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Supplementary Materials for

Nanowired human cardiac organoid transplantation enables highly efficient and effective recovery of infarcted hearts

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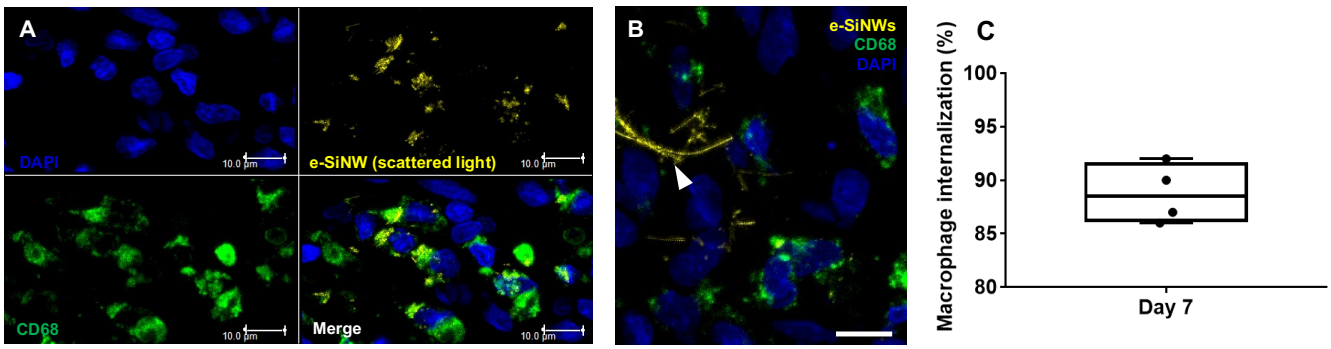


Fig. S1. Macrophage response to injected e-SiNWs post-transplantation. (A) Internalization of e-SiNWs was evidenced by colocalization of e-SiNWs with CD68-positive (green) macrophages 7 days post-transplantation. (B) Some larger e-SiNWs (arrow) were not internalized due to size limitations. Scale bars = 10 µm. (C) Quantification of % nanowire colocalization with $CD68^{+}$ macrophages in rat myocardium at Day 7 post-transplantation. N=average from 3 regions of interest in 4 independent hearts.

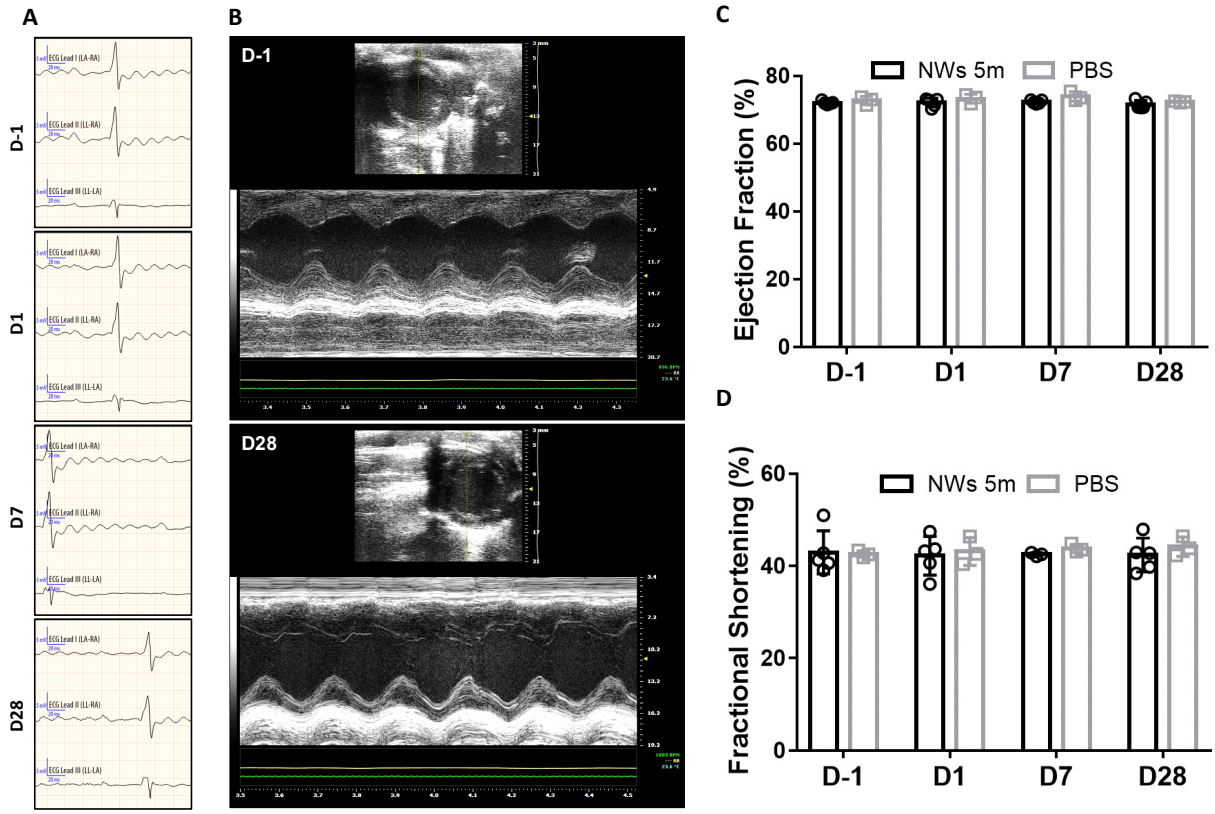
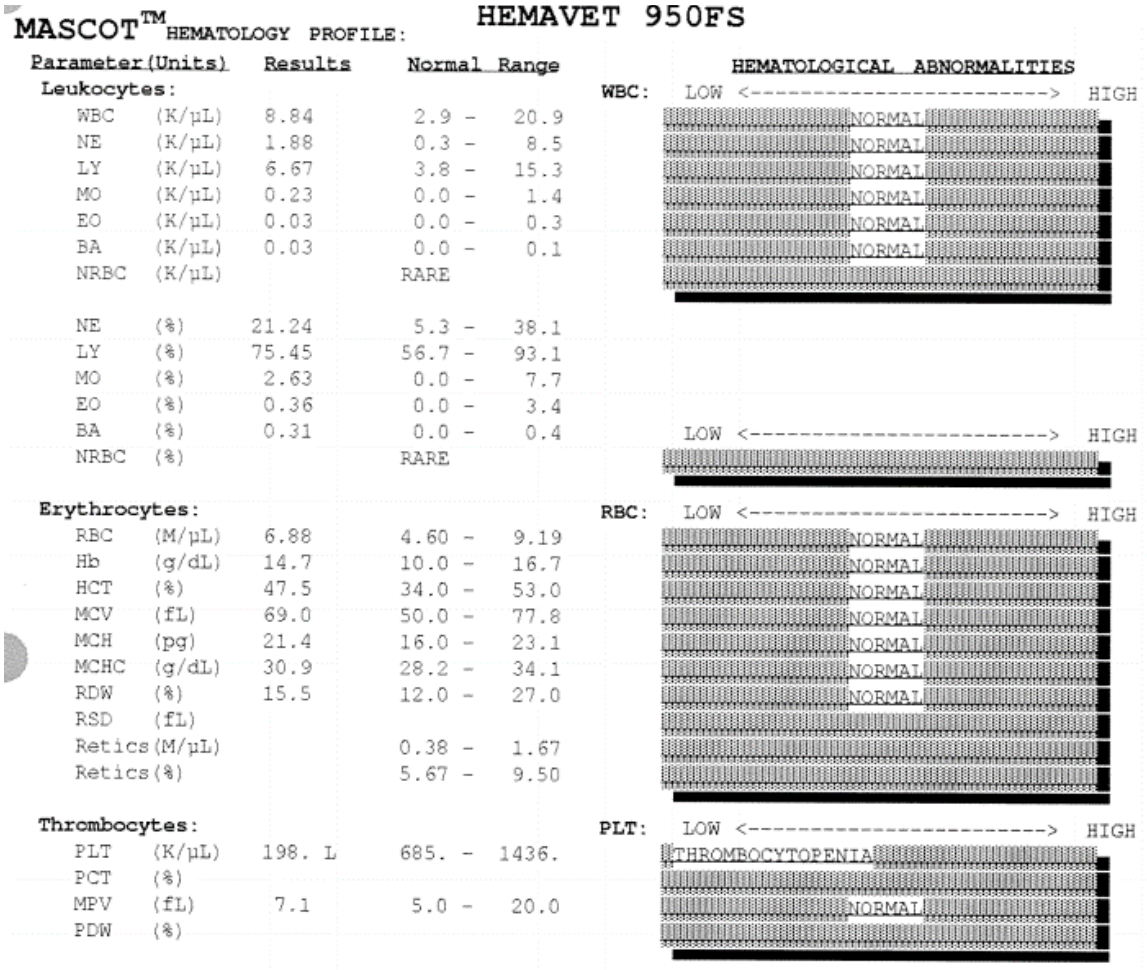


Fig. S2. Cardiac functional compatibility analysis after e-SiNWs injection into adult rat myocardium. (A) Representative ECG profiles of rats with 5 million e-SiNWs injection in the left ventricular myocardium at time points of before injection (D-1) and after injection (D1, D7 and D28). (B) Representative echocardiogram images in short axis of rat hearts with 5 million e-SiNWs injection in the left ventricular myocardium at time points of D-1 and D28. (C) Ejection fraction and (D) fractional shortening were used for quantitative analysis of cardiac function after e-SiNWs and PBS injection. Error bars represent standard deviation (N=4 rats).



MASCOT™ HEMATOLOGY PROFILE:

HEMAVET 950FS

Parameter	(Units)	Results	Normal Range
Leukocytes:			
WBC	(K/µL)	8.84	2.9 - 20.9
NE	(K/µL)	1.88	0.3 - 8.5
LY	(K/µL)	6.67	3.8 - 15.3
MO	(K/µL)	0.23	0.0 - 1.4
EO	(K/µL)	0.03	0.0 - 0.3
BA	(K/µL)	0.03	0.0 - 0.1
NRBC	(K/µL)		RARE
NE	(%)	21.24	5.3 - 38.1
LY	(%)	75.45	56.7 - 93.1
MO	(%)	2.63	0.0 - 7.7
EO	(%)	0.36	0.0 - 3.4
BA	(%)	0.31	0.0 - 0.4
NRBC	(%)		RARE
Erythrocytes:			
RBC	(M/µL)	6.88	4.60 - 9.19
Hb	(g/dL)	14.7	10.0 - 16.7
HCT	(%)	47.5	34.0 - 53.0
MCV	(fL)	69.0	50.0 - 77.8
MCH	(pg)	21.4	16.0 - 23.1
MCHC	(g/dL)	30.9	28.2 - 34.1
RDW	(%)	15.5	12.0 - 27.0
RSD	(fL)		
Retics	(M/µL)		0.38 - 1.67
Retics	(%)		5.67 - 9.50
Thrombocytes:			
PLT	(K/µL)	198. L	685. - 1436.
PCT	(%)		
MPV	(fL)	7.1	5.0 - 20.0
PDW	(%)		

HEMATOLOGICAL ABNORMALITIES

WBC: LOW <----------> HIGH — NORMAL

LOW <----------> HIGH

RBC: LOW <----------> HIGH — NORMAL

PLT: LOW <----------> HIGH — THROMBOCYTOPENIA, NORMAL

Fig. S3. Representative blood test result. The hematology profile of rat blood sample collected from tail vein was performed by Drew Scientific HemaVet 950FS Auto Blood Analyzer. The boxed value was used to construct Supplementary Table 1.

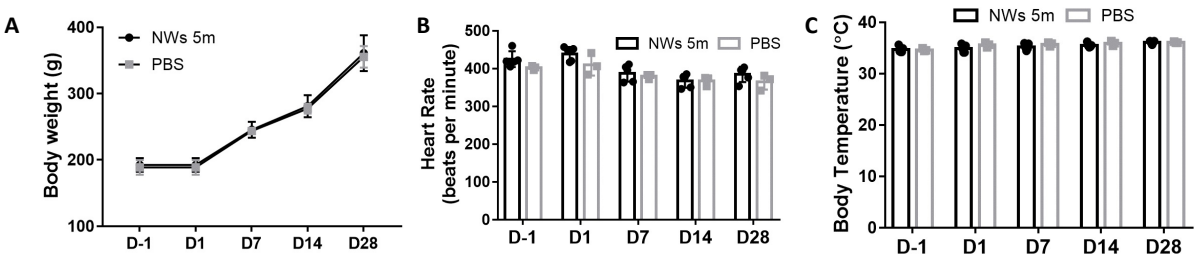


Fig. S4. Normal physiological condition of rats after receiving NWs injection. (A) The rats with 5 million e-SiNWs or PBS (control) intramyocardial injection were weighed at time points of before injection (D-1) and after injection (D1, D7 and D28) to monitor the body growth. Error bars represent standard deviation. N=5 rats for e-SiNWs injection group and N=3 rat for PBS injection group. (B) Heartbeat rate and (C) rectal temperature were recorded for the rats with 5 million e-SiNWs or PBS (control) intramyocardial injection at time points of before injection (D-1) and after injection (D1, D7 and D28) to monitor the physiological condition. Error bars represent standard deviation. N=5 rats for e-SiNWs injection group and N=3 rat for PBS injection group.

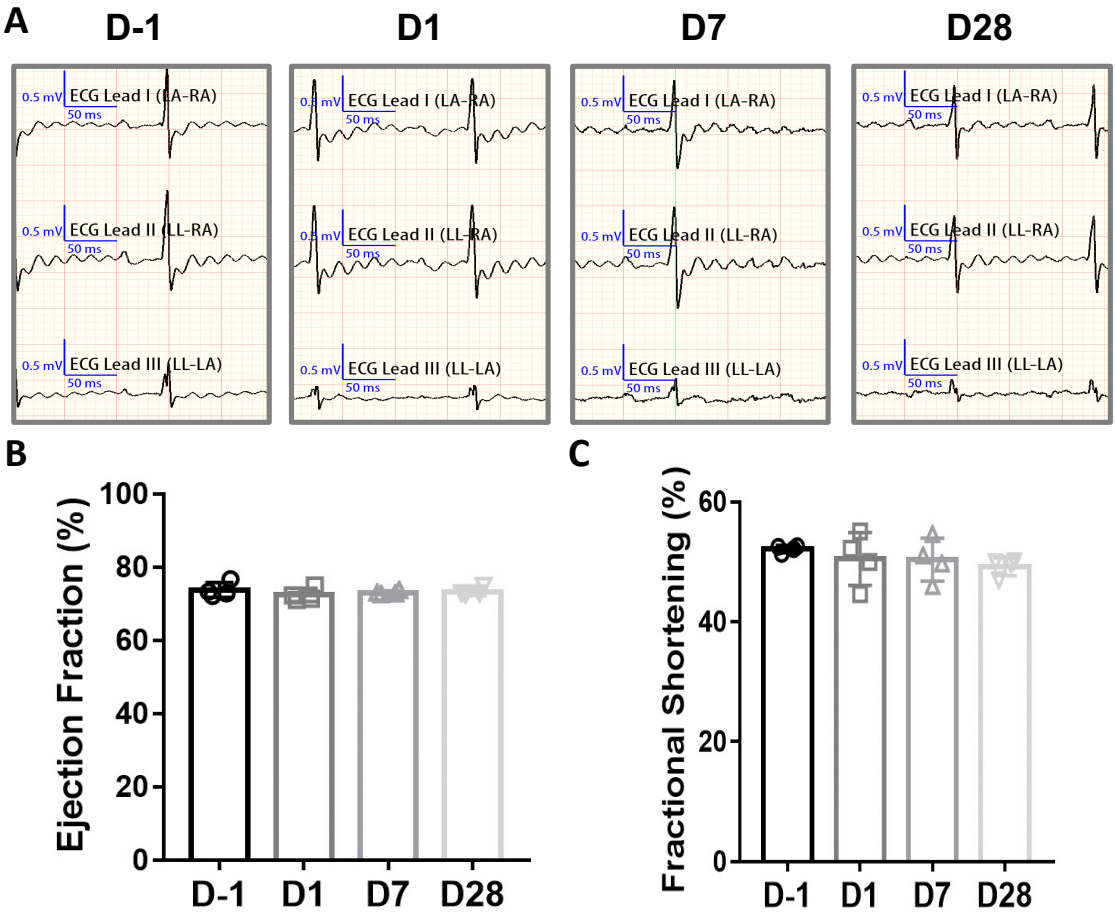


Fig. S5. Normal electrophysiology of rat hearts after nanowired hiPSC cardiac spheroid transplantation. (A) Representative ECG profiles of rats with nanowired cardiac spheroids injection in the left ventricular myocardium at time points of before injection (D-1) and after injection (D1, D7 and D28) indicate healthy cardiac electrical signal propagation with no arrhythmias. (B) Ejection fraction and (C) fractional shortening as quantitative analysis for cardiac function for spheroids injection do not show deleterious effects of nanowired spheroid injection. Error bars represent standard deviation (N=4).

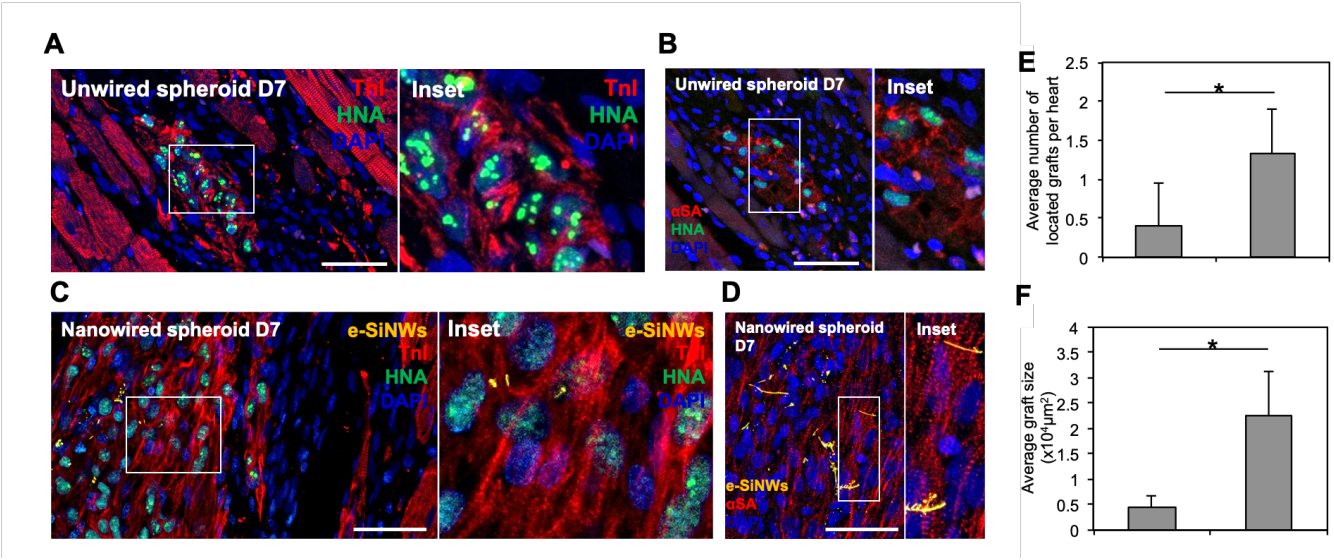


Fig. S6. hPSC-CM contractile development in unwired and nanowired spheroid transplantation. (A) Representative immunofluorescent staining of TnI in grafted unwired cardiac spheroids at day 7 post-transplantation. (B) Representative immunofluorescent staining of αSA in grafted unwired cardiac spheroids at day 7 post-transplantation. (C) Representative immunofluorescent staining of TnI in grafted nanowired cardiac spheroids at day 7 post-transplantation. (D) Representative immunofluorescent staining of αSA in grafted nanowired cardiac spheroids at day 7 post-transplantation. (E) Average number of spheroid grafts found at day 7. (F) Average spheroid graft size at day 7. (N=4 hearts, two-tailed Student's t-test) Data represents mean ± s.d. Scale: (A-D) = 50 μm.

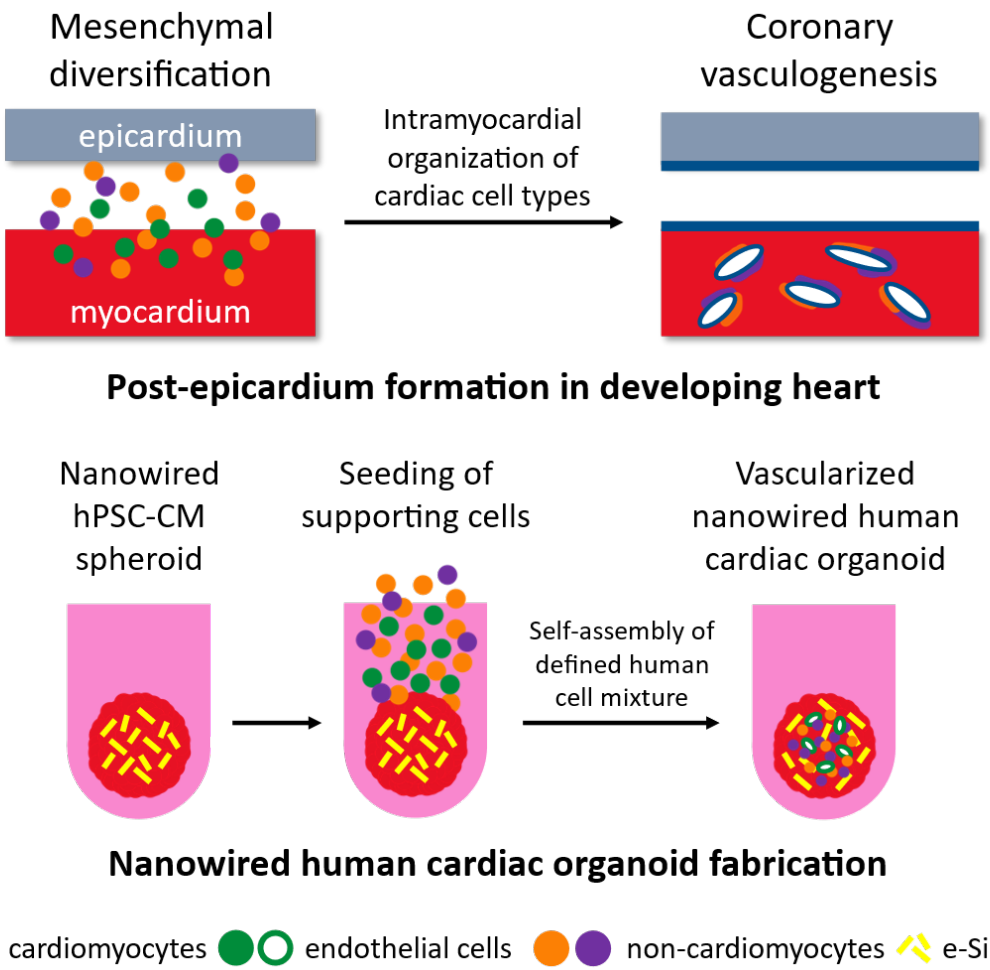


Fig. S7. Developmental inspiration for biomimetic fabrication of nanowired human cardiac organoids. After 10 days of spheroid culture with or without nanowires, supporting cells were seeded onto the pre-formed spheroids. This sequential seeding approach was inspired by epicardial cell infiltration and mesenchymal diversification during post-epicardium formation, which produces most of the non-cardiomyocyte lineages of the mature heart.

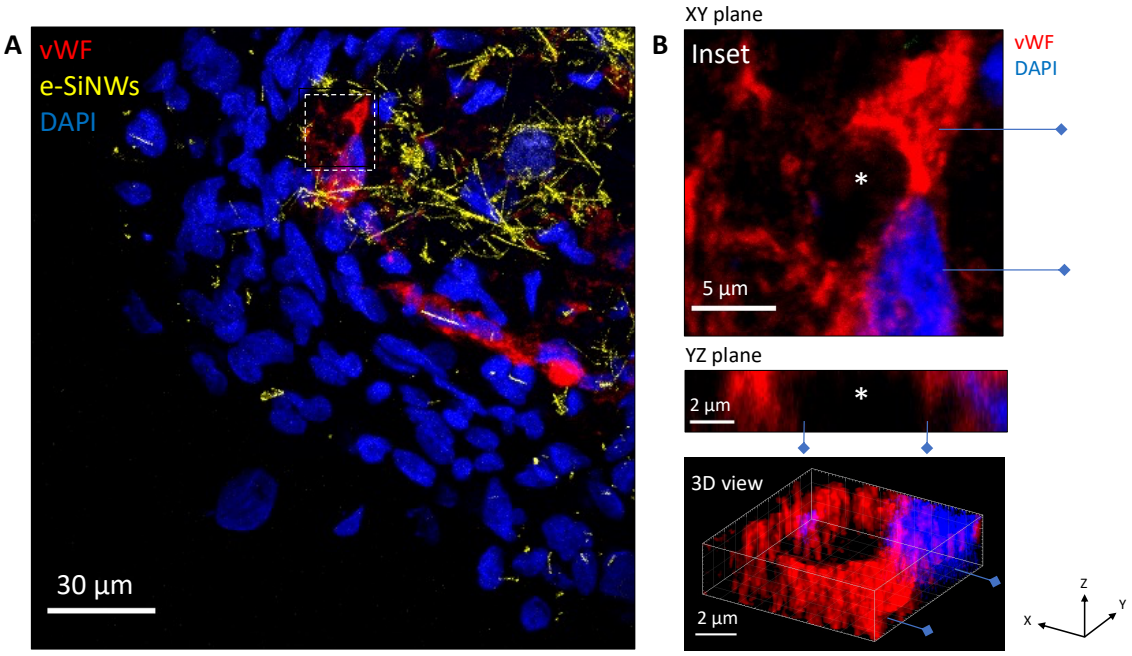


Fig. S8. Lumenized vascular morphogenesis in nanowired human cardiac organoids. (A) Overview of vWF-positive vascular tube formation in nanowired cardiac organoids at day 7 of organoid culture. (B) High-magnification 3D visualization of capillary-scale lumenized vasculature within nanowired organoids. Blue lines serve as consistent reference points among planes.

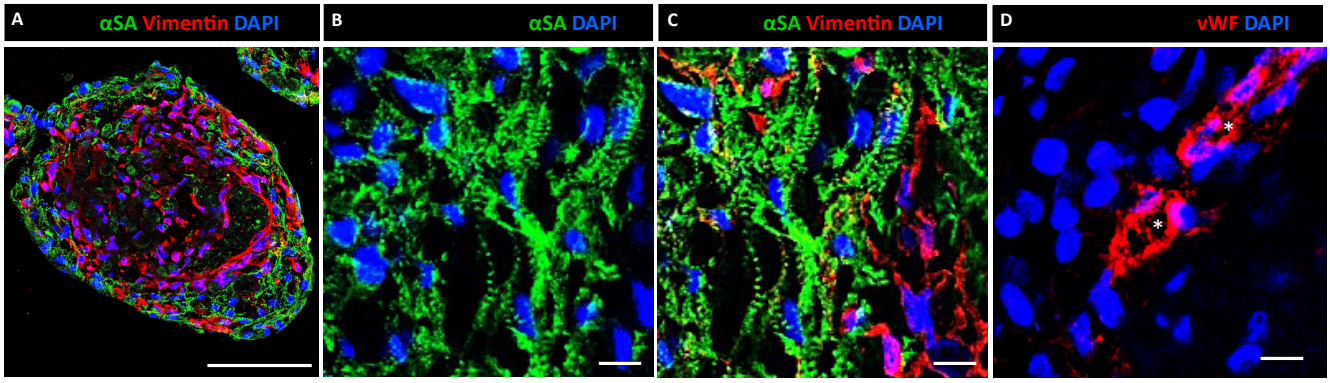


Fig. S9. Unwired human cardiac organoids. (A) Representative image of cell organization within unwired cardiac organoids. hiPSC-CMs (green) organize on the peripheral region while mesenchymal cells (red) mainly organize in the interior. (B) Sarcomere development of pre-injection unwired cardiac organoids. (C) Mesenchymal cells are distributed across cardiomyocyte regions and endothelial cells (D) form lumen-like structures (*). Scale bars: (A) = 100 μm; (B-D) = 10 μm.

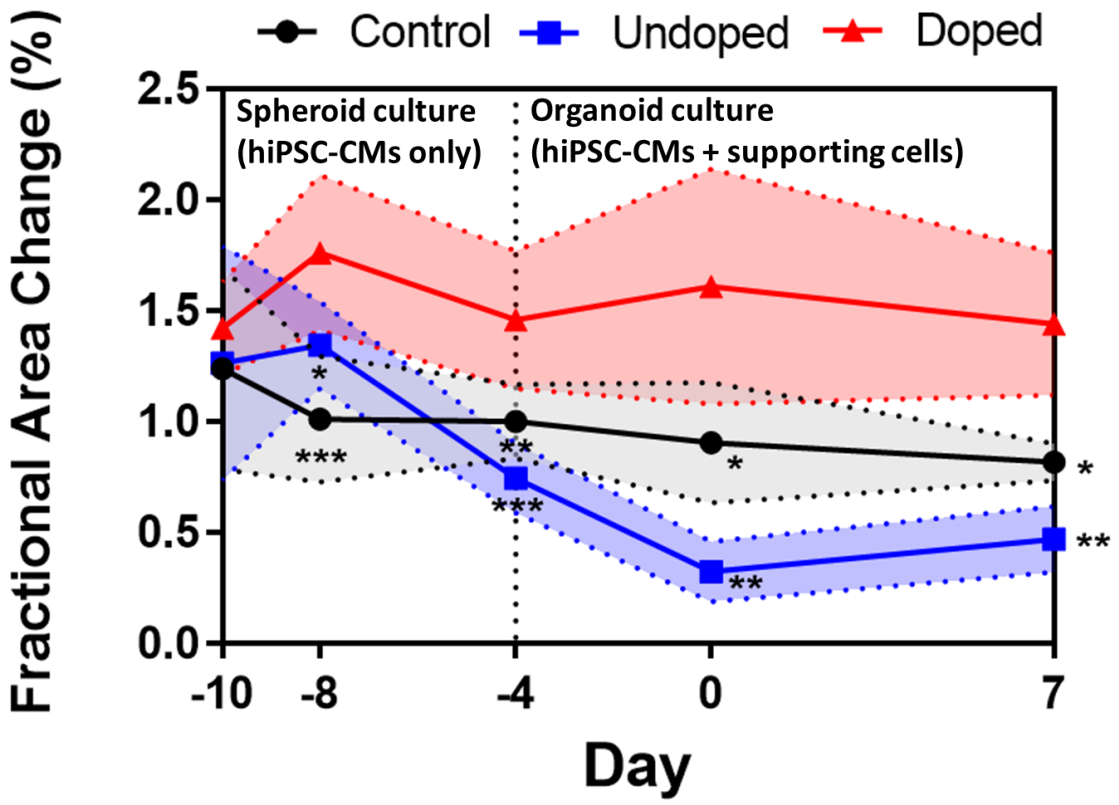


Fig. S10. Conductivity-dependent improvements in nanowired cardiac microtissue contractility. Fractional area changes of unwired and nanowired cardiac spheroids and organoids with conductive (doped) and non-conductive (undoped) silicon nanowires (1:1 nanowires:CMs). Data is represented as mean ± SD, with SD represented as shaded area above and below connecting line N≥5 independent organoids per condition. Asterisks indicate significant difference from Doped, One-way ANOVA, *p<0.05, **p<0.01, ***p<0.005.

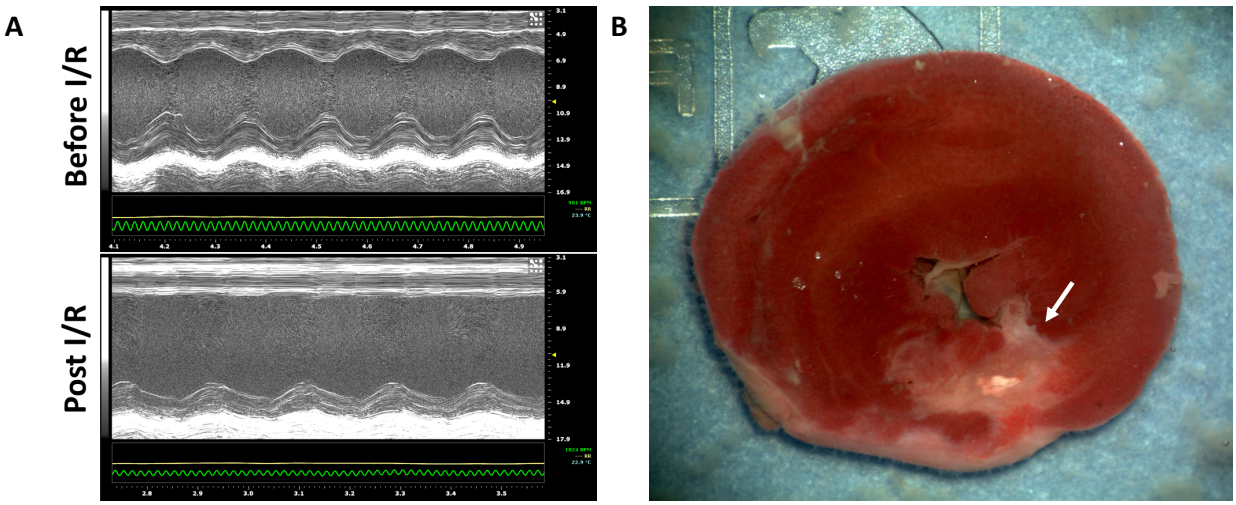


Fig. S11. Ischemia-reperfusion injury of adult rat hearts. (A) Representative M-mode echocardiograph pre/post-I/R surgery: EF (77%/49%), FS (47%/26%), LVEDD (6.9mm/6.7mm), and LVESD (3.7mm/5.0mm). (B) Representative 2,3,5-Triphenyltetrazolium Chloride (TTC) staining of I/R injured rat hearts (pale area indicates infarcted tissue, arrow).

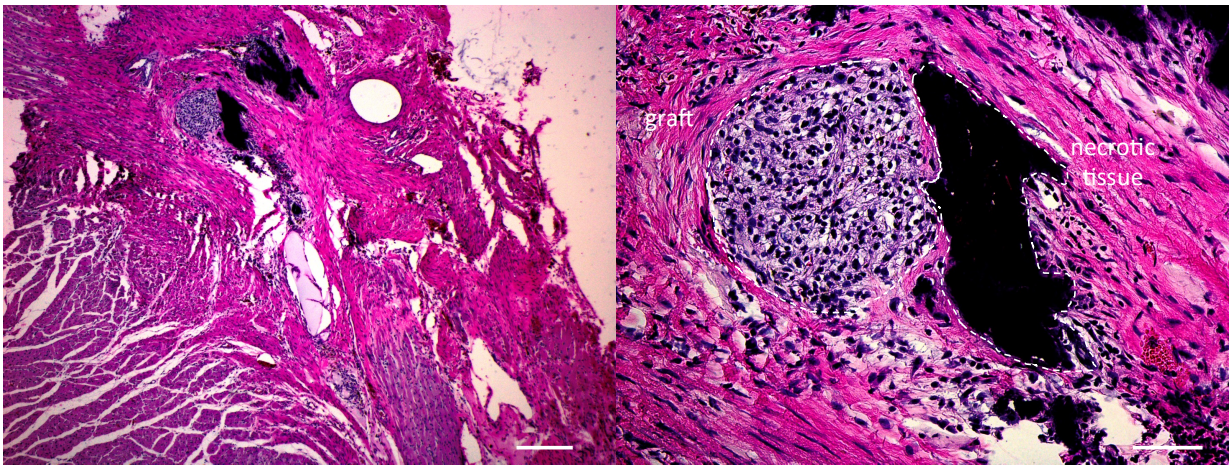


Fig. S12. Peri-necrotic engraftment of nanowired cardiac organoids after 28 days. H&E stain of nanowired organoid graft (outlined) directly adjacent to necrotic host myocardium (black, outlined) 28 days post-transplantation. Scale: left = 200 μm; right = 50 μm.

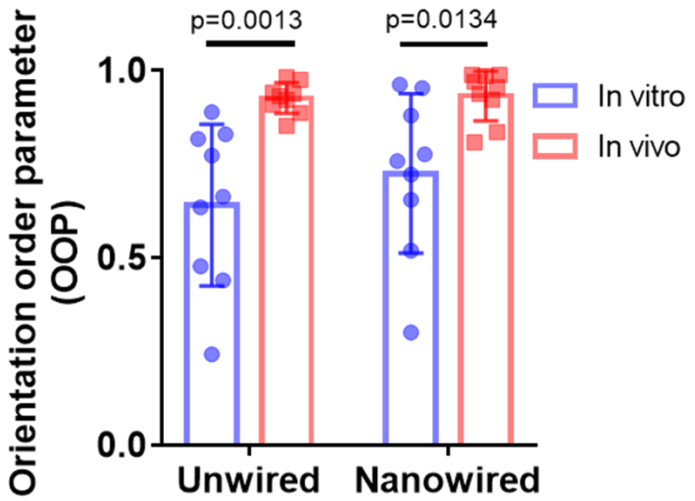


Fig. S13. Alignment of hiPSC-CMs in cardiac organoids *in vitro* and *in vivo*. Orientation order parameter (OOP) was calculated for cardiac organoids with or without nanowires based on the alignment of contractile proteins αSA and TnI, respectively. Data represents mean ± S.D. N=9 regions of interest across three organoids for each group. Two-tailed Student's t-test was used to compared in vitro to in vivo.

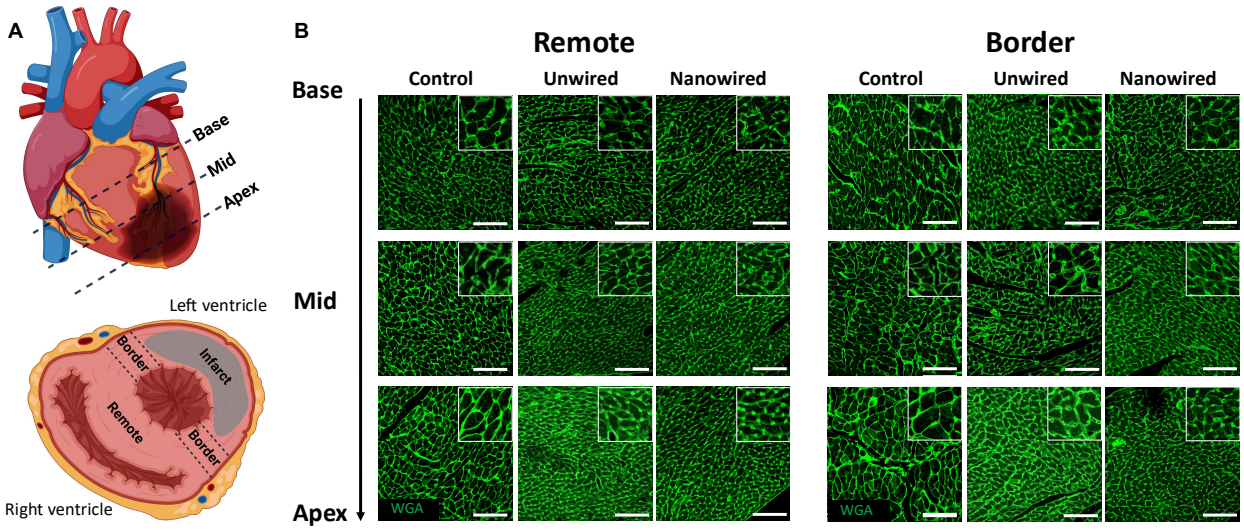


Fig. S14. Whole-heart hypertrophy analysis. (A) Paraffin embedded formalin fixed rat hearts were serially cross sectioned from base to apex and equidistant sections were randomly chosen from "base", "mid", and "apex" regions of the left ventricle for immunofluorescent staining. (B) Wheat germ agglutinin (WGA) stains glycoproteins in cell membranes and was used to quantify cardiomyocyte area in remote and border zones from base to apex in N=5 rat hearts each. Scale = 100 μm.

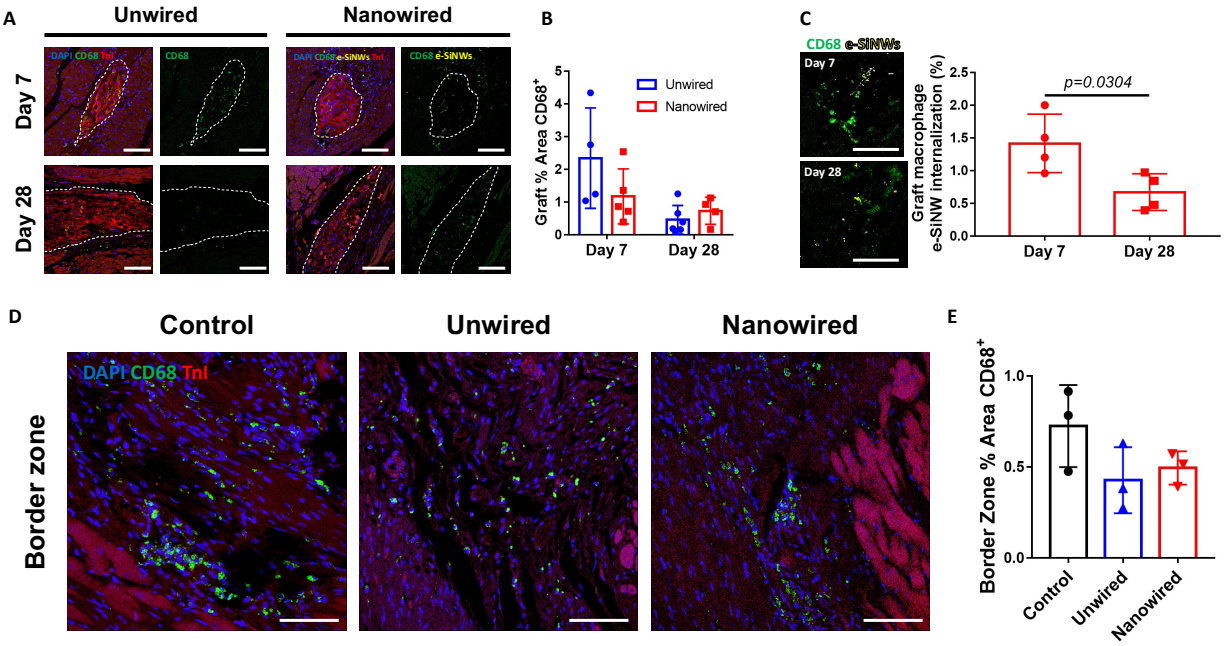


Fig. S15. Nanowired human cardiac organoids do not exacerbate host or graft inflammation in infarcted myocardium. (A) Representative images of macrophage (CD68) infiltration in engrafted cardiac organoids at day 7 and 28 post-transplantation. Scale = 100 μm. (B) Percent area coverage of organoid grafts by CD68+ macrophages. Data are presented as mean ± s.d. (N≥4 grafts across 3 hearts per condition). (C) Internalization of e-SiNWs by CD68+ macrophages within nanowired organoid grafts at Days 7 and 28. Scale = 50 μm. Data are presented as mean ± s.d. (N=average of 3 regions of interest from 4 grafts across 4 hearts per timepoint). Two-tailed Student's t-test was used for comparing unwired and nanowired graft % macrophage area and graft macrophage e-SiNW internalization at day 7 and day 28. (D) Representative images of macrophage presence in the border zone at day 28. Scale = 100 μm (E) Percent area coverage of border zone by macrophages. Data are presented as mean ± s.d. (N=average from 3 regions of interest in border zones of 3 independent hearts from each condition). Two-tailed Student's t-test was used for comparing control, unwired, and nanowired groups.

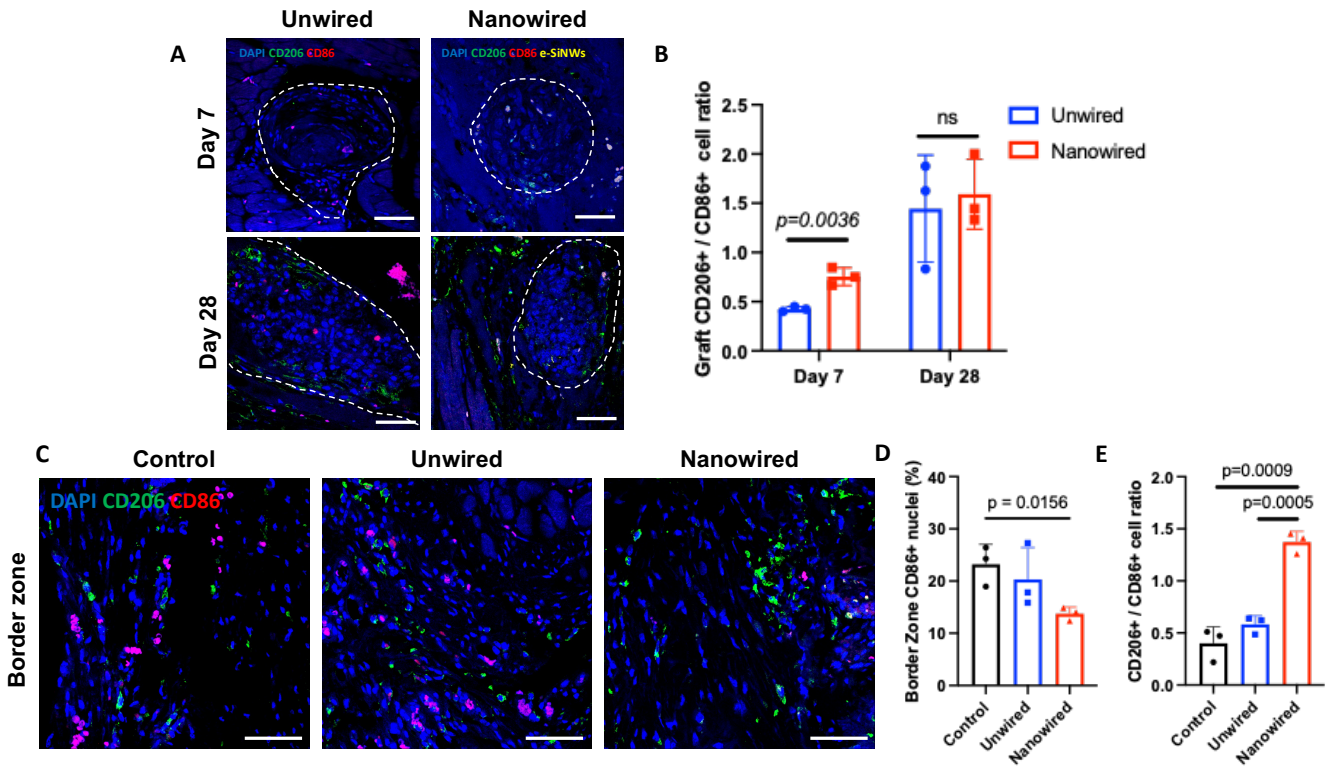


Fig. S16. Nanowired human cardiac organoid transplantation-associated macrophage phenotypes in infarcted myocardium. (A) Representative images of CD86+ and CD206+ macrophage infiltration in engrafted cardiac organoids at day 7 and 28 post-transplantation. Scale = 50 μm, inset = 10 μm. (B) Ratio of CD206+ to CD86+ macrophages in organoid grafts. N≥3 grafts across 3 hearts per condition. Data are presented as mean ± s.d. (N=average of 3 regions of interest from 4 grafts across 4 hearts per timepoint). Two-tailed Student's t-test was used for comparing unwired and nanowired graft macrophage ratios at day 7 and day 28. (C) CD86+ and CD206+ macrophage populations in the border zone at day 28. Scale = 50 μm (D) Percent CD86+ macrophages relative to total nuclei in the border zone. (E) Ratio of CD206+ to CD86+ macrophages in the border zone. Data are presented as mean ± s.d. (N=average of 3 regions each from base to apex in each of 3 hearts per condition, two-tailed Student's t-test). All data are presented as mean ± s.d.

Table S1. Total white blood cell counts for nanowires biocompatibility test. Hematology profiles of rats' blood samples with 5 million e-SiNWs or PBS (control) intramyocardial injection at time points of before injection (D-1) and after injection (D1, D7 and D28) indicate minimal inflammation response post-surgery. N=5 rats for e-SiNWs injection group and N=3 rat for PBS injection group.

	Total White Blood Cells (k/ul) (Normal range: 2.9~20.9)				
Group	Pre-surgery	Post-surgery			
	Day -1	Day 1	Day 7	Day 14	Day 28
5m e-SiNWs (N=5)	15.80	16.82	16.06	15.76	14.60
	23.48	13.38	19.88	17.84	11.74
	7.76	12.98	18.66	12.18	11.36
	8.86	11.76	18.52	19.76	19.74
	16.10	11.90	19.74	13.96	13.32
PBS (N=3)	13.04	12.14	17.22	20.32	19.66
	16.46	17.24	12.32	22.78	19.20
	17.86	19.86	12.86	18.46	17.58

Table S2. Echocardiographic measurements.

Timepoint	Parameter	Control	Unwired	Nanowired
Pre-I/R	Fractional shortening (%)	49.8±2.1	50.4±2.2	50.0±2.6
24 hours post-I/R		25.1±2.9	27.3±3.2	26.4±1.7
7 days post-injection		29.0±4.3	34.5±5.9	40.9±4.7
28 days post-injection		26.0±3.9	36.3±5.1	42.7±3.4
Pre-I/R	LVEDD (mm)	6.0±0.5	6.4±0.4	6.4±0.3
24 hours post-I/R		6.1±0.7	5.7±0.7	5.4±0.7
7 days post-injection		7.1±0.8	6.7±0.9	6.3±0.4
28 days post-injection		7.2±0.5	7.3±0.8	7.0±0.3

Pre-I/R	LVESD (mm)	3.0±0.3	3.2±0.2	3.2±0.3
24 hours post-I/R		4.6±0.6	4.2±0.6	4.0±0.5
7 days post-injection		5.1±0.9	4.4±0.9	3.7±0.5
28 days post-injection		5.4±0.6	4.6±0.7	4.0±0.4
7 days post-injection	Fractional shortening, change from post-I/R (%)	3.9±4.5	7.2±7.5	14.5±5.4
28 days post-injection		0.9±5.2	9.0±5.1	16.3±4.5
7 days post-injection	LVEDD, change from pre-I/R (mm)	1.0±0.7	0.9±1.0	0.9±0.6

28 days post-injection		1.2±0.3	0.9±0.1	0.6±0.2
7 days post-injection	LVESD, change from pre-I/R (mm)	0.5±0.8	0.2±1.1	0.3±0.5
28 days post-injection		2.3±0.5	1.5±0.8	0.8±0.3
7 days post-injection	Fractional shortening, recovery of post-I/R injury (%)	14.5±16.2	30.1±30.5	60.2±16.2
28 days post-injection		2.2±22.0	39.5±23.4	68.7±18.4

Abbreviations: I/R, ischemia/reperfusion; LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter.

Table S3: Details of the antibodies used in this study.

Antigen	Antibody	type	Company	Catalog number	Lot number	Dilution
Vimentin	Rabbit	polyclonal	Abcam	ab92547	GR3258719-11	1:200
CD68	Mouse	monoclonal	BioRad	MCA1957	0515	1:200
CD86	Mouse	monoclonal	Novus Biologicals	NBP225208	942-1P221206	1:200
CD206	Rabbit	polyclonal	Proteintech	50-173-2199	00114009	1:200
pan Troponin I	Rabbit	polyclonal	Invitrogen	710580	1997919	1:200
Connexin-43	Rabbit	polyclonal	Sigma	C6129		1:200
Von Willebrand Factor	Rabbit	polyclonal	Abcam	ab6994	GR3180938-1	1:200
Sarcomeric alpha-actinin	Rabbit	polyclonal	Abcam	ab52917	GR3174517-4	1:200
CD31	Mouse	Monoclonal	BD Biosciences	550274	5170510	1:200
N-cadherin	Mouse	monoclonal	BD	610921	4357631	1:200
Laminin	Mouse	monoclonal	Sigma	L9393		1:200
Collagen I	Rabbit	polyclonal	Abcam	ab34710		1:200
Human Nuclear	Mouse	monoclonal	Abcam	ab191181	GR3192103-3	1:200
Isolectin B4 Alexa488 Conjugate	Not specified	Not specified	Vector	FL-1201-.5	ZB0406	1:50
DAPI	n/a	n/a	Invitrogen	R37606		1:10
Live/dead staining kit	n/a	n/a	Life Tech	L3224		EthD-1 (1:500) / calcein (1:2000)
TUNEL staining kit	n/a	n/a	Roche	11684795910		enzyme solution: label solutioN=1:9
Alexa Fluor 546	Goat anti-mouse	Polyclonal	Invitrogen	A-11030	2026145	1:200
Alexa Fluor 647	Goat anti-rabbit	Polyclonal	Invitrogen	A32733	2047630	1:200