Electronic Supplementary Material (ESI) for Biomaterials Science

Supporting Information

A Nanopatterned Cell-Seeded Cardiac Patch Prevents Electro-Uncoupling and Improves the Therapeutic Efficacy of Cardiac Repair

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Materials and methods

Mechanical testing of patch

The mechanical properties of aligned or randomly oriented EPs were performed using uniaxial load testing equipment (H1KH-0048; Tinius Olsen) with a crosshead speed of 5 mm/min. EP was cut into strips of 3 cm long and 1 cm wide. Samples were stretched under

ambient conditions until failure in the preferred direction of the fiber orientation. Force-elongation data were used to generate stress–strain curves.

Gene expression

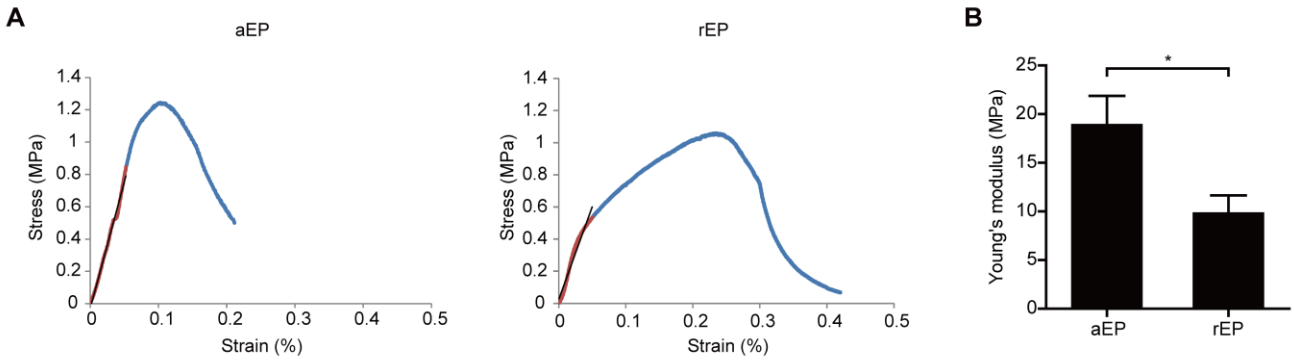
CMs were cultured on aligned or randomly oriented EPs in 24 well plates at density of 2×10^5 cells/well for 3 days. Total RNA was extracted from the CMs using TRIzol (Invitrogen) and converted to cDNA using RevertAid Reverse Transcriptase (Fermentas) as previously described.[1] The expression patterns were determined using real-time PCR.

Western blot

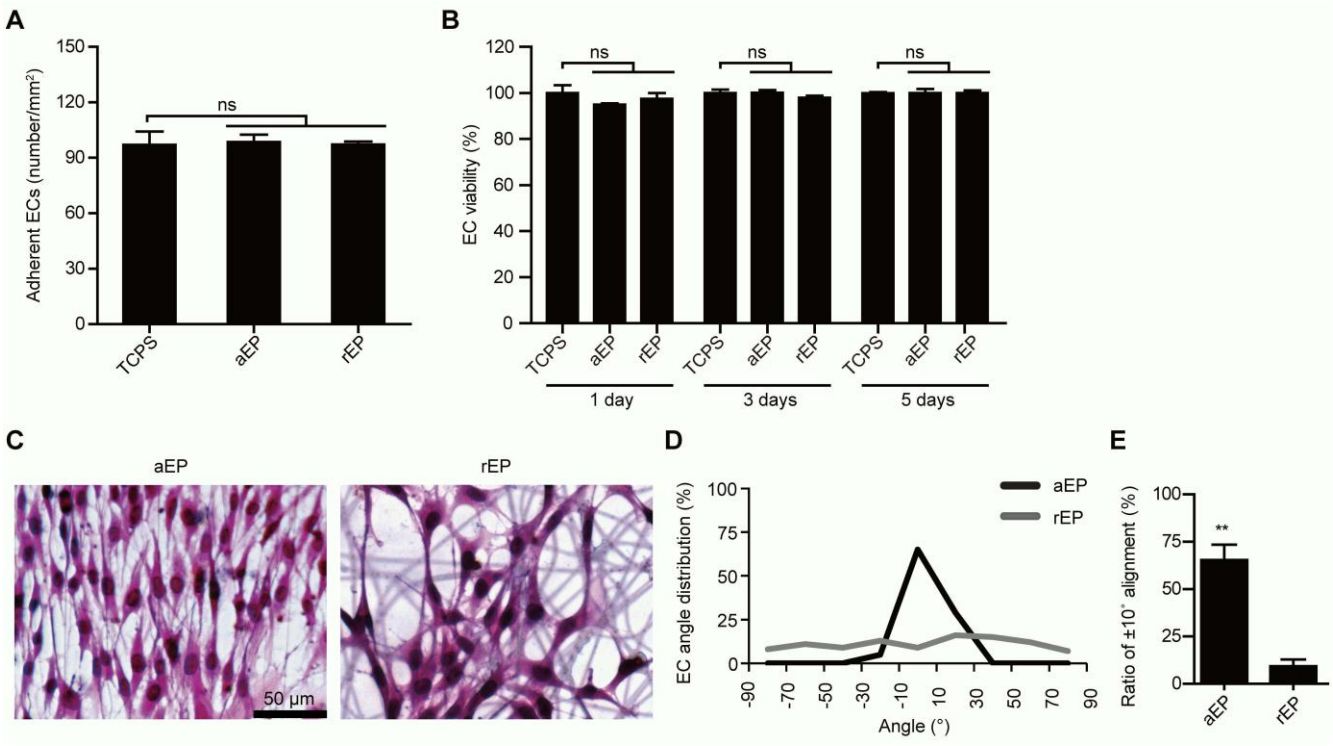
CMs were cultured on aligned or randomly oriented EPs in 24 well plates at density of 2×10^5 cells/well for 3 days. Protein was extracted using a RIPA buffer as previously described.[2] The protein extract was incubated with connexin-43 primary antibodies (Abcam) at 4°C overnight. The signal was detected with an enhanced chemiluminescence detection kit (Millipore).

References

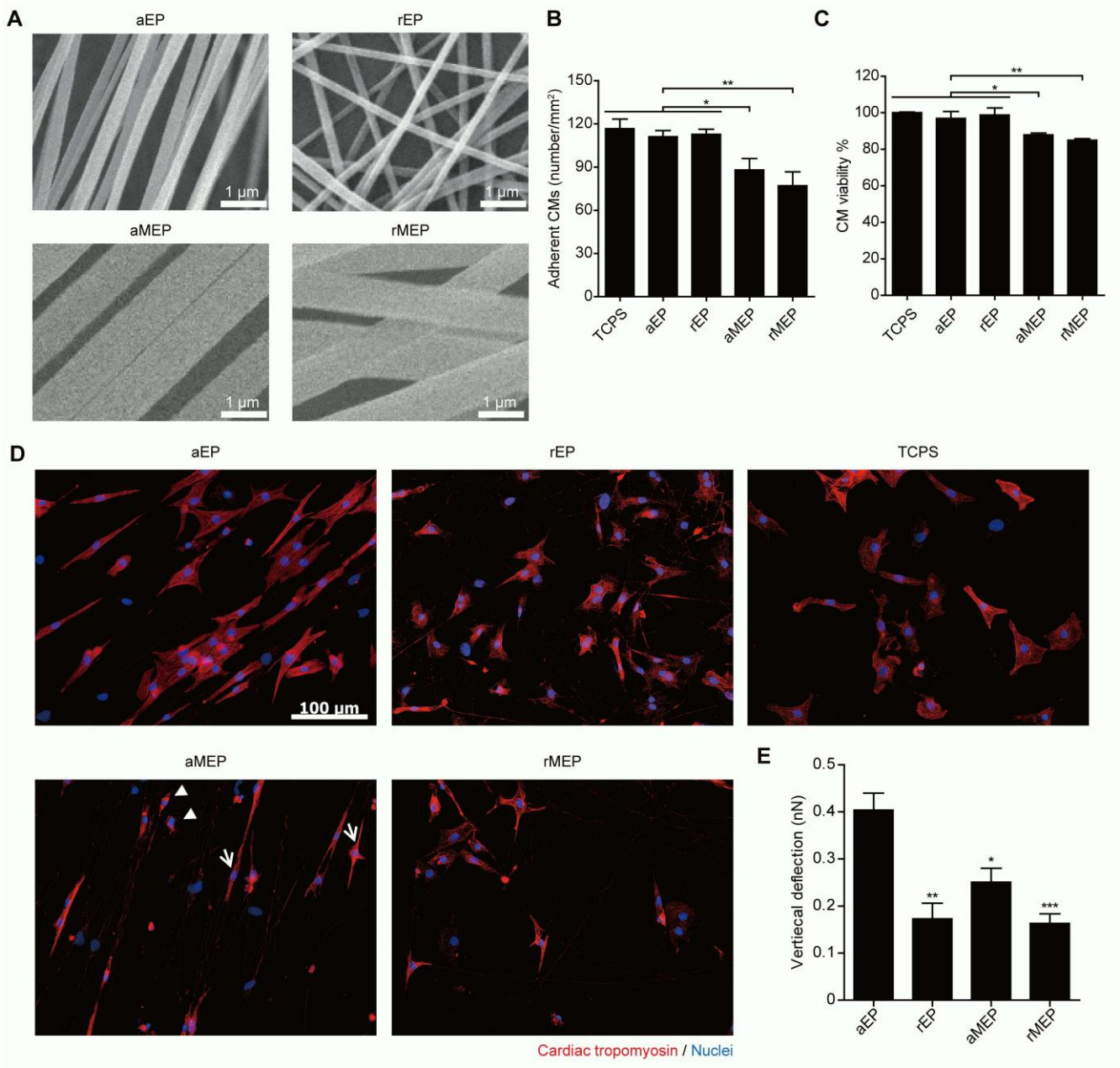
1. C. H. Chen, S. S. Wang, E. I. H. Wei, T. Y. Chu and P. C. H. Hsieh, *Mol. Ther.*, 2013, **21**, 670-679.
2. M. Y. Chang, Y. J. Yang, C. H. Chang, A. C. L. Tang, W. Y. Liao, F. Y. Cheng, C. S. Yeh, J. J. Lai, P. S. Stayton and P. C. H. Hsieh, *J. Controlled Release*, 2013, **170**, 287-294.



Supplemental Fig. 1. The anisotropic or unpatterned nanofibrous electrospun patches are mechanically identical. (A) Stress-strain curves and **(B)** mechanical properties of aligned or unaligned EPs. The data are presented as the mean ± SEM (*n* = 4 per group). *P < 0.05.

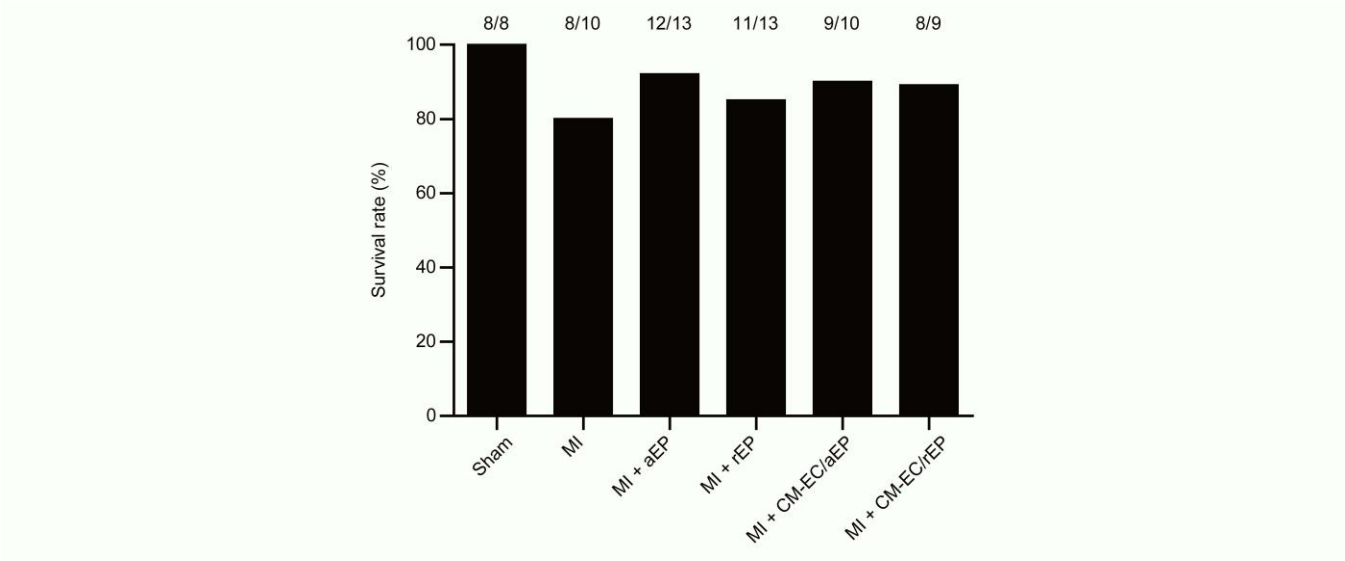


Supplemental Fig. 2. Endothelial cells align in an orderly manner when cultured on an aligned electrospun patch. (A) Cell adhesion of ECs cultured under various conditions. The data are presented as the mean ± SEM (*n* = 3–4 per group). **(B)** The cell viability of ECs cultured on various substrates. The data are presented as the mean ± SEM (*n* = 6–9 per group). **(C)** Representative H&E staining images of ECs 3 days after culturing on aEP or rEP. **(D)** The angle distribution of ECs on various substrates. **(E)** The ratio of ±10° alignment for each group. The data are presented as the mean ± SEM (*n* = 3–4 per group). ns, not significant; $^{**}P < 0.01$.



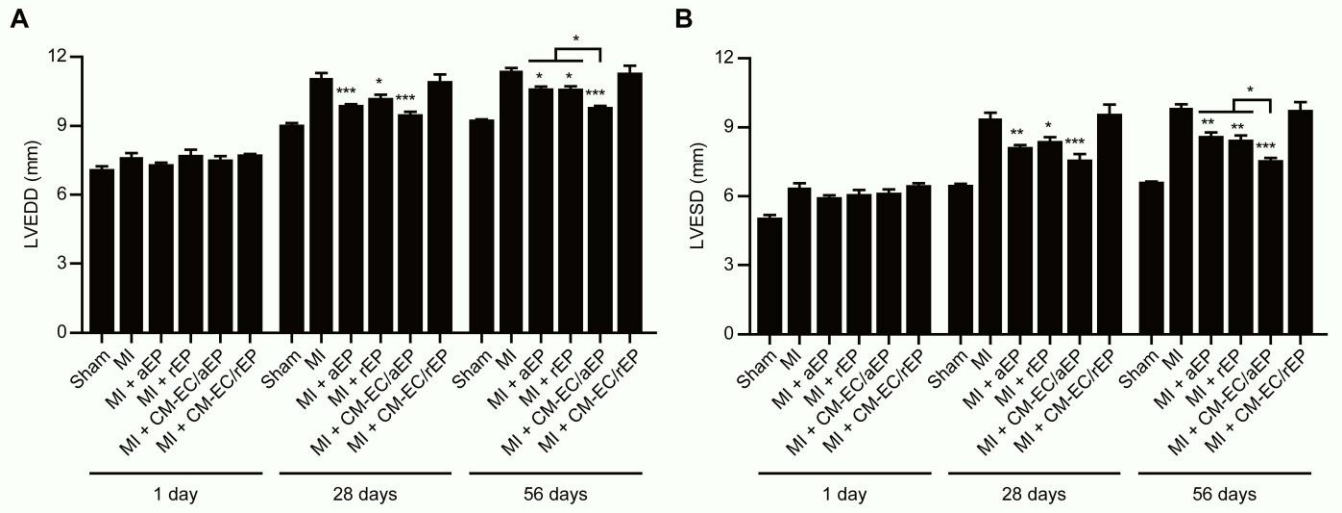
Supplemental Fig. 3. Cardiomyocytes showed worse cell-adhesive tendency and non-toxicity, abnormal cell morphology, and lower beating force on anisotropic or unpatterned microfibrous electrospun patches than on anisotropic nanofibrous electrospun patches. (A) Representative SEM images of aligned and randomly oriented electrospun patches (aEP, rEP, aMEP and rMEP, respectively). **(B)** Cell adhesion of CMs cultured under various conditions. **(C)** The cell viability of CMs cultured on various substrates.

(D) Representative immunostaining images of CMs cultured alone on various substrates for 3 days. Abnormally elongated or round shaped CMs are indicated by arrows or arrowheads. **(E)** Beating vertical deflection detected with atomic force microscopy. The data are presented as the mean ± SEM (n = 5–10 per group). ns, not significant; *P < 0.05, **P < 0.01, ***P < 0.001.

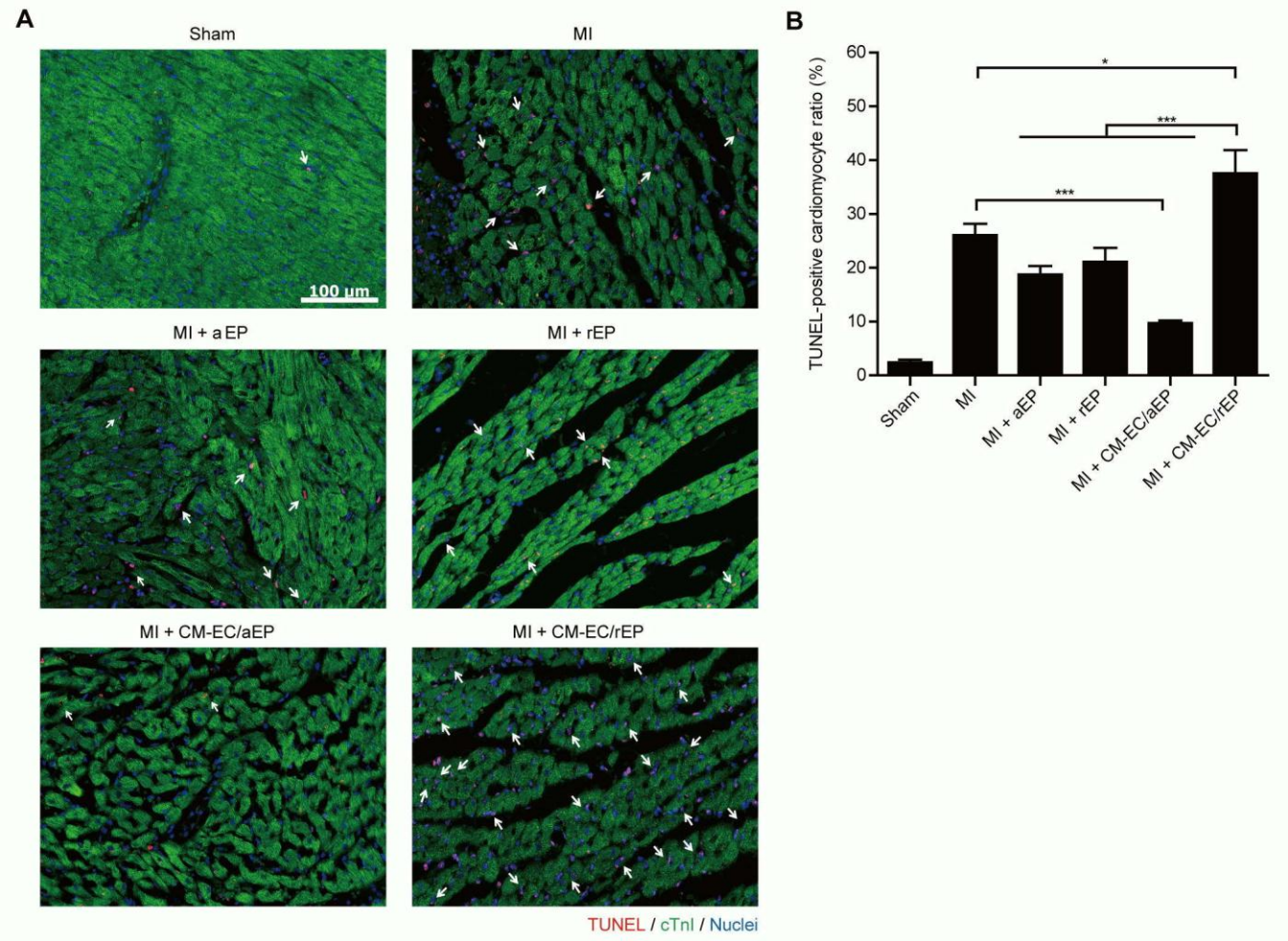


Supplemental Fig. 4. The mortality rate is not evident after surgical patch implantation.

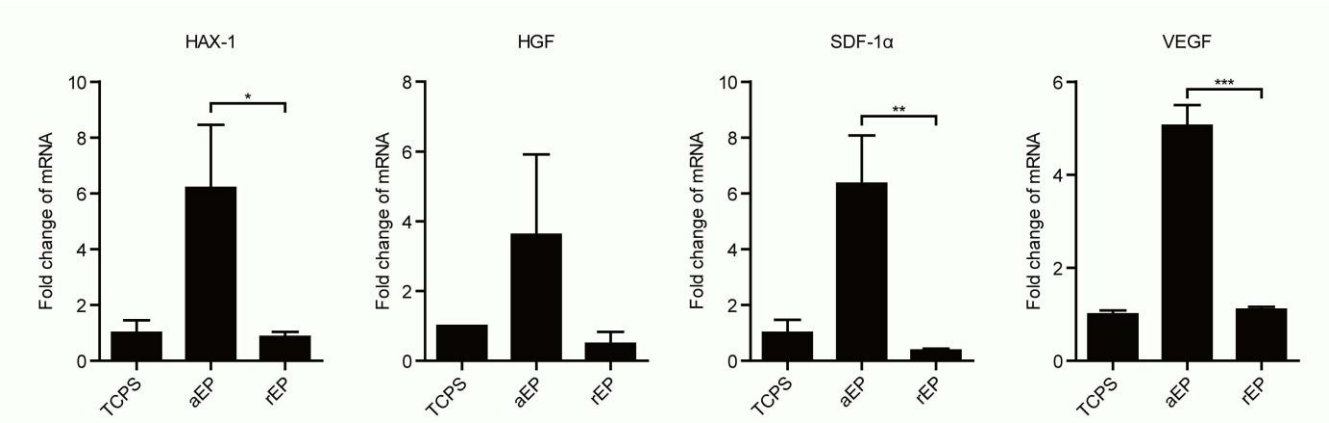
The survival rate of rats undergoing various treatments at 2 months post-MI is shown.



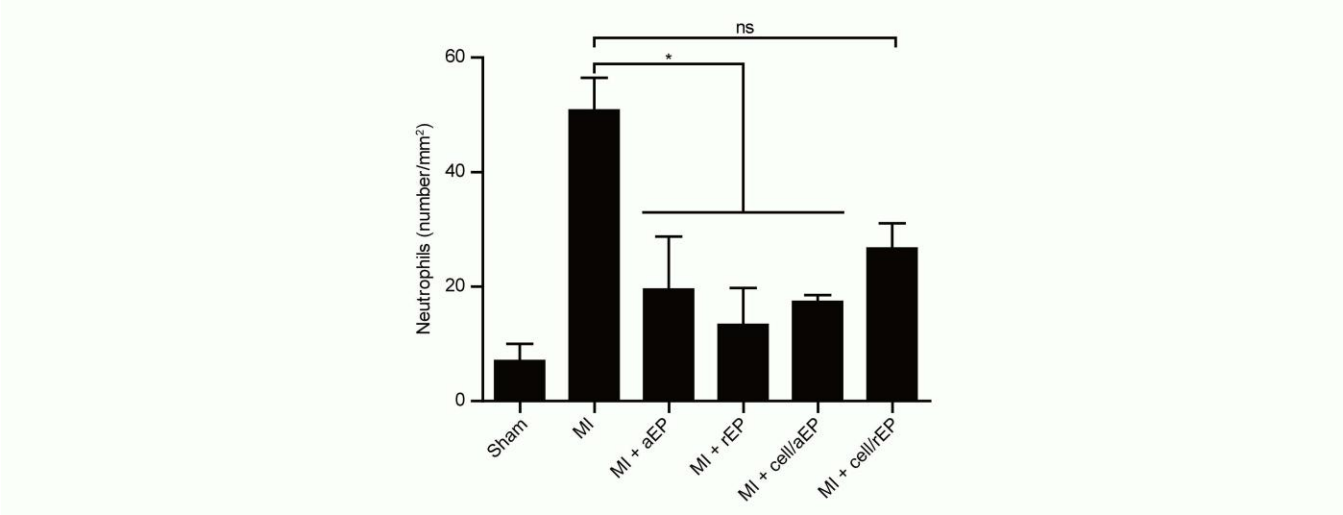
Supplemental Fig. 5. Transplantation of cells with the aligned electrospun patch retards cardiac dilatation after infarction. The left ventricular dimension was examined at 1 day, 1 month and 2 months after MI using echocardiography. **(A)** The LVEDD and **(B)** LVESD were quantified. The data are presented as the mean ± SEM (*n* = 9–13 per group). **P* < 0.05, ***P* < 0.01, ****P* < 0.001, compared with MI unless otherwise indicated in brackets.



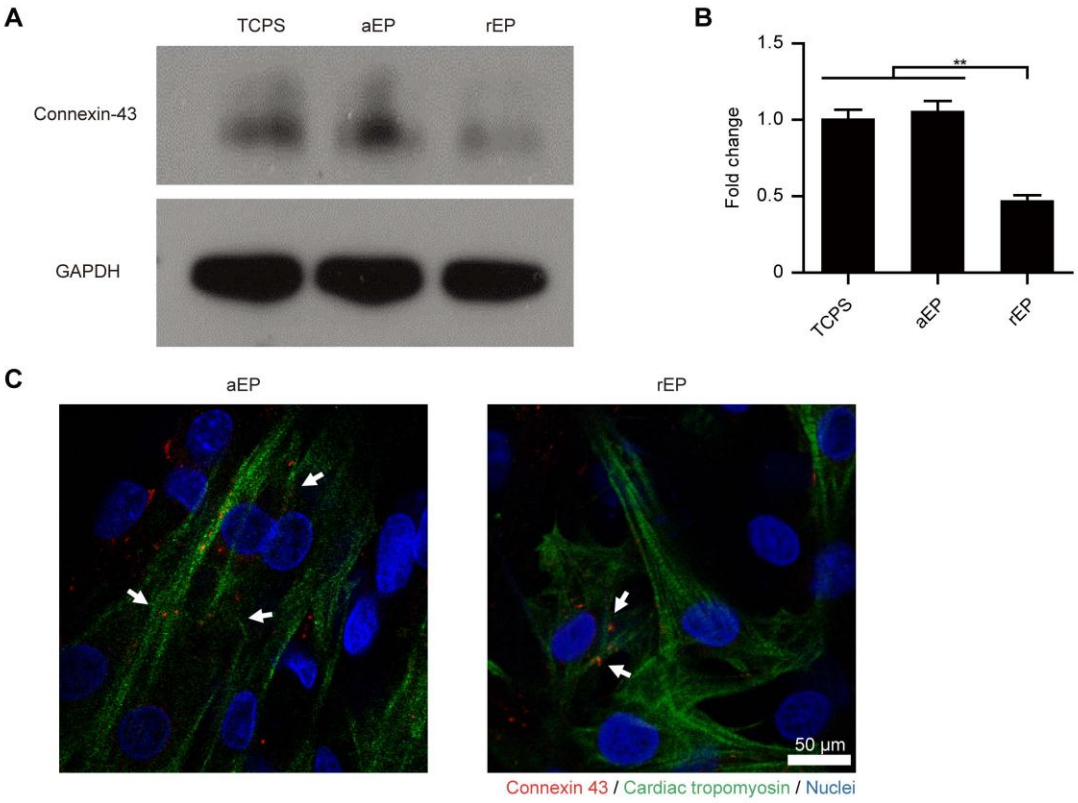
Supplemental Fig. 6. Transplantation of cells with an aligned electrospun patch protects the host cardiomyocytes from apoptosis at 1 day after infarction. (A) Representative immunostaining images of cardiomyocytes (cTnI; green) and nuclei (DAPI; blue) overlapped with TUNEL (terminal deoxynucleotidyl transferase dUTP nick-end labeling) staining (red) at the peri-infarct region in each group. $TUNEL^+$ CMs are indicated by arrows. **(B)** Quantification of $TUNEL^+$ CMs in each group. The data are presented as the mean ± SEM (n = 4–6 per group). *P < 0.05, ***P < 0.001.



Supplemental Fig. 7. Higher gene expression of beneficial factors on cardiomyocytes seeded on aEP than in control. The data are presented as the mean ± SEM (*n* = 3 per group). **P* < 0.05, ***P* < 0.01, ****P* < 0.001.



Supplemental Fig. 8. Cardiac patch implantation does not increase leukocyte infiltration at the implanted regions of the infarcted myocardium at 1 day after infarction. The level of leukocyte infiltration in each group. The data are presented as the mean ± SEM (*n* = 3–4 per group). ns, not significant; **P* < 0.05.



Supplemental Fig. 9. Higher expression of connexin 43 on cardiomyocytes seeded on aEP than in control. (A) Representative images of connexin 43 expression on cardiomyocytes seeded on various substrates. **(B)** Quantification of connexin 43 expression in each group. The data are presented as the mean ± SEM (*n* = 3 per group). ***P* < 0.01. **(C)** Immunostaining of CMs (cTnI; green) and nuclei (DAPI; blue) overlapped with connexin 43 (red) cultured on aEP or rEP for 3 days. Connexin 43 expression is indicated by arrows.