Supporting Information

Elastomeric Conductive Hybrid Hydrogels with Continuous Conductive Networks

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EXPERIMENTS SECTION

Materials and reagents. Gelatin, methacrylic anhydride (94%), acrylamide, pyrrole (Py) (99%), DA, and ammonium persulfate were purchased from Aladdin (Shanghai, China). Actin-Tracker Green and a CCK-8 kit were purchased from Beyotime (Shanghai,

China). 4',6-Diamidino-2-phenylindole (DAPI, Sigma), calcein (AM), and propidium iodide (PI) were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Mesenchymal stem cells (MC3T3-E1) were purchased from American Type Culture Collection. All other chemicals were of analytical grade and were used without further purification.

Preparation of GelMA-PAM biohybrid hydrogels. The synthesis of GelMA was reported previously.[1] GelMA (5% w/v) and Irgacure 2959 (1% w/v) were dissolved in phosphate-buffered saline (PBS) and maintained at 60 °C for approximately 30 min. Acrylamide monomers, with the same weight as that of GelMA, were dissolved in the solution at room temperature. Next, 160 μL of a precursor solution were placed dropwise into a PDMS mold. Then, the GelMA-PAM biohybrid hydrogel (GP hydrogel) was prepared by irradiation with UV light (700 mW/cm^2) for 90 s. The samples were stored in PBS for 24 h to remove unreacted photoinitiators and monomers.

Preparation of the conductive hydrogels. The GelMA-PAM hydrogel samples were immersed in 10 mL of a 0.01 M HCl solution containing 0.1 M (0.5 M and 1.0 M) pyrrole monomer and 0.03 g (0.15 g and 0.3 g DA, respectively) DA for 12 h. Then, 10 mL of deionized water containing 0.228 g ammonium persulfate (APS) (APS: Py=1: 1) was slowly added dropwise to the above solution, and the solution was stirred for 4 h at 4 °C. The mixture was kept at 4 °C for 12 h. Dark black 0.1 M (0.5 M and 1.0 M) DA-PPy-GP hydrogels were purified in PBS for 24 h to remove any residual unreacted pyrrole and by products. An unmodified GP hydrogel was used as the control sample.

Characterization. Scanning electron microscopy (SEM) images were obtained from a field-emission scanning electron microscope (FE-SEM, ZEISS Ultra 55, Germany). The samples were precooled in liquid nitrogen, freeze-dried at -80 °C, pasted on a sample table and covered with a metal spray coating for 60 s. SEM can show the surface morphology, which can be used to further analyze the mechanical properties. Fourier transform infrared (FTIR) spectroscopy was achieved using an FTIR spectrometer from Thermo Fisher, USA. The infrared (IR) spectra of the hydrogels were obtained using the attenuated total reflectance method after the samples were dried. The glass transition temperature of the freeze-dried hydrogels were measured by Differential

Scanning Calorimetry (DSC) machine (DSC-TYW) in N_2 atmosphere, heating rate was 10℃/min. The UV-vis spectra of the hydrogels were obtained with a UV-vis spectrophotometer (UV2450, Japan). The test was carried out by adhering the sliced parts of hydrogel (Φ 8 mm × 3 mm) to a glass sheet (2 cm × 2 cm) and collecting the solid UV-vis spectrum. The PPy powders were compressed into the same size tablets as those in the control group.

Mechanical properties. The rheological properties of the hydrogels were evaluated using a rotary rheometer (Physica MCR301, Anton Paar). The elastic (G') and loss (G'') moduli were obtained from frequency-modulus curves between 0.1 and 10.0 Hz at a 2.0% strain amplitude (linear region). The swelling rate was measured. The prepared GP hydrogels, PPy-GP hydrogels and DA-PPy-GP hydrogels (3 mm in thickness and 8 mm in diameter), were immersed in PBS for 24 h at room temperature to reach an equilibrium swelling state. After the excess water on the surface of the hydrogels was dried with filter paper, the hydrogels were freeze-dried until no change in sample mass was detected. The mass swelling ratio was calculated using equation:

$$Swelling\ ratio\ (\%) = \frac{W_S - W_d}{W_d} \times 100$$

Where W_S and W_d are the hydrated weight and dehydrated weight, respectively.

The porosity of the hydrogels was measured by ethanol substitution method,[2] it was determined by the amount of ethanol absorbed by dry hydrogels (3 mm in thickness and 8 mm in diameter), after 24 h immersion in ethanol, using the following equation:

$$\text{Porosity (\%)} = \frac{W_2 - W_1}{d_{ethanol} \times V_{hydrogel}} \times 100$$

Where W_1 is the weight of dry hydrogel and W_2 is the weight of wet hydrogel, $d_{ethanol}$ is the ethanol density at room temperature, $V_{hydrogel}$ is the volume of the wet hydrogel. The $V_{hydrogel}$ was calculated by the actual thickness and diameter of the samples. Test 5 times, calculate the means.

The mechanical properties of the conductive hydrogels (Φ 8 mm × 3 mm) were

evaluated with a dynamic mechanical analysis (DMA) machine (Q800, TA Company, USA). After the samples were immersed in PBS at room temperature for 24 h to reach swelling equilibrium, they were placed between two plates of a compression tester and compressed by the upper plate at a strain rate of 1 N/min; the diameter of the swollen hydrogels was measured. The compression modulus was calculated from the slope of the stress-strain curve in the 0 to 15% strain range. Cyclic loading-unloading curves were obtained by setting the strain as 65% and the compression time as 1 min; the process was repeated 5 times.

The electrical properties. The electrical properties of the conductive hydrogels were evaluated by electrochemical impedance spectroscopy (EIS) at frequencies from 0.01 Hz to 100 kHz and an amplitude of 5 mV (Electrochemical Workstation, ZAHNER, Germany). Cyclic voltammetry (CV) was performed in 0.067 M PBS using potentials between -0.8 and 0.4 V at a sweep rate of 50 mV/s. A three-electrode system was equipped with a working electrode (hydrogels on conductive glass, ITO), a counter electrode (a platinum electrode), and a reference electrode (a saturated calomel electrode). The precursor solution was placed dropwise on the ITO and then UV-irradiated to coat the ITO. *I-V* curves were collected using a two-electrode system (the hydrogel was placed between two platinum electrodes) using potentials between -0.2 and 0.2 V. The electrical conductivity of the conductive hydrogels was tested by the four-probe method (RTS-8, Guangzhou Four-point Probe Technology Company, China) with a sourcemeter. Cylinders of hydrogel samples with a thickness of 3 mm were placed on a plate after the water on the surface of the hydrogel was removed with filter paper, and then probes were used to compress the hydrogel sample. In theory, the conductivity could be calculated by the following equation.

$$\sigma = (\frac{1}{Rt})$$

Where σ is conductivity, R is resistance, t is the thickness of the sample.[3, 4.]

Before the test, we should gently adjust the height of the probes until the current indicator appeared, which means the probes slightly contacted the surface of sample. Then, we need to input the actual thickness of the cylinder sample, choose the proper

current range, the conductivity of the hydrogel was obtained.

Cytocompatibility of the hydrogels. The cytocompatibility of the hydrogels (3 mm in thickness and 8 mm in diameter) toward MC3T3-E1 cells was tested using live/dead assays, cytoskeleton staining and CCK-8 assays. Before cell seeding, the hydrogels were sterilized with 75% ethanol for 12 h and washed with DPBS approximately 6 times for 24 h to remove the remaining ethanol. Finally, the hydrogels were placed into 48-well plates, immersed in 500 μL α-MEM with 10% fetal bovine serum and 1% penicillin-streptomycin (Hyclone) for 12 h. The cytotoxicity of the hydrogel samples was tested by a live/dead assay. In the *in vitro* study, the cell density was diluted to 6 × 10^4 cells/mL, and then the cell suspension was seeded on the hydrogels. The cells were stained with AM (live) and PI (dead) after being cocultured for 24 h with the GP, PPy-GP and DA-PPy-GP hydrogels. The samples were observed with an Olympus IX73 inverted microscope equipped with an Olympus U-HGLGPS fluorescent light source and an Olympus XM10 camera (Shinjuku, Tokyo, Japan). The cell adhesion behaviors on the samples were studied by cytoskeleton staining. The cell seeding density was approximately 4 × 10^4 cells/mL. After the cells were incubated on the hydrogels for 24 h, the cell cytoskeleton was stained with F-actin and DAPI, and the fluorescence of the samples was observed with a laser scanning confocal microscope (LSM-780, Zeiss company, Germany). The proliferation of the MC3T3-E1 cells on the hydrogels was observed by CCK-8 assays. The cell seeding density was calculated to be 4 × 10^4 cells/mL. The CCK-8 assays were carried out after the cells were incubated for 1, 4 and 7 d with fresh medium containing 10% CCK-8 kit reagent solution preincubated at 37 °C for 3 h. Then, the optical density (OD) at 450 nm was measured using a microplate reader (MK3, Thermo Company, USA).

Pressure-sensitive resistance measurements. The resistance measurements of the DA-PPy-GP hydrogel (10 mm in thickness and 10 mm in diameter) were carried out under a reciprocal compression force with a digit multimeter (Keithley DMM 7510) connected to two copper foils, which made a sandwiched structure with the ends of the cylindrical gel sample.

Statistical analysis. All data are expressed as the mean ± standard deviation. Statistical analysis was performed using Origin statistical software. The differences in the data were compared using one-way analysis of variance (ANOVA).

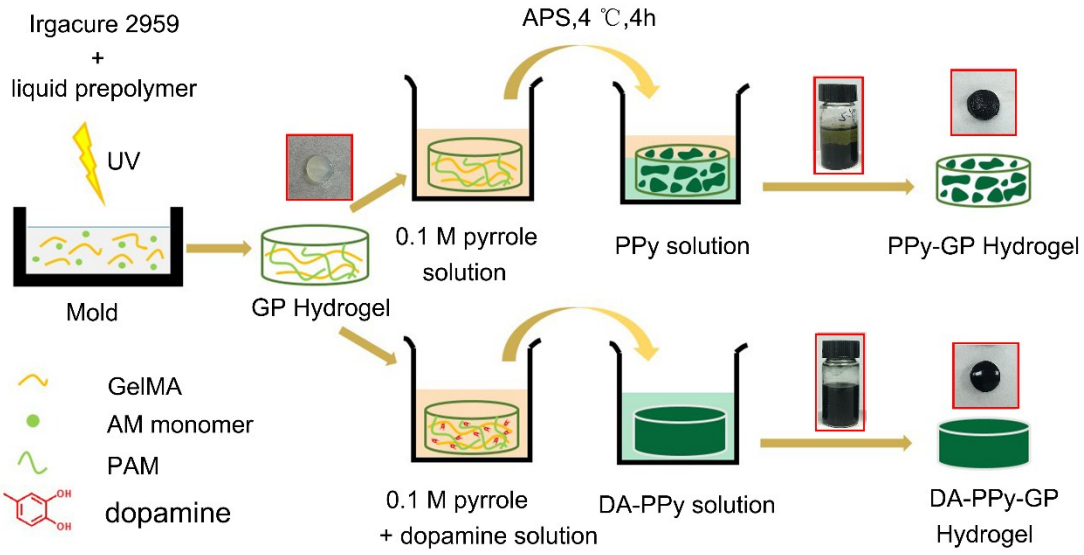

Fig. S1 The detailed preparation process of a hybrid hydrogel composed of GelMA-PAM hydrogel (GP hydrogel) and conductive polymers was performed using an *in situ* polymerization method.

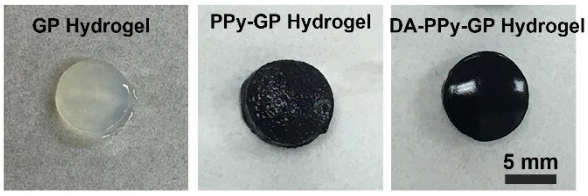

Fig. S2 The actual pictures of the hydrogels.

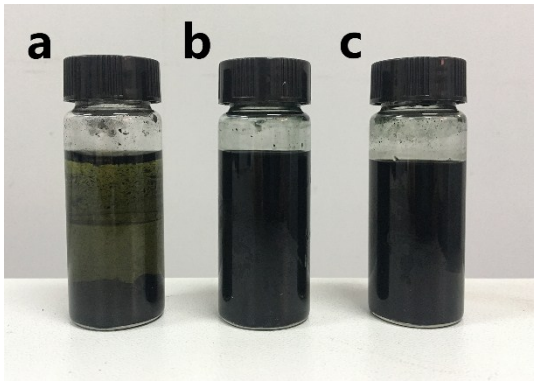

Fig. S3 Digital photograph of DA-PPy aqueous suspensions. a) pure PPy, b) 0.5 M DA-

PPy, c) 1.0 M DA-PPy.

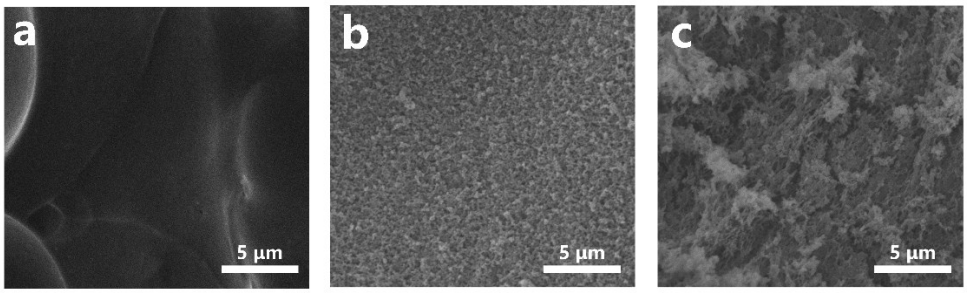

Fig. S4 High-magnification SEM images of a) pure GP hydrogel, b) 0.5 M DA-PPy-GP hydrogel, and c) 1.0 M DA-PPy-GP hydrogel.

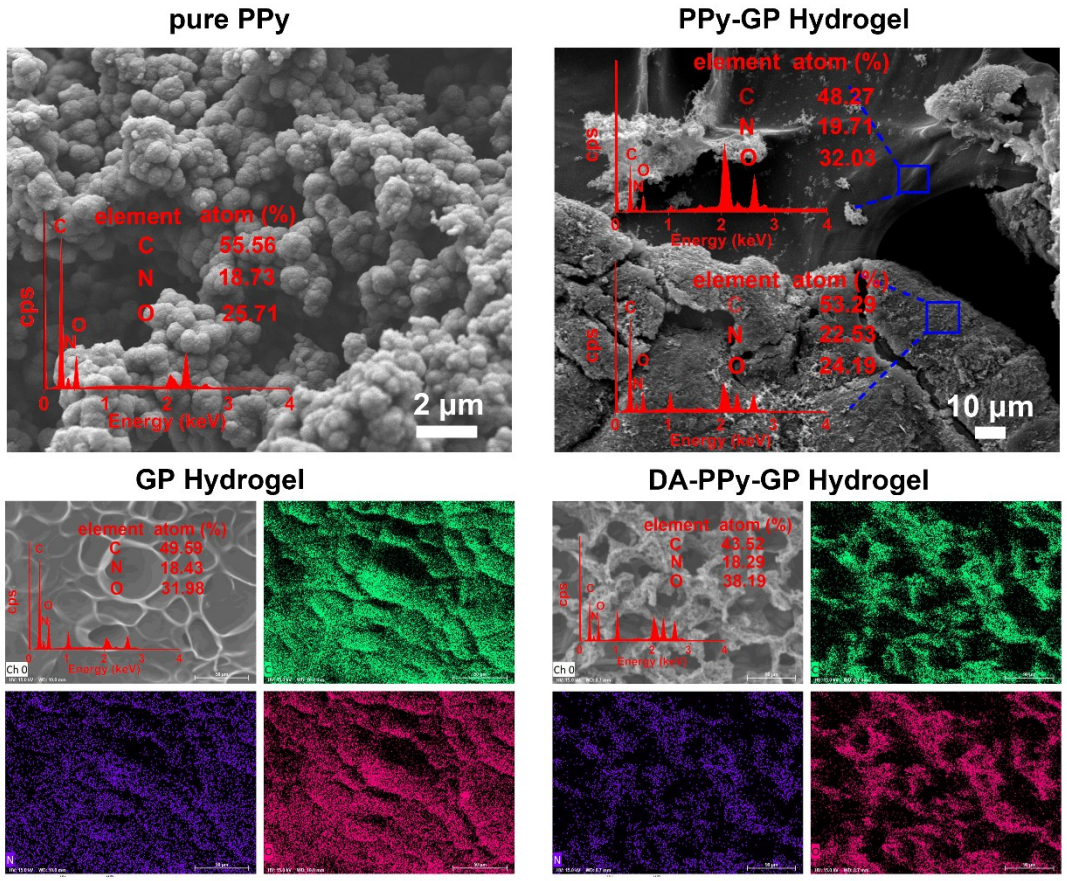

Fig. S5 EDS analysis of the PPy, PPy-GP hydrogel, GP hydrogel, and DA-PPy-GP hydrogel.

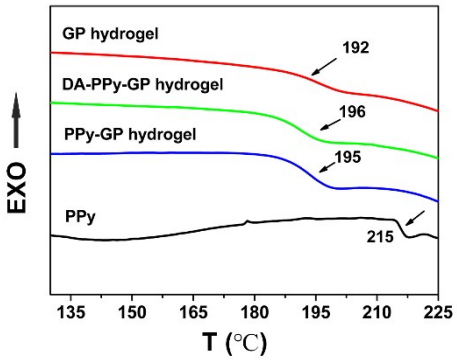

Fig. S6 DSC spectra of the PPy, GP, PPy-GP and DA-PPy-GP hydrogels.

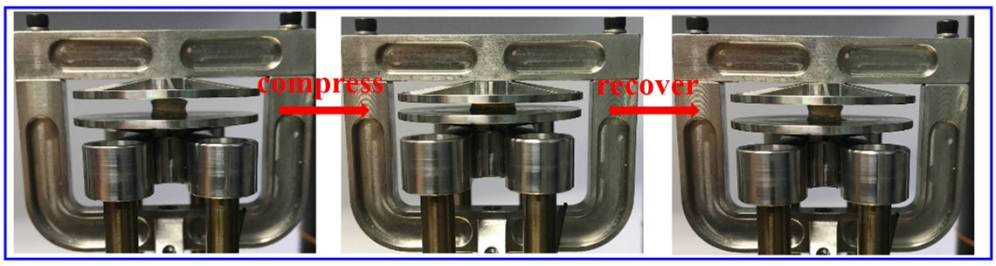

Fig. S7 Digital images of the GP hydrogel, which recovered its original shape after the 70% compression strain.

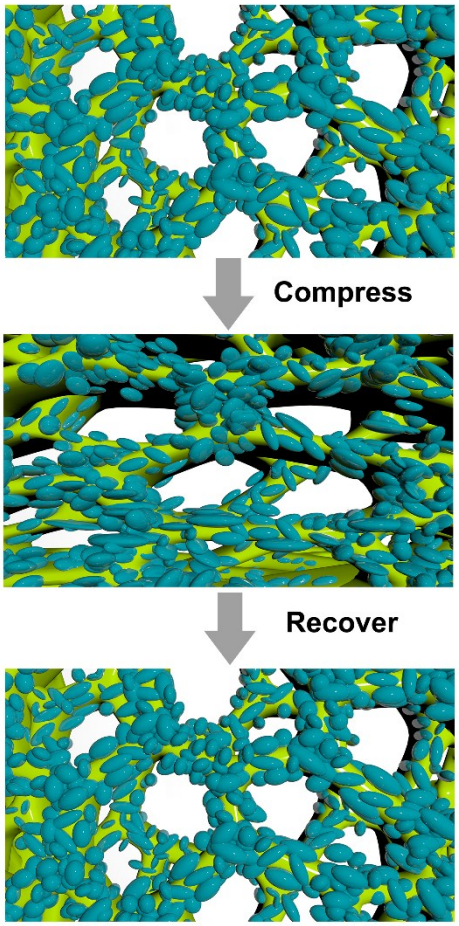

Fig. S8 Schematic of the deformation of the DA-PPy-GP hydrogel under appropriate compressive pressure.

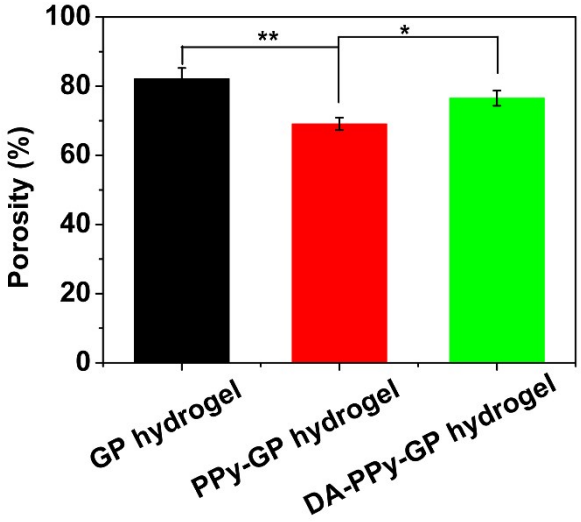

Fig. S9 The porosity of the hydrogels.

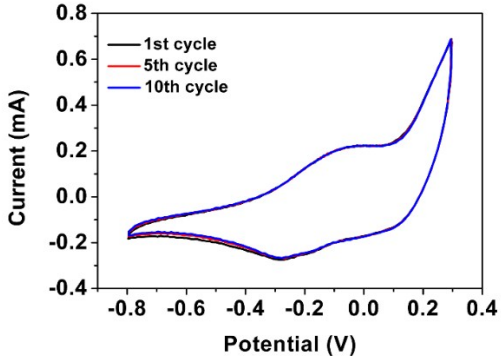

Fig. S10 Multiple CV cycles of the electrochemical stability DA-PPy-GP hydrogel, showing the current response remained stable upon multiple doping/dedoping cycles.

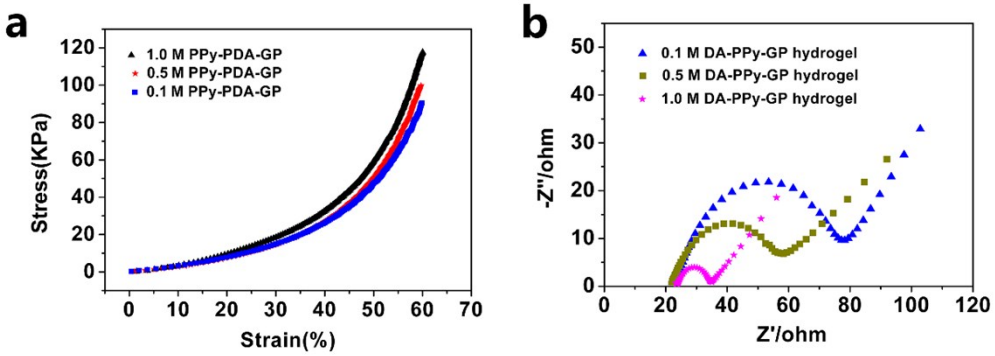

Fig. S11 Mechanical and electrical properties of the conductive hydrogels with different PPy concentrations. a) Compressive stress-strain curves. b) Nyquist curves.

Tab. S1 Conductivity and compression modulus of the hydrogels

Samples	Comp. modulus (KPa)	Conductivity (10^{-4} S/cm)
0.1 M DA-PPy-GP Hydrogel	4.28 ± 0.18	7.07±0.84
0.5 M DA-PPy-GP Hydrogel	4.56 ± 0.31	8.74±0.63
1.0 M DA-PPy-GP Hydrogel	4.88 ± 0.14	9.33±1.03

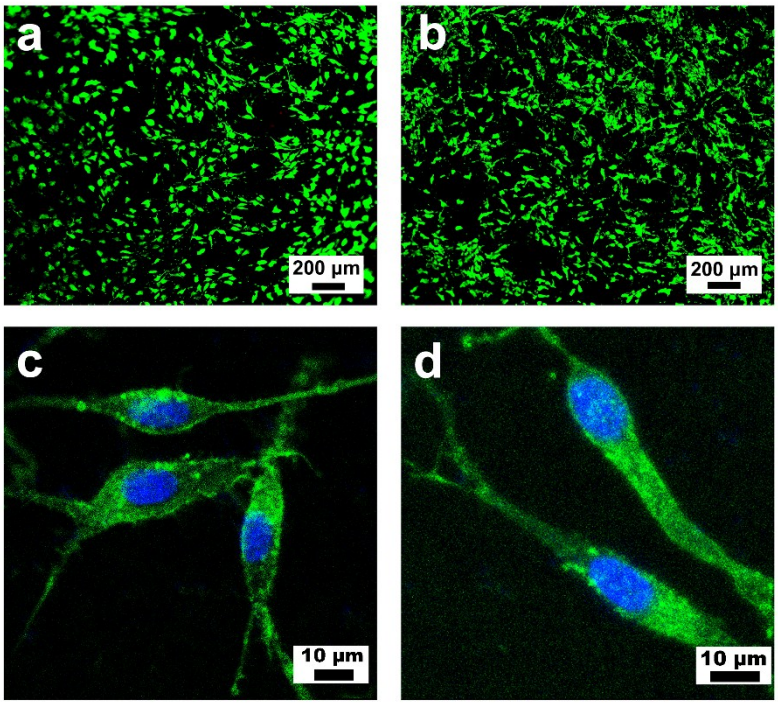

Fig. S12 Biocompatibility assessment of the conductive hydrogels. A live/dead cell staining was conducted after the MC3T3-E1 cells were incubated on the surfaces of a) 0.5 M and b) 1.0 M DA-PPy-GP hydrogels for 24 h. MC3T3-E1 cell spreading behavior was studied by cytoskeletal staining after 24 h of incubation on the surfaces of c) 0.5 M and d) 1.0 M DA-PPy-GP hydrogels.

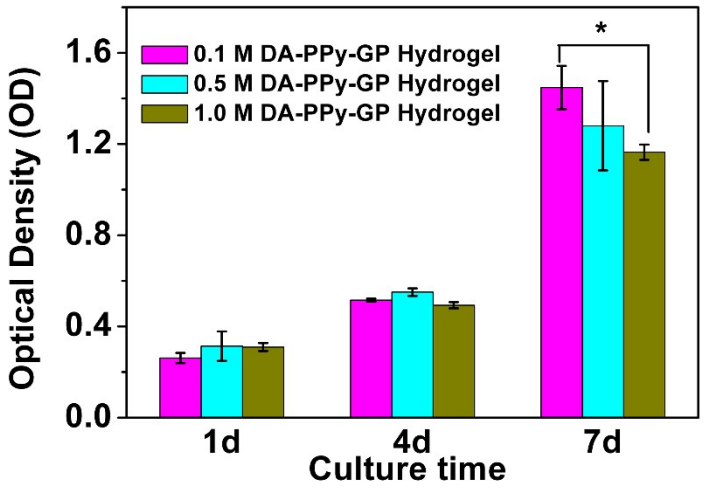

Fig. S13 MC3T3-E1 cell proliferation on the hydrogels was assessed by a CCK-8 assay.

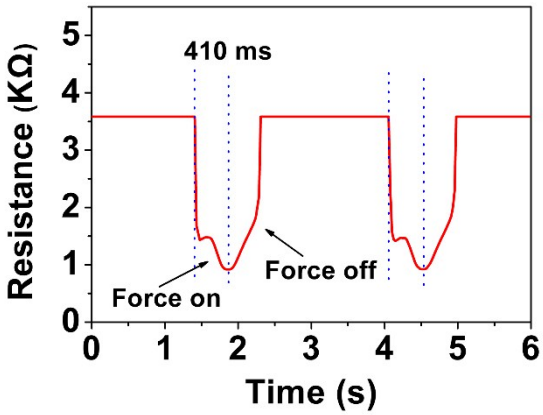

Fig. S14 The response time for the force sensor of the DA-PPy-GP hydrogel.

Reference

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