111	2.69321812
0.08671007	0.01709565	0.00241583	11.79206442	2.74887080
0.08782102	0.01747404	0.00245147	12.05250172	2.79808473
0.08893181	0.01763653	0.00252063	12.16663626	2.85094438

0.09004304	0.01778939	0.00248807	12.27439039	2.85160183
0.09115381	0.01807422	0.00260459	12.48394415	2.95753287
0.09226134	0.01838441	0.00263393	12.69045841	2.99229801
0.09337475	0.01846812	0.00266550	12.76258060	3.03270744
0.09448225	0.01871067	0.00273582	12.92864777	3.09328767
0.09559127	0.01903008	0.00277587	13.15834699	3.15720621
0.09669603	0.01923822	0.00280778	13.29233103	3.17683222
0.09781975	0.01935221	0.00284371	13.39087909	3.23559445
0.09891757	0.01970612	0.00291408	13.63631277	3.30271912
0.10003193	0.01989372	0.00295540	13.76975096	3.35214357
0.10113536	0.02014084	0.00302601	13.94486625	3.41670441
0.10225720	0.02038244	0.00305218	14.11509317	3.46054781
0.10335691	0.02059369	0.00308996	14.26183433	3.49696262
0.10446690	0.02087881	0.00318181	14.46972675	3.58610116
0.10557743	0.02110433	0.00323900	14.62538021	3.63468411
0.10669213	0.02123129	0.00325140	14.72258012	3.66916392
0.10779319	0.02161425	0.00328800	14.98160104	3.71608451
0.10890140	0.02191839	0.00340855	15.19862786	3.81670733
0.11001727	0.02205069	0.00341325	15.29831417	3.84269844
0.11112333	0.02233596	0.00348373	15.50284382	3.91296643
0.11223037	0.02261065	0.00354856	15.69681492	3.97958435
0.11335419	0.02292325	0.00357157	15.90844481	4.00998969
0.11445504	0.02309500	0.00362623	16.03064550	4.06196853
0.11557456	0.02333707	0.00368154	16.21003596	4.13177668
0.11666797	0.02357453	0.00378772	16.40189438	4.24842299
0.11778557	0.02392335	0.00380041	16.63351911	4.26863931
0.11888806	0.02414979	0.00384548	16.78377092	4.30488654
0.11999835	0.02443575	0.00393711	16.99289421	4.39904350
0.12111172	0.02460813	0.00399969	17.12547077	4.46742792
0.12221534	0.02485176	0.00404716	17.30458105	4.53141911
0.12332014	0.02531921	0.00411441	17.61008738	4.58151213
0.12443410	0.02553471	0.00418269	17.77881545	4.66763365
0.12554470	0.02580883	0.00420476	17.96612993	4.69595979
0.12665966	0.02600926	0.00429101	18.11231743	4.77481647
0.12776695	0.02626891	0.00439809	18.30994684	4.88499460
0.12887084	0.02659066	0.00444642	18.54704026	4.95433346
0.12998927	0.02700574	0.00448417	18.81865038	4.99077817
0.13110221	0.02720149	0.00453058	18.95788491	5.04051397
0.13220513	0.02745257	0.00461779	19.14489229	5.12626090
0.13331006	0.02765653	0.00469497	19.29116803	5.19442271
0.13442195	0.02795145	0.00473681	19.51304456	5.26428272
0.13553756	0.02846625	0.00481521	19.86574439	5.34987862
0.13665093	0.02870608	0.00489387	20.03492598	5.41867813
0.13775831	0.02890758	0.00493900	20.17939718	5.46546916

0.13886786	0.02917631	0.00501082	20.37617726	5.54485115
0.13997737	0.02944422	0.00508299	20.57712628	5.63030445
0.14107879	0.02975536	0.00514794	20.80490725	5.71168606
0.14218648	0.03023039	0.00525006	21.12377120	5.79459846
0.14329595	0.03043816	0.00532195	21.28521380	5.87174475
0.14440105	0.03072266	0.00536408	21.48456740	5.92565602
0.14551411	0.03104997	0.00543260	21.71392767	5.99381151
0.14663102	0.03125803	0.00550648	21.87419619	6.07580856
0.14774361	0.03171296	0.00557431	22.18442689	6.14648932
0.14884088	0.03194788	0.00567017	22.36116039	6.24107302
0.14995512	0.03224650	0.00576550	22.58073276	6.33404062
0.15106563	0.03254421	0.00580990	22.78710262	6.38478174
0.15218451	0.03287362	0.00588440	23.02261420	6.46808422
0.15329137	0.03320910	0.00590209	23.25376255	6.50303337
0.15438984	0.03355842	0.00603033	23.51149080	6.62644935
0.15549918	0.03387814	0.00610828	23.73884434	6.70620875
0.15661542	0.03421464	0.00617009	23.96969508	6.77251116
0.15772498	0.03449456	0.00629254	24.18864402	6.89114159
0.15883677	0.03481091	0.00634660	24.41322574	6.95627232
0.15993959	0.03517677	0.00643312	24.66713967	7.04040782
0.16105818	0.03558448	0.00646857	24.95117902	7.09438224
0.16216786	0.03588953	0.00656371	25.18191951	7.19451963
0.16327435	0.03624348	0.00667304	25.43877112	7.30418807
0.16438336	0.03655702	0.00670504	25.65258200	7.34621664
0.16548153	0.03689202	0.00680683	25.89304174	7.44153078
0.16659809	0.03727831	0.00685783	26.16626900	7.51069886
0.16770850	0.03757642	0.00695367	26.38514397	7.60368404
0.16882219	0.03804212	0.00703078	26.70412539	7.68806122
0.16992816	0.03838526	0.00712645	26.95570611	7.78615559
0.17103924	0.03861110	0.00720186	27.12763940	7.86546301
0.17214900	0.03899161	0.00727822	27.39293861	7.94425936
0.17325812	0.03932717	0.00736702	27.63888774	8.04377656
0.17436964	0.03977620	0.00743811	27.94981077	8.12067916
0.17547819	0.04019563	0.00749699	28.24566595	8.19576966
0.17658653	0.04043585	0.00759388	28.42873871	8.29227936
0.17769092	0.04077201	0.00770342	28.67690845	8.40320092
0.17880506	0.04121729	0.00781820	28.99512430	8.51899389
0.17991293	0.04162619	0.00785803	29.27287602	8.56579719
0.18102165	0.04188263	0.00793926	29.46292725	8.64912014
0.18213090	0.04230155	0.00804259	29.76023317	8.75062495
0.18325258	0.04267880	0.00813500	30.03702625	8.85448483
0.18435362	0.04298723	0.00818807	30.25154251	8.91501533
0.18546781	0.04342965	0.00821789	30.54791220	8.95309897
0.18657175	0.04375955	0.00838803	30.80446216	9.11592363

0.18768380	0.04425263	0.00845403	31.15250729	9.19918926
0.18878584	0.04459147	0.00850401	31.38746568	9.25512283
0.18989762	0.04488643	0.00858836	31.60634591	9.34659237
0.19100322	0.04530423	0.00872276	31.90587641	9.47267501
0.19212034	0.04575577	0.00877878	32.21533837	9.53696742
0.19322843	0.04614227	0.00885498	32.49761048	9.62768692
0.19434326	0.04647845	0.00896145	32.74142385	9.72885068
0.19544418	0.04681977	0.00905316	32.99581594	9.83632691
0.19655667	0.04723062	0.00913522	33.28113802	9.91488321
0.19766042	0.04774900	0.00921170	33.63986237	10.00204669
0.19877404	0.04808498	0.00930352	33.89062082	10.10633836
0.19987697	0.04852103	0.00935951	34.18925556	10.16650789
0.20099354	0.04890238	0.00947003	34.47492302	10.29033077
0.20209981	0.04932843	0.00956045	34.77422997	10.38242291
0.20321700	0.04970401	0.00963808	35.03795364	10.46250107
0.20433502	0.05015968	0.00971080	35.35059204	10.53915895
0.20544118	0.05052166	0.00984564	35.62439679	10.67209349
0.20654593	0.05101040	0.00991937	35.96843681	10.76519622
0.20765923	0.05134836	0.01002719	36.22395344	10.87949176
0.20876746	0.05171056	0.01008925	36.47246837	10.94180566
0.20987281	0.05213592	0.01015554	36.77162738	11.01832500
0.21097714	0.05255077	0.01026216	37.07245657	11.12939337
0.21208784	0.05306310	0.01036172	37.42618752	11.22906097
0.21320127	0.05346734	0.01043752	37.71091944	11.31125615
0.21429972	0.05379991	0.01057512	37.96707448	11.44702335
0.21541076	0.05419576	0.01062652	38.24427261	11.51123365
0.21652653	0.05469887	0.01072761	38.59065915	11.60879708
0.21763957	0.05509056	0.01078092	38.86501037	11.67722227
0.21873764	0.05552120	0.01091309	39.17243646	11.80027010
0.21984943	0.05584754	0.01101821	39.43071089	11.92424824
0.22096100	0.05626972	0.01109051	39.72996539	12.00827050
0.22207709	0.05681434	0.01118050	40.09457490	12.09011062
0.22318481	0.05723034	0.01125679	40.38828915	12.17315794
0.22428724	0.05767487	0.01134240	40.70795353	12.26960865
0.22540358	0.05807903	0.01144314	41.00597675	12.38286738
0.22651959	0.05847428	0.01154670	41.29388793	12.48986990
0.22762714	0.05892601	0.01165295	41.61018285	12.59552934
0.22874609	0.05942350	0.01174187	41.96006421	12.69007000
0.22984199	0.05991622	0.01183810	42.31049296	12.79725757
0.23095414	0.06035438	0.01190160	42.61341688	12.86738607
0.23206243	0.06080583	0.01198175	42.93177984	12.95274032
0.23317373	0.06120654	0.01207467	43.22561332	13.06322125
0.23427826	0.06166080	0.01221004	43.55600060	13.19359934
0.23538361	0.06210185	0.01228260	43.87058578	13.28187118

0.23649638	0.06255991	0.01237358	44.19220874	13.37722990
0.23761348	0.06299879	0.01244228	44.50023849	13.45523171
0.23872073	0.06348633	0.01249408	44.83694070	13.52378940
0.23983137	0.06388665	0.01260316	45.12800935	13.63411342
0.24093377	0.06434651	0.01263353	45.43625149	13.67578182
0.24204673	0.06478876	0.01276661	45.76630638	13.81547420
0.24315351	0.06530687	0.01281325	46.11830485	13.87175131
0.24426623	0.06577775	0.01289859	46.44893093	13.96509836
0.24536803	0.06614407	0.01297737	46.71289973	14.04892910
0.24647630	0.06663721	0.01308625	47.06373376	14.15912469
0.24759416	0.06713524	0.01315393	47.40604301	14.23855813
0.24871222	0.06765417	0.01329007	47.78517771	14.37934142
0.24982466	0.06806627	0.01337757	48.08362640	14.48089388
0.25091873	0.06847249	0.01338486	48.35386895	14.50283519
0.25203208	0.06898372	0.01343526	48.70687493	14.56685804
0.25314577	0.06941694	0.01351971	49.01314341	14.65838115
0.25425769	0.06986520	0.01363015	49.33641310	14.76950605
0.25535621	0.07033819	0.01375159	49.68031481	14.90089016
0.25647382	0.07086511	0.01381100	50.03714105	14.96591773
0.25757852	0.07130291	0.01392176	50.35279185	15.07778988
0.25869490	0.07171079	0.01397863	50.64295053	15.15450949
0.25980911	0.07226699	0.01408890	51.03851456	15.27368872
0.26091023	0.07275723	0.01418254	51.38623659	15.37689753
0.26201935	0.07328305	0.01429664	51.74799284	15.47826757
0.26312529	0.07372761	0.01439610	52.07907926	15.59805571
0.26423765	0.07419386	0.01441959	52.40159914	15.64585599
0.26534581	0.07473679	0.01448681	52.77755976	15.72446903
0.26645372	0.07513637	0.01462073	53.05812129	15.84129289
0.26756056	0.07563833	0.01471123	53.41566889	15.94321209

Table S2. NHT average tension/stress versus strain.

NHT Average Tension/Stress vs Strain				
Strain Zeroed (mm/mm)	Tension (N/mm)	SE	Stress (kPa)	SE
0.00000000	0.00381355	0.00047537	2.24432947	0.33409264
0.00108457	0.00386963	0.00051122	2.27478897	0.34785722
0.00217403	0.00400603	0.00057254	2.35870560	0.38924440
0.00323237	0.00406895	0.00056435	2.39300725	0.37629922
0.00428583	0.00415407	0.00061904	2.43443456	0.38933452
0.00534094	0.00438092	0.00066806	2.57099346	0.42918517
0.00638987	0.00432565	0.00068325	2.53640886	0.42574780
0.00743952	0.00448214	0.00075912	2.63046480	0.47024609
0.00848513	0.00466051	0.00075880	2.73438273	0.47206437
0.00954015	0.00472198	0.00080772	2.77435653	0.50582191
0.01060066	0.00481133	0.00087339	2.81641484	0.52117611
0.01163984	0.00505269	0.00090130	2.95689142	0.53701614
0.01269667	0.00507386	0.00096221	2.97596896	0.58216448
0.01375339	0.00497997	0.00097067	2.91827596	0.57844240
0.01479839	0.00528290	0.00099285	3.09718062	0.59541766
0.01584298	0.00544031	0.00107804	3.19015052	0.64125901
0.01690560	0.00545831	0.00107468	3.19760608	0.63556847
0.01795723	0.00558899	0.00114773	3.26533927	0.66219750
0.01900914	0.00572717	0.00127426	3.34826678	0.73529290
0.02006021	0.00577625	0.00123186	3.38348177	0.71887847
0.02111284	0.00594643	0.00128485	3.47705872	0.74005315
0.02216340	0.00608413	0.00136193	3.56421836	0.79019613
0.02321500	0.00619011	0.00141559	3.62345402	0.81606342
0.02426310	0.00626043	0.00149706	3.65864201	0.85313263
0.02531563	0.00645059	0.00147834	3.77067786	0.84509502
0.02637128	0.00663198	0.00156226	3.88222281	0.89650840
0.02742701	0.00665181	0.00163068	3.89141070	0.93160396
0.02847482	0.00689975	0.00166761	4.03126862	0.94628383
0.02952401	0.00695192	0.00172658	4.06545231	0.98199679
0.03058368	0.00703996	0.00178512	4.12020114	1.01722492
0.03161702	0.00728029	0.00183580	4.25156963	1.03653752
0.03267721	0.00735724	0.00189098	4.29892257	1.06773792
0.03373004	0.00751380	0.00197545	4.39477722	1.11401286
0.03478206	0.00765406	0.00202174	4.47546319	1.14341466
0.03582820	0.00780791	0.00208810	4.56206875	1.17714943
0.03688153	0.00800309	0.00215372	4.67480215	1.20907266
0.03793528	0.00803468	0.00224685	4.69268442	1.25794530
0.03898725	0.00827829	0.00230072	4.83709338	1.29232522

0.04003251	0.00842595	0.00237880	4.92119873	1.33059628
0.04109464	0.00859629	0.00245533	5.02072187	1.37251583
0.04214492	0.00874794	0.00248832	5.11113525	1.39570849
0.04319912	0.00881029	0.00259242	5.14323104	1.45131582
0.04424908	0.00894333	0.00269612	5.21948215	1.50382651
0.04529723	0.00925409	0.00273639	5.40384397	1.52642069
0.04634972	0.00933881	0.00283287	5.45310587	1.58213769
0.04739914	0.00947895	0.00290557	5.52940931	1.61564148
0.04844845	0.00976227	0.00295267	5.70008635	1.64358211
0.04950408	0.00985882	0.00306145	5.75172665	1.70146902
0.05054901	0.00992331	0.00313045	5.78945248	1.74154277
0.05160265	0.01024730	0.00319328	5.98145127	1.77739699
0.05265562	0.01043710	0.00332160	6.08946508	1.84339110
0.05371532	0.01059408	0.00340225	6.17464955	1.88077935
0.05475832	0.01083336	0.00347641	6.31590933	1.92427655
0.05580933	0.01106718	0.00361263	6.45772627	2.00361438
0.05686481	0.01107491	0.00365296	6.45599166	2.01999985
0.05791742	0.01128344	0.00368479	6.58115345	2.03987053
0.05897022	0.01156931	0.00381323	6.74598605	2.10874169
0.06001716	0.01165139	0.00392047	6.79030976	2.16386973
0.06107484	0.01180997	0.00395514	6.88521633	2.18386937
0.06212619	0.01203720	0.00405194	7.01716216	2.23827895
0.06317491	0.01216608	0.00412471	7.09303038	2.27826308
0.06422746	0.01238047	0.00419708	7.20970286	2.31069925
0.06527721	0.01252243	0.00424533	7.29846854	2.34265137
0.06632958	0.01265967	0.00432560	7.37997773	2.38785404
0.06737527	0.01285694	0.00444108	7.48546242	2.44199855
0.06842544	0.01304517	0.00447355	7.59953177	2.46470306
0.06948352	0.01318058	0.00457939	7.67730761	2.52012552
0.07053083	0.01332603	0.00469784	7.75566353	2.58097634
0.07158727	0.01353525	0.00470815	7.88370479	2.59150155
0.07263748	0.01375215	0.00480267	8.00836803	2.64074233
0.07369228	0.01376912	0.00485615	8.01457321	2.66663056
0.07474402	0.01397364	0.00491116	8.13372484	2.69592371
0.07579641	0.01416265	0.00501259	8.23529707	2.74765154
0.07684723	0.01429954	0.00510610	8.32448614	2.80283422
0.07790018	0.01448383	0.00514045	8.43315967	2.82439601
0.07894364	0.01464923	0.00520563	8.52005805	2.85227034
0.07999523	0.01477995	0.00531979	8.59561285	2.91407644
0.08104875	0.01488095	0.00535080	8.65808981	2.93356172
0.08210907	0.01506763	0.00543854	8.76436758	2.97888131
0.08315419	0.01528540	0.00557253	8.88594116	3.04996875
0.08420469	0.01548325	0.00557644	9.00569558	3.05593249
0.08525902	0.01561261	0.00567485	9.08089514	3.10841293

0.08630968	0.01576738	0.00578692	9.16079657	3.16100866
0.08735518	0.01591781	0.00582805	9.25033847	3.18532959
0.08840510	0.01608255	0.00587605	9.34551179	3.21209042
0.08945817	0.01632056	0.00597007	9.48761424	3.26686928
0.09051268	0.01637305	0.00606090	9.51520341	3.31211614
0.09156779	0.01658614	0.00612255	9.63691338	3.34455579
0.09262454	0.01685925	0.00621411	9.79715374	3.39376938
0.09367102	0.01691242	0.00628231	9.82010093	3.42656990
0.09472557	0.01715394	0.00635738	9.96801840	3.47101374
0.09577040	0.01733401	0.00641548	10.07203263	3.50439468
0.09681913	0.01746445	0.00655091	10.13734176	3.56976022
0.09787525	0.01763466	0.00661013	10.24155481	3.60716483
0.09892427	0.01772989	0.00664573	10.29471302	3.62452156
0.09997718	0.01793794	0.00678788	10.41764689	3.70003715
0.10102780	0.01812877	0.00688829	10.52413013	3.75488364
0.10209310	0.01832149	0.00692702	10.63029731	3.77116031
0.10313347	0.01841355	0.00699924	10.68849078	3.81099323
0.10418337	0.01864227	0.00707893	10.81958480	3.85582549
0.10523519	0.01889751	0.00714318	10.96688812	3.89025797
0.10628657	0.01899652	0.00726149	11.02238679	3.95192074
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0.10838860	0.01938008	0.00741111	11.24235675	4.03297191
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0.11259897	0.01998001	0.00771121	11.58252918	4.19175142
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0.11470556	0.02048192	0.00790721	11.87551653	4.29719033
0.11575649	0.02054703	0.00794166	11.90931528	4.31432944
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0.11890407	0.02112360	0.00822192	12.23941372	4.46386712
0.11995327	0.02125420	0.00836681	12.31655962	4.54288609
0.12100899	0.02148338	0.00843449	12.45114577	4.58082788
0.12205895	0.02169624	0.00846459	12.57087664	4.59489782
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0.12626793	0.02244055	0.00888812	12.99548974	4.81782565
0.12731478	0.02266233	0.00893746	13.12377086	4.84835762
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0.12941854	0.02293474	0.00909389	13.27951170	4.92991669
0.13047340	0.02316055	0.00920203	13.40662770	4.98958517
0.13152527	0.02334375	0.00930857	13.51420576	5.04698320

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0.13468998	0.02401066	0.00954028	13.89687288	5.16893935
0.13573188	0.02418102	0.00963276	13.98909306	5.21654549
0.13678258	0.02436170	0.00974452	14.09761748	5.27641351
0.13783853	0.02455768	0.00984162	14.20868298	5.33019538
0.13888185	0.02478594	0.00992418	14.33319473	5.37114454
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0.14098785	0.02526504	0.01011519	14.61517119	5.47490709
0.14204181	0.02537566	0.01014578	14.67936016	5.49165550
0.14309194	0.02554422	0.01025621	14.76512858	5.54595555
0.14413895	0.02582171	0.01035875	14.93506915	5.60321905
0.14519635	0.02598158	0.01040416	15.02612215	5.63059701
0.14625345	0.02623223	0.01053195	15.17033914	5.69710627
0.14729843	0.02641788	0.01061270	15.27952222	5.73987361
0.14835073	0.02662402	0.01069679	15.39401412	5.78574354
0.14940444	0.02682425	0.01079949	15.50792420	5.83965748
0.15046132	0.02691543	0.01087926	15.55989580	5.88199575
0.15150371	0.02720062	0.01099515	15.72228697	5.94344841
0.15255432	0.02747352	0.01104350	15.88142453	5.96814880
0.15360846	0.02765663	0.01116549	15.98490224	6.03556071
0.15465787	0.02790047	0.01129227	16.12359707	6.10227075
0.15570837	0.02808691	0.01136596	16.22736487	6.14032373
0.15676147	0.02820889	0.01143729	16.30195914	6.18073666
0.15781157	0.02855678	0.01156309	16.50433453	6.24693911
0.15886177	0.02874314	0.01166036	16.60574710	6.29609646
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0.16097242	0.02904844	0.01182249	16.77998942	6.38668017
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0.16412808	0.02980200	0.01210072	17.22161523	6.53630859
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0.16623453	0.03014045	0.01223015	17.40711808	6.60493461
0.16728416	0.03030209	0.01229951	17.50920426	6.64371464
0.16832573	0.03044442	0.01228156	17.58661267	6.62960107
0.16937766	0.03060634	0.01228408	17.68417157	6.63235166
0.17043618	0.03073174	0.01226437	17.76449199	6.62599067
0.17148607	0.03091075	0.01236724	17.86634031	6.68173704
0.17253477	0.03118015	0.01246926	18.01811690	6.73464132
0.17358929	0.03142333	0.01247367	18.16088489	6.73713494
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0.17569026	0.03181966	0.01275590	18.37777541	6.88412306
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0.17779160	0.03230699	0.01293530	18.66840634	6.98362786

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0.17990041	0.03278938	0.01315251	18.93929708	7.09613594
0.18095194	0.03296407	0.01319876	19.04008283	7.12247267
0.18200289	0.03322376	0.01331091	19.19006749	7.18430940
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0.18410051	0.03367662	0.01351301	19.45210143	7.28967072
0.18515044	0.03392460	0.01363727	19.58651445	7.35557388
0.18620093	0.03414430	0.01373006	19.71595124	7.40740048
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0.19146363	0.03533839	0.01418163	20.40275620	7.64805149
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0.22091305	0.04168089	0.01621173	24.05336852	8.72850242
0.22196257	0.04188955	0.01631184	24.16987010	8.78340389
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0.22826936	0.04305985	0.01654154	24.85284016	8.90850801
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0.23037251	0.04338423	0.01655655	25.03908155	8.91725865
0.23142671	0.04360308	0.01657166	25.17277797	8.92686688
0.23247484	0.04381748	0.01664781	25.30005921	8.97187090
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