Multiscale Anisotropic Scaffolds Enable a Biomimetic Electro-Mechanical Myocardial Platform for Drug Discovery and Heart Repair

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This file includes: Supplementary Table 1 to 3; Supplementary Fig. S1 to 8.

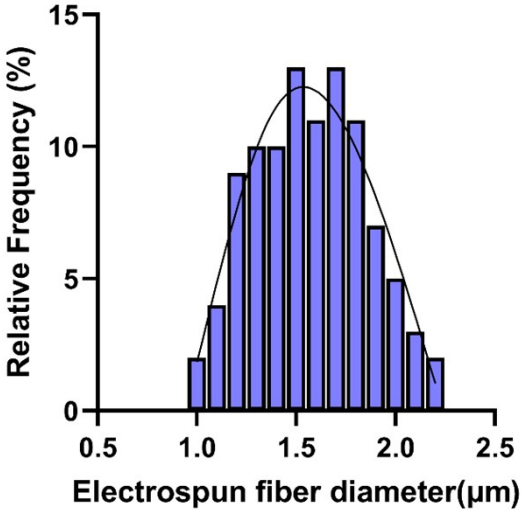

Fig. S1. Fiber diameter distribution of the electrospun scaffold.
Histogram showing the fiber diameter distribution measured from SEM images of the electrospun scaffold (n = 100 fibers).

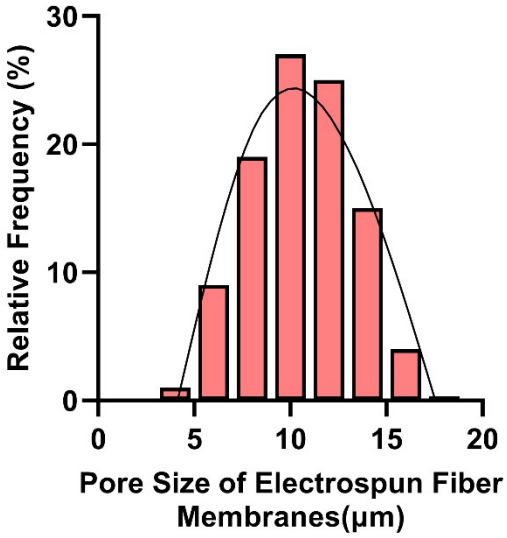

Fig. S2. Pore size distribution of the electrospun scaffold.
Histogram showing the pore size distribution measured from SEM images of the electrospun scaffold (n = 100 pores).

Table S1. Structural characterization of GP-D-AM scaffolds.

Parameter	Value (Mean ± SD)
Fiber Diameter	1.58±0.32μm
Pore Size	10.42±2.65μm
Porosity	81.0±1.5%
Microgroove Width	628.3±9.4μm
Microgroove Depth	86.6±3.4μm
Scaffold Thickness	224.8 ± 9.5μm

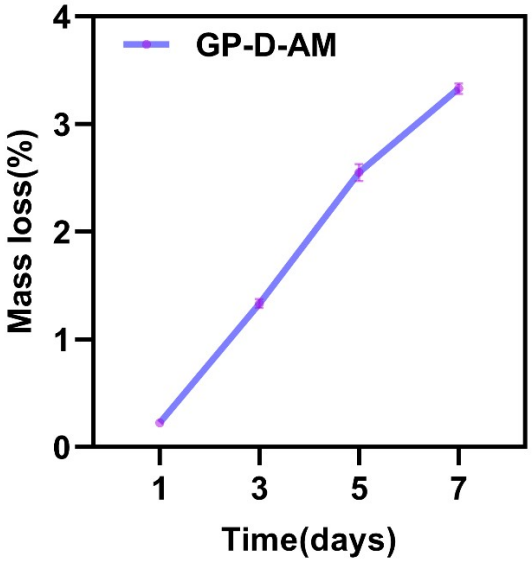

Fig. S3. In vitro degradation behavior of GP-D-AM scaffolds.
Mass loss percentage of GP-D-AM scaffolds after incubation in complete culture medium for 1, 3, 5, and 7 days. The scaffolds exhibited a gradual and relatively slow degradation profile during the 7-day culture period. Data are presented as mean ± SD (n = 3).

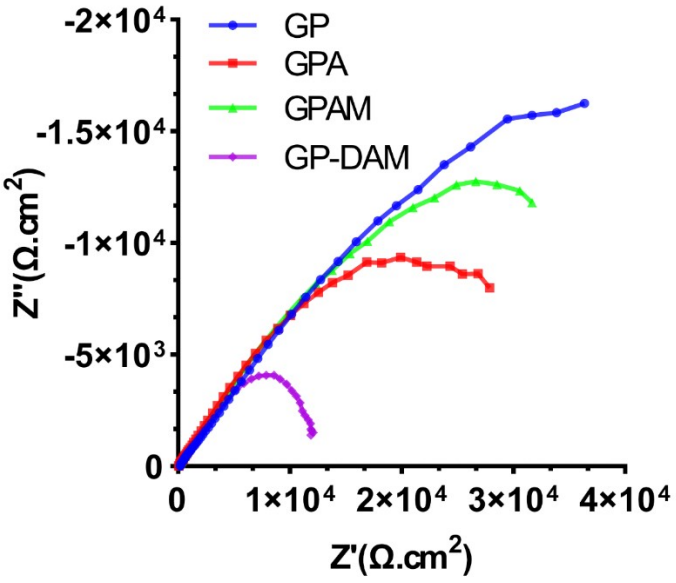

Fig. S4. Electrical conductivity characterization of GP, GPA, GPAM, and GP-D-AM scaffolds.
Resistivity curves of the different scaffolds. GP, GPA, GPAM, and GP-D-AM represent scaffolds composed of gelatin (G) and poly(ε-caprolactone) (PCL), with anisotropic structure (A), microstructured pattern (M), and dual modification with polypyrrole and polydopamine (D).

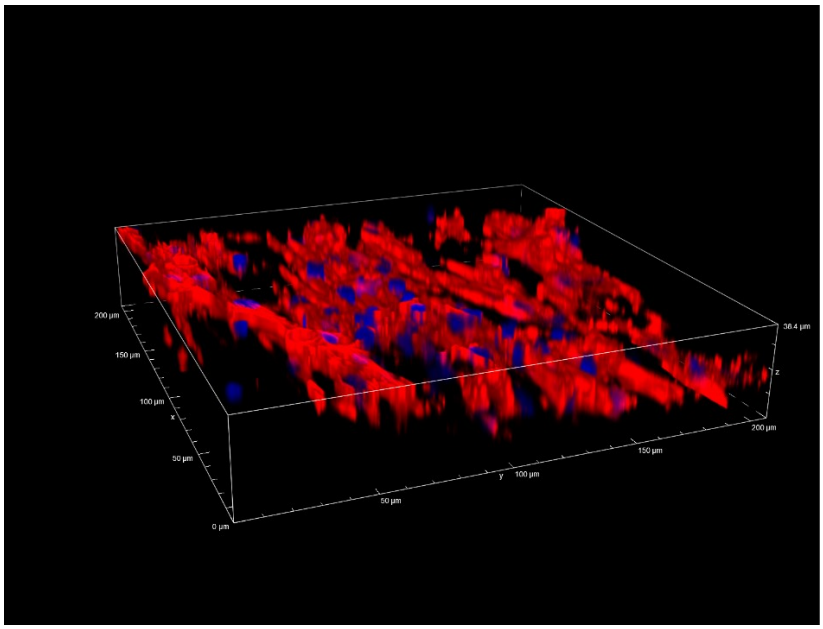

Fig. S5. Confocal Z-stack images showing cardiomyocyte infiltration within the engineered GP-D-AM cardiac patches.

Cardiomyocytes were stained with phalloidin to visualize the cytoskeleton. Z-stack reconstruction revealed multilayer cell distribution within the scaffold, with an observed cellular stacking depth of 32.7 ± 5.0µm (n = 3), indicating partial cell infiltration into the porous fibrous network.

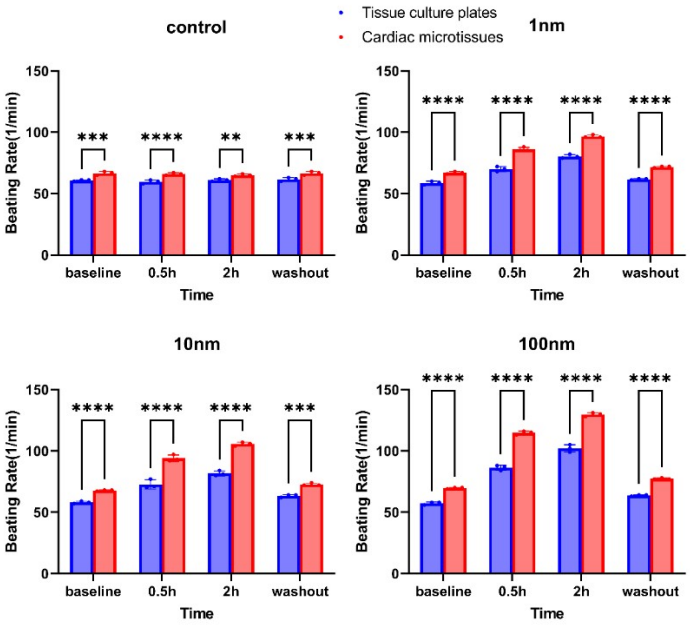

Fig. S6. Beating frequency analysis following isoproterenol stimulation.
Beating frequency of cardiomyocytes cultured in tissue culture plates and engineered cardiac microtissues after 7 days of culture following the addition of different concentrations of isoproterenol. Contractile activity was analyzed using optical flow analysis.

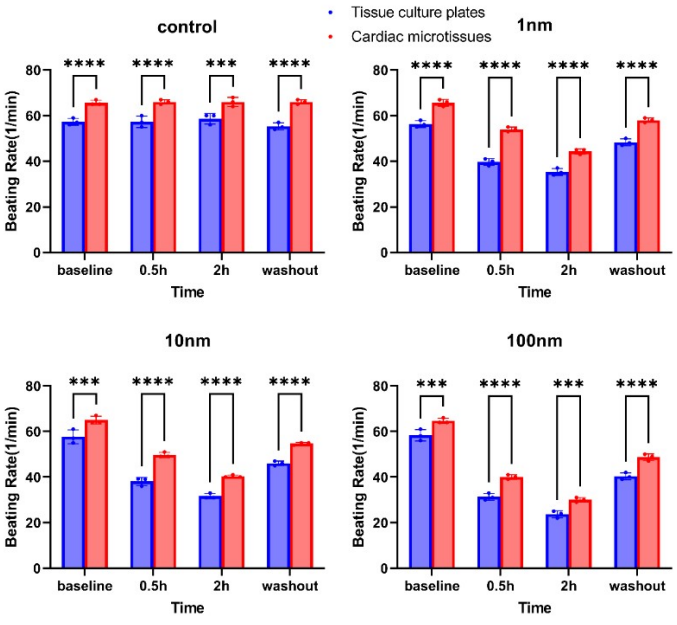

Fig. S7. Beating frequency analysis following verapamil stimulation.
Beating frequency of cardiomyocytes cultured in tissue culture plates and engineered cardiac microtissues after 7 days of culture following the addition of different concentrations of verapamil. Contractile activity was analyzed using optical flow analysis.

Table S2. Response of cardiac microtissues to different concentrations of isoproterenol.

Group	Beating Rate(1/min)	Movement(pixel/frame)
Control	65 ± 2.16	13.53 ± 0.60
1nM	75.67 ± 2.62	15.94 ± 0.43
10nM	98 ± 2.83	17.77 ± 0.33
100nM	132.67 ± 2.83	21.17 ± 0.54

Table S3. Response of cardiac microtissues to different concentrations of verapamil.

Group	Beating Rate(1/min)	Movement(pixel/frame)
Control	64 ± 2.83	13.53 ± 0.60
1nM	42 ± 1.63	8.46 ± 0.19
10nM	30.67 ± 1.70	7.10 ± 0.51
100nM	24.33 ± 1.25	5.87 ± 0.30

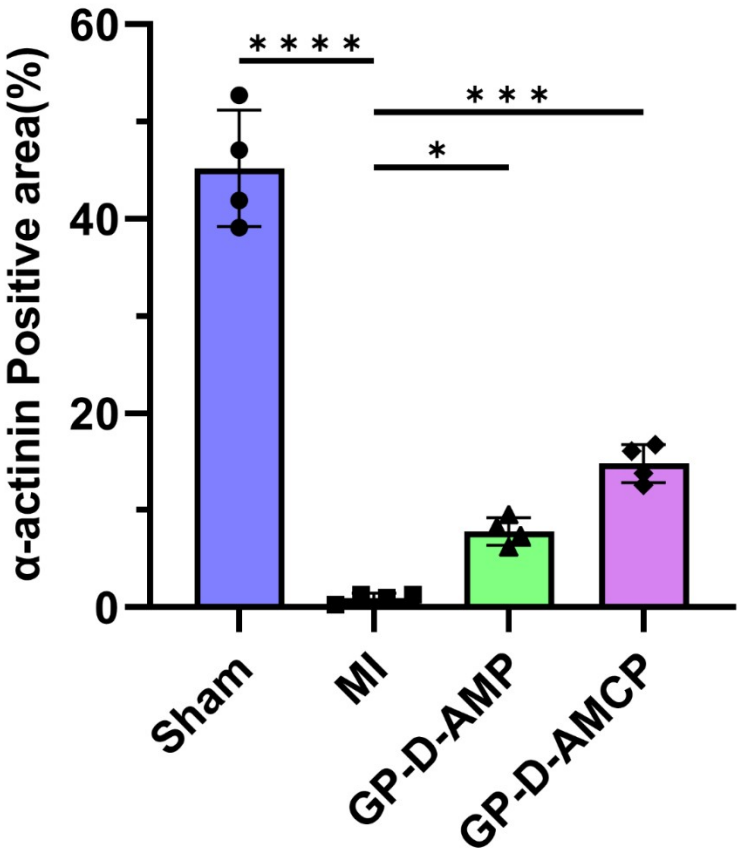

Fig. S8. Quantitative analysis of α-actin–positive areas in myocardial tissue sections. Quantification of α-actin–positive areas in myocardial tissue sections from different experimental groups at 4 weeks after patch transplantation, indicating differences in myocardial regeneration among groups.