Electronic Supplementary Material (ESI) for Lab on a Chip.

Enhanced Cardiomyocyte Structural and Functional Anisotropy through Synergetic Combination of Topographical, Conductive, and Mechanical Stimulation

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Cell culture and neonatal rat ventricular myocytes (NRVM isolation)

All animal experiments were performed following protocols approved by the Animal Ethics Committee of Chonnam National University in accordance with the Principles of Laboratory Animal Care and specific national laws (license number: CNU IACUC-YB-2022-29). Isolation of neonatal rat ventricular myocytes(NRVM) was performed as previously described. Ventricular myocytes were harvested from 1-day-old Sprague-Dawley rats and dissociated into single cells by incubating cardiac tissue with enzyme solution (Collagenase 0.4 mg mL^{-1}, Northington, Pancreatin 0.6 mg mL^{-1}, Sigma-Aldrich) for 2 hours at 37 °C with shaking. The single-cell suspension was separated into fibroblast and cardiomyocyte layers by centrifugation with Percol solution. The cardiomyocyte layer was pre-plated to obtain highly pure cardiomyocytes. The culture substrate was coated with fibronectin (50 mg ml^{-1}, Corning) for 1 hour at 37 °C to increase the attachment of cardiomyocytes. Cardiomyocytes were cultured at a density of 800/mm^2 and the culture medium was replaced every 3 days in a stage-top incubator. Contraction of cardiomyocytes started 24 hours after culturing and synchronization occurred after 48 hours. Cardiomyocytes were grown in a CO_2 incubator for the first 3 days after culturing, and then placed in a stage-top incubator for mechanical stimulation from day 4 to day 10. Figure S3 shows a prepared PDMS well plate with and without AgNW-embedded samples. The PDMS was made hydrophilic by O_2 plasma asher treatment, and ECM coating was performed to promote cardiomyocyte attachment.

Immunocytochemical staining analysis

Fluorescent staining was performed to evaluate the maturity of cardiomyocytes. The cultured cardiomyocytes were treated with paraformaldehyde (3.7%, Sigma-Aldrich) and Triton X (0.1%, Sigma-Aldrich) at room temperature for 10 minutes, followed by washing with phosphate-buffered saline(PBS, Takara). They were then treated with 3% bovine serum albumin(BSA, Sigma-Aldrich) at room temperature for 40 minutes. Primary antibodies(monoclonal anti α-sarcomere actinin and connexin43) were cultured with 1% BSA solution at room temperature for 120 minutes. Secondary antibodies(Alexa-Flour 488, 568 goat anti-mouse IgG modify) were cultured with 1% BSA solution at room temperature for 90 minutes. Finally, DAPI(4',6-Diamidino-2-phenylindole) solution was added for nuclear staining and cultured at 37 °C for 15 minutes. Stained images were measured using a confocal microscope, and the alignment of cardiomyocytes under mechanical stimulation and the expression of connexin43 protein after AgNW treatment, a conductive material, were analyzed.

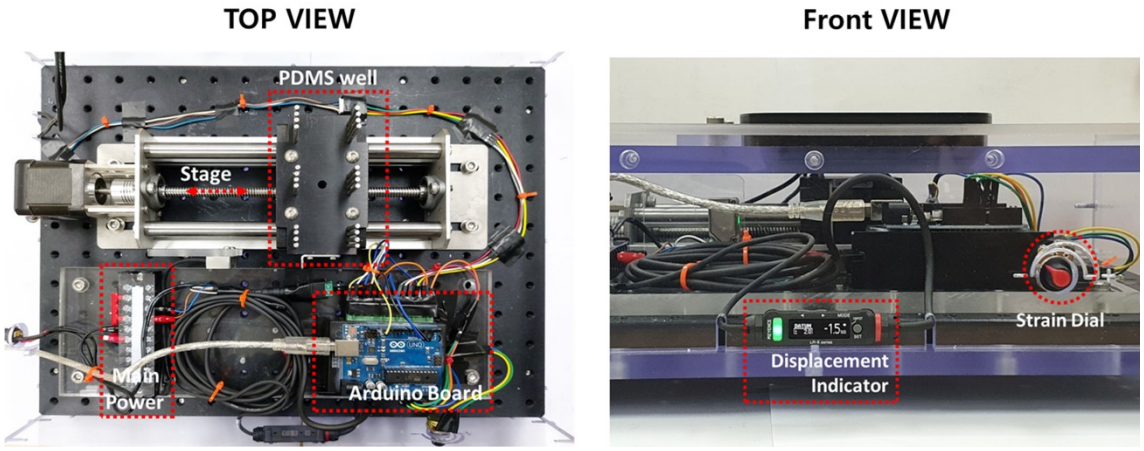

Fig. S1. Photographs show the top and front view of the proposed mechanical stimulation system.

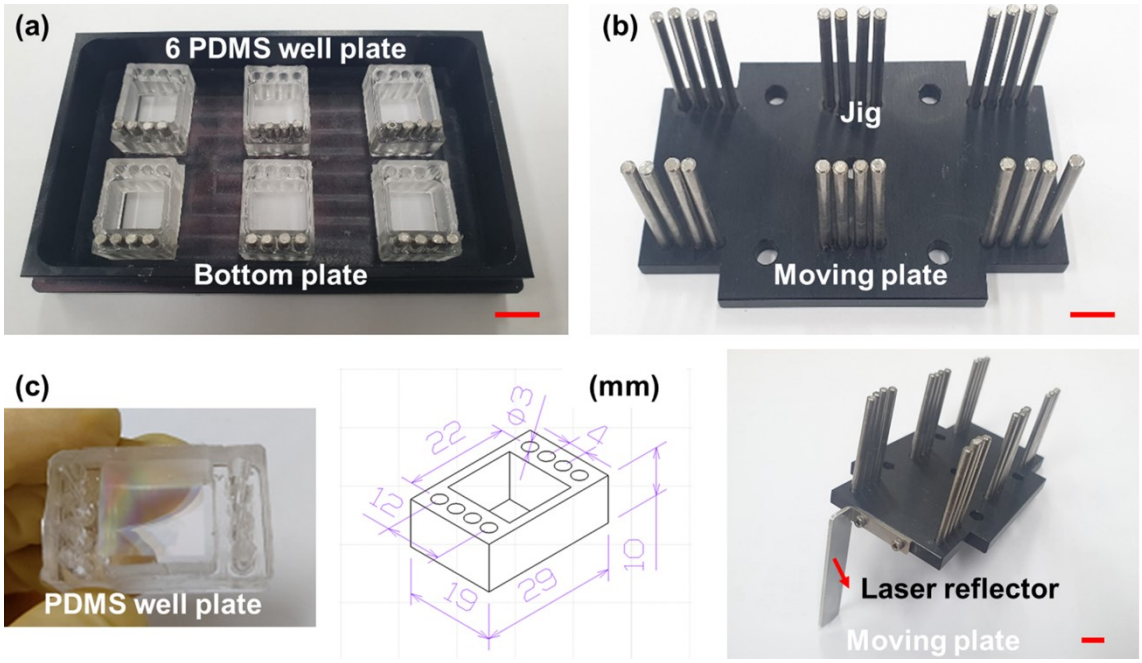

Fig. S2. Photographs show the various parts of the mechanical stimulation system such as (a) PDMS well plate, (b) moving plate, (c) PDMS well plate and laser reflecting sensor in the moving plate. (Scale bar: 1 mm)

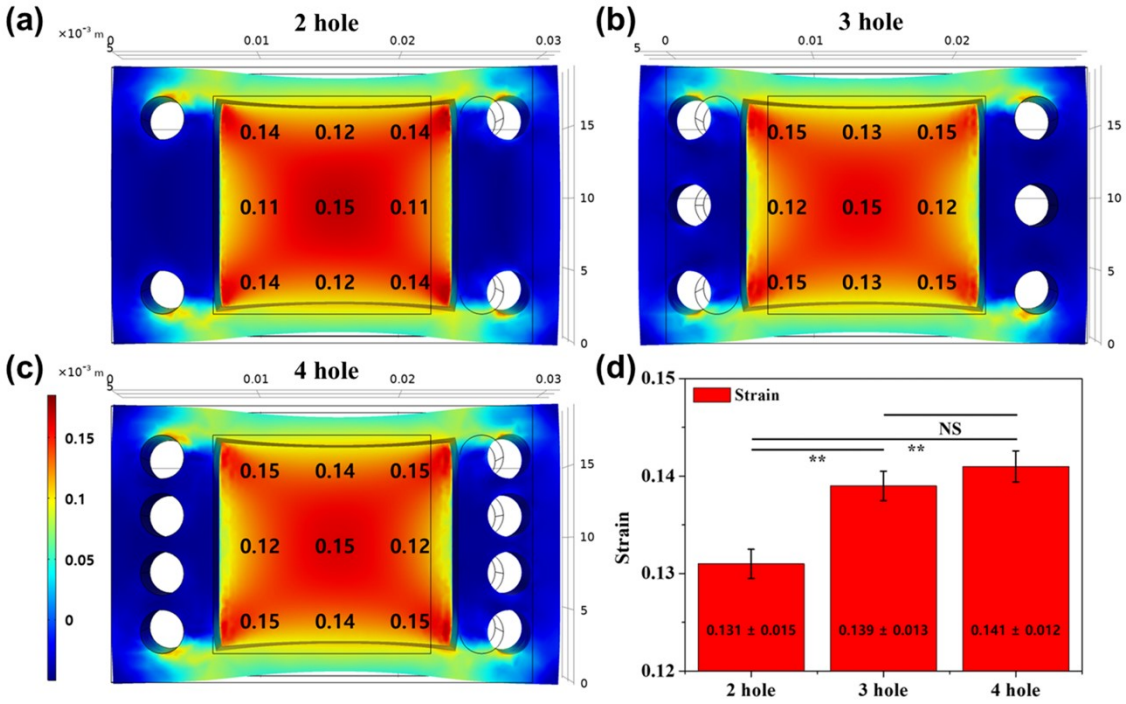

Fig. S3. (a-c) Finite Element Method (FEM) simulation analysis of the proposed PDMS membrane with 15 mm in length x 15 mm in width x 100 μm in thickness, employed to evaluate stress distribution across the membrane. (d) Bar plot depicting strain distribution on the PDMS membrane according to number of jigs. Data represented as mean ± standard deviation (n = 6). The bars and error bars indicate the mean ± standard deviation (n = 6). *P < 0.05, **P < 0.01.

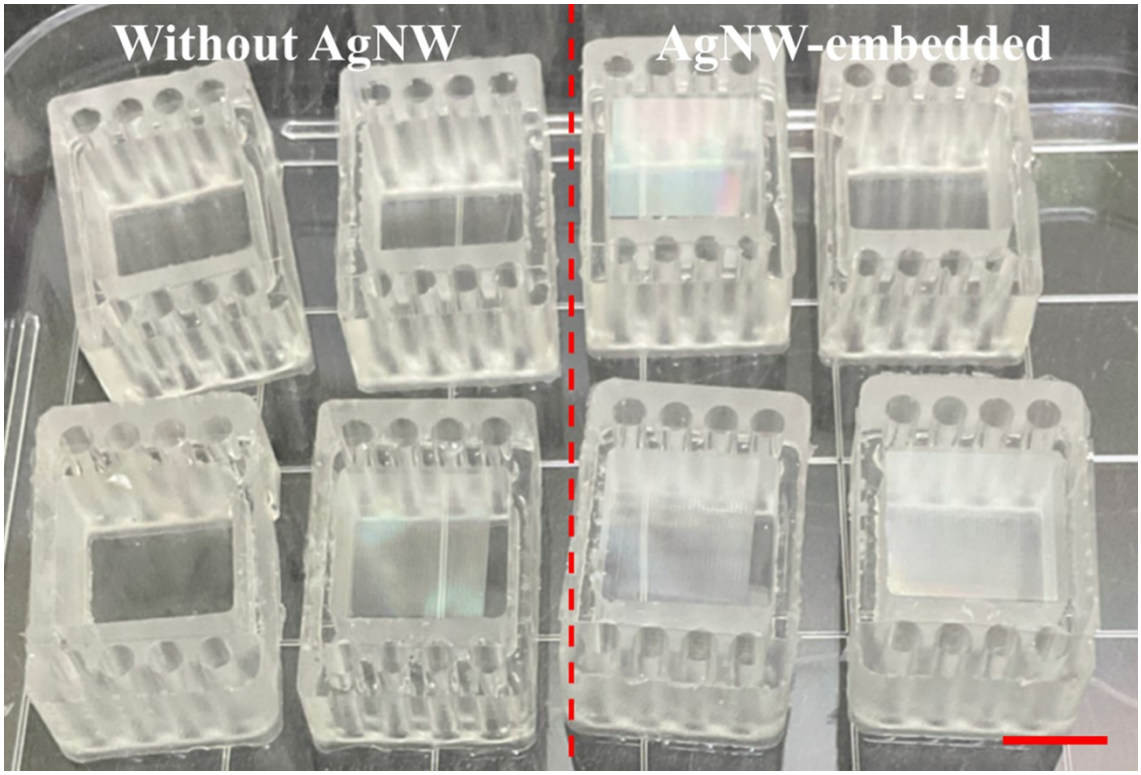

Fig. S4. Photograph shows the fabricated PDMS functional well plate, presented in two variations: one without and another with AgNWs embedded within the microgrooved PDMS membrane (Scale bar: 1 mm).

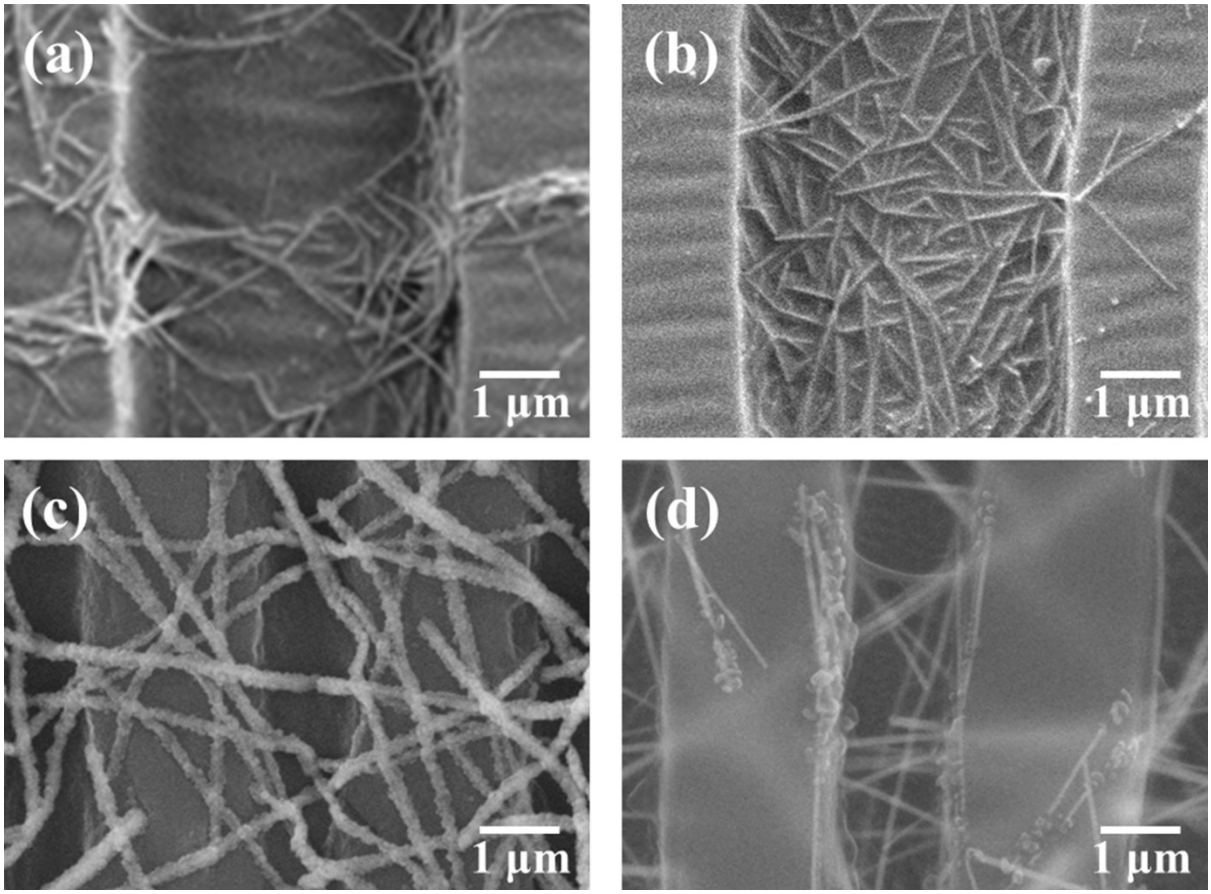

Fig. S5. FE-SEM analysis of the AgNWs-S-PDMS and AgNWs-E-PDMS membrane before (a, b) and after (c, d) exposed to oxygen plasma treatment at 100 W for 1 min.

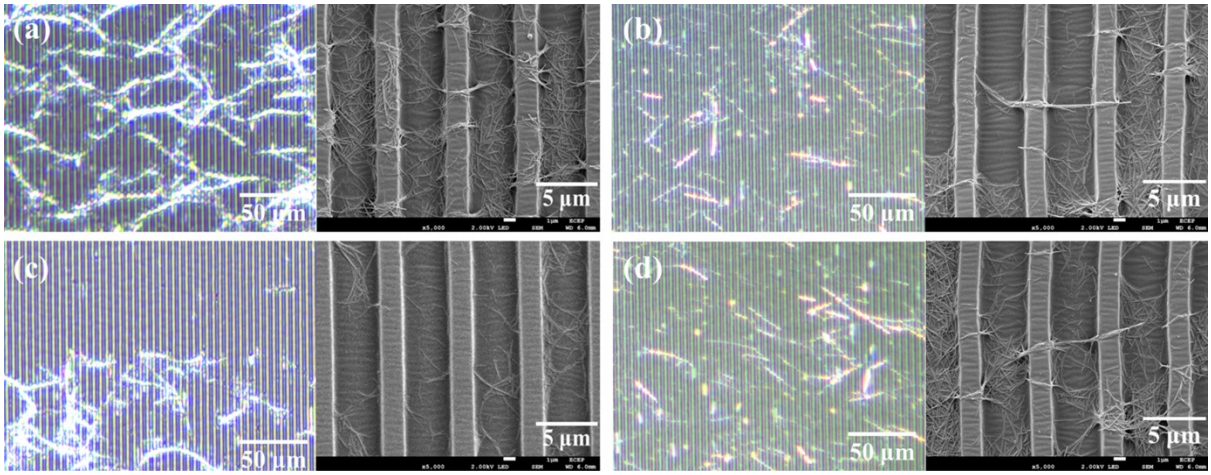

Fig. S6. Optical and FE-SEM analysis of the AgNWs-S-PDMS and AgNWs-E-PDMS membrane before (a, b) and after (c, d) peeling test using 3 M adhesive tape.

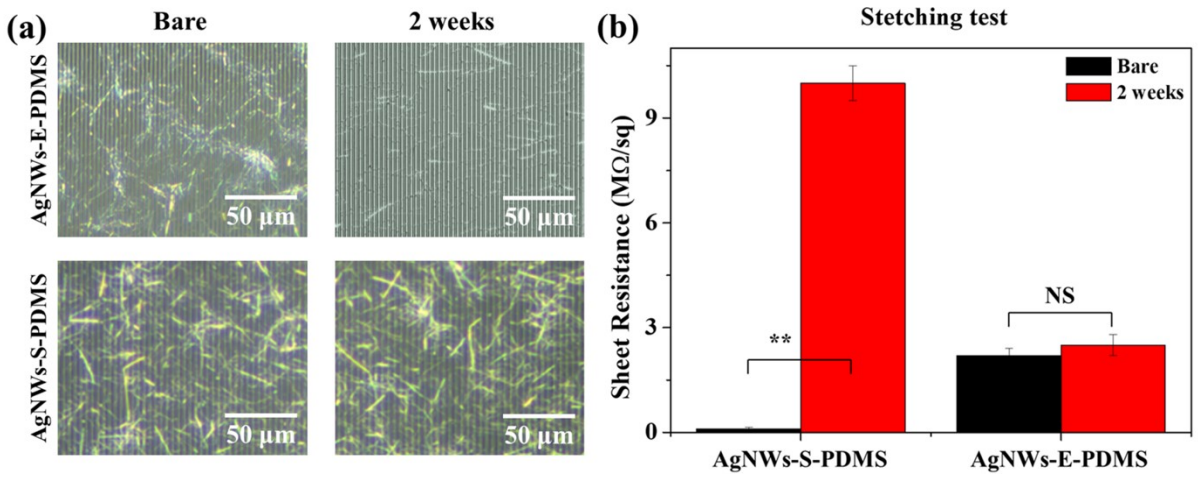

Fig. S7. Structural integrity and sheet resistance of the AgNWs-S-PDMS and AgNWs-E-PDMS membranes before and after sustained cyclic mechanical stretching at 1 Hz for 2 weeks. The bars and error bars indicate the mean ± standard deviation (n = 6). NS (Non-significant), *P < 0.05, **P < 0.01.

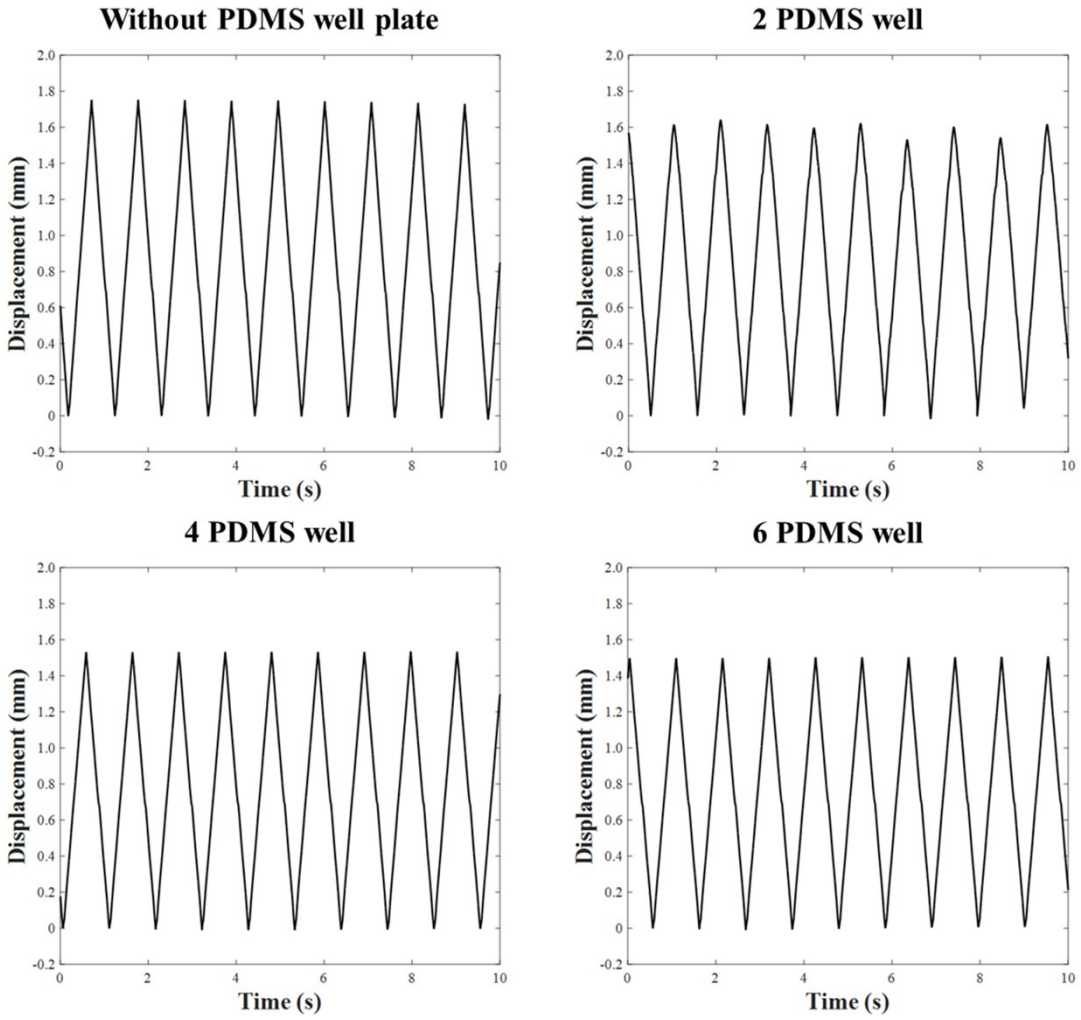

Fig. S8. Real-time traces illustrating the displacement variations of the moving plate, corresponding to the number of PDMS well plates, under a 10% applied tensile stress with 1 Hz mechanical stimulation.

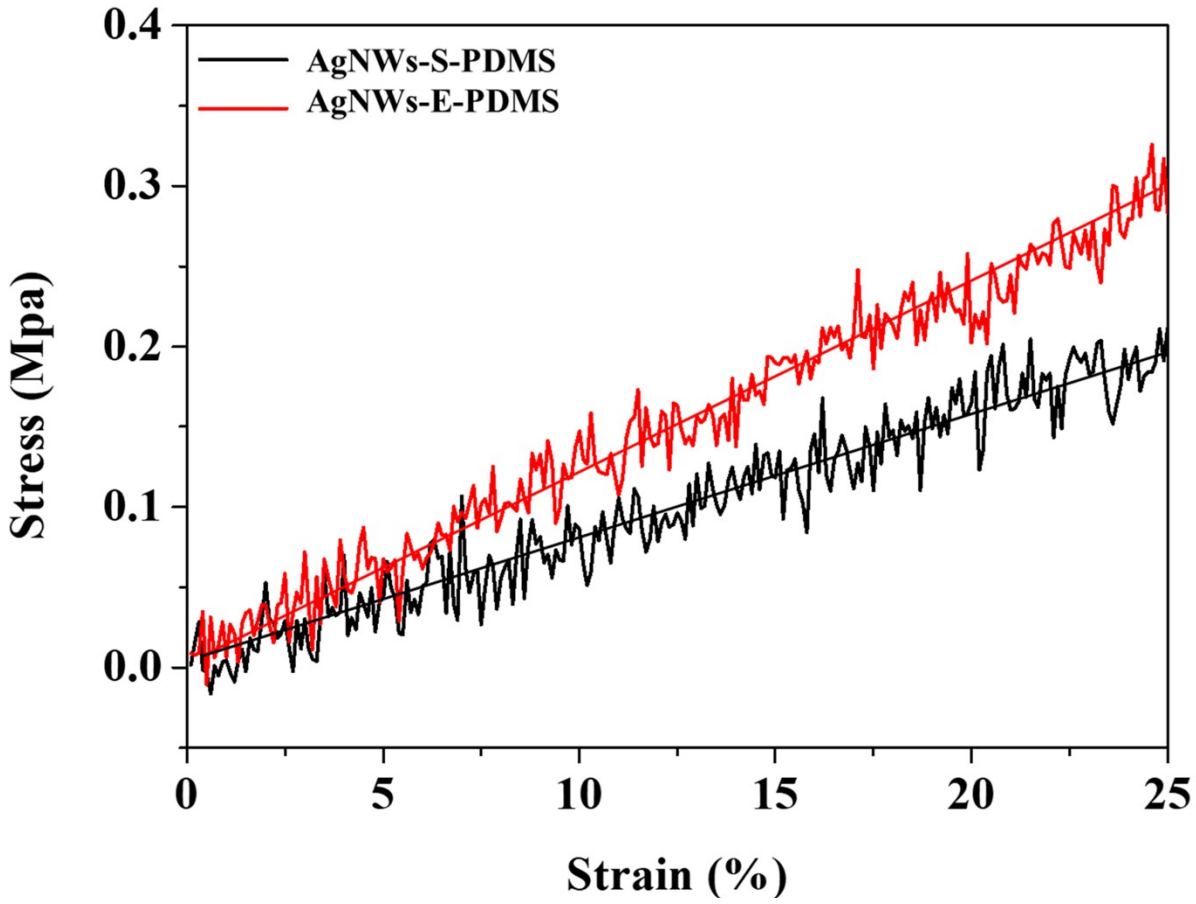

Fig. S9. Stress-strain profile demonstrate the tensile strength of the AgNWs-S-PDMS and AgNWs-E-PDMS membranes.

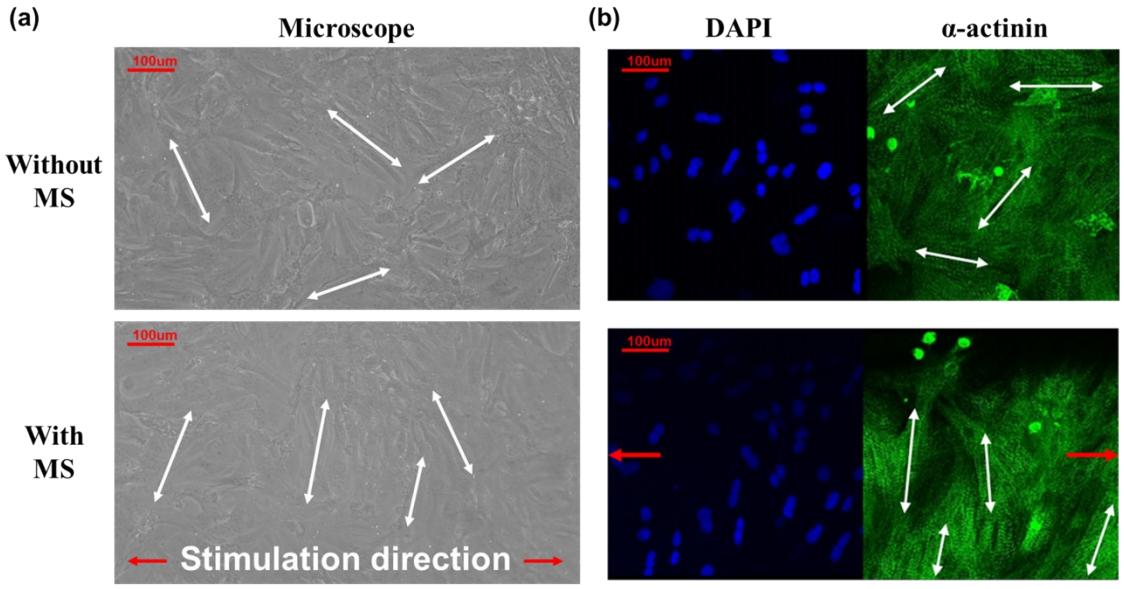

Fig. S10. Effect of mechanical stimulation on the Analysis of cardiomyocyte characteristics under mechanical stimulation; (a) Microscopic images of day 10 cardiomyocytes under mechanical stimulation, (b) Fluorescent staining images. (DAPI: blue, α-actinin: green)

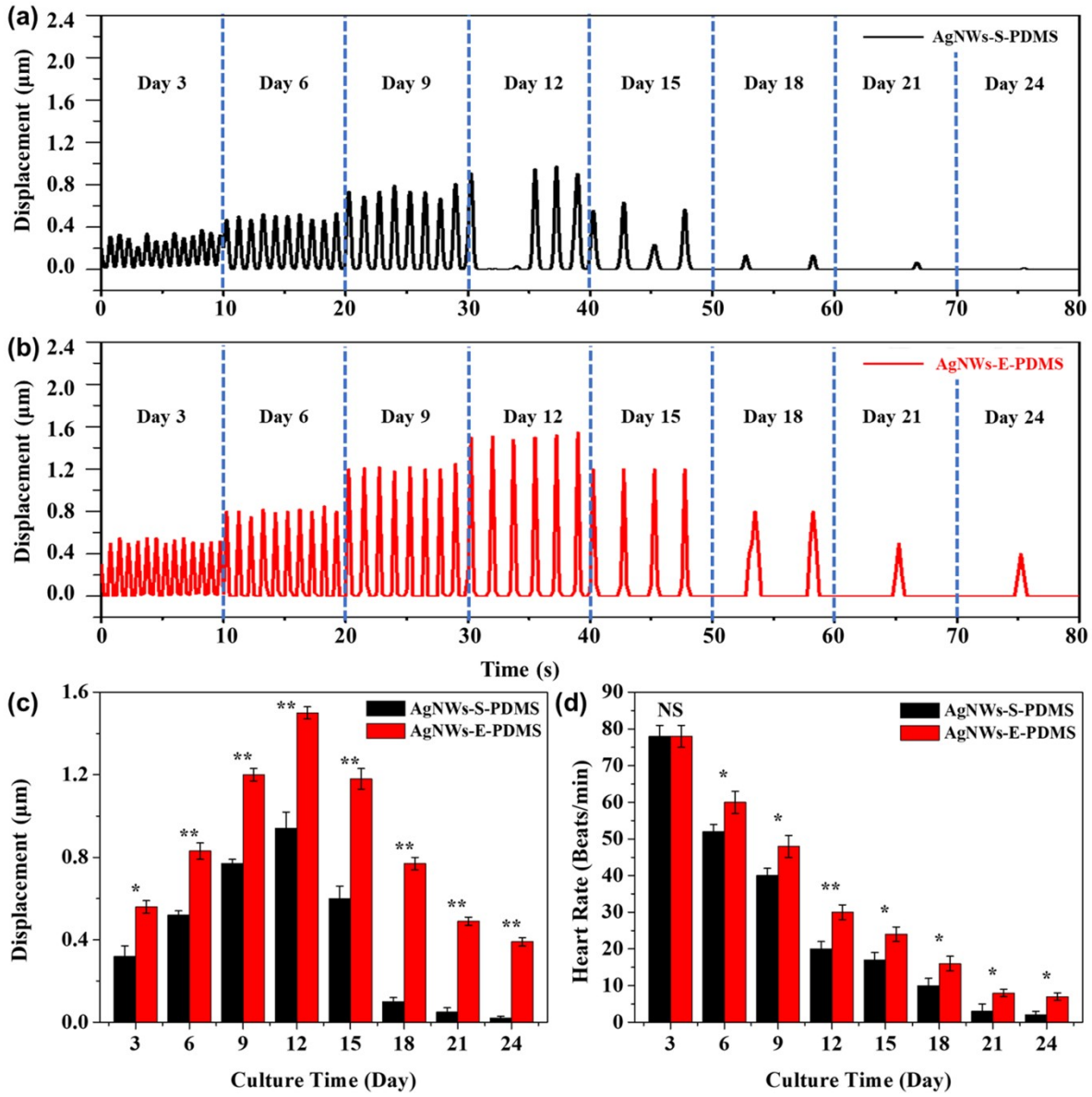

Fig. S11. (a, b) Real-time traces shows the contractility of the cardiomyocytes cultured on the AgNWs-S-PDMS and AgNWs-E-PDMS membrane on different culture period. (c, d) Bar plots show the normalized contraction force of the and heart rate of the cardiomyocytes cultured on AgNWs-S-PDMS and AgNWs-E-PDMS membrane according to the culture period. The bars and error bars indicate the mean ± standard deviation (n = 6). NS (Non-significant), *P < 0.05, **P < 0.01.