Supplementary information

Design and fabrication of the IDA-Pt electrodes

IDA-Pt electrodes were fabricated such that the effective electrode dimensions were 8 × 12 mm^2 as shown in Figure 1. The band electrode was 50 µm wide and had a 50 µm gap. The electrodes were patterned on the glass slide (thickness 1 mm; Matsunami Co., Japan) by the conventional photolithography procedure. Briefly, hexamethyldisilazane and S1818 were poured onto a glass slide, and the slide was baked at 90ºC for 10 min. The slide was then irradiated with UV light through a mask aligner (MA-20; Mikasa Co. Ltd., Tokyo, Japan) and developed at MF CD-26. A Ti adhesive layer was then seeded onto the glass slide followed by a Pt film (100 nm thickness). The electrode design was revealed by the lift-off technique. Eventually, a passivation layer of SU-8 photoresist was patterned on the device such that only the electrode bands remained intact. The photoresist layer was then hard baked to create an inert polymer resin. The geometrical parameters and electric characteristics of the device are provided in the following parts.

Fabrication of the PDMS stamp

A micropattern of the SU-8 photoresist (50 μm ridge and 50 μm groove) was made on the silicon wafer according to the conventional photolithography method. PDMS prepolymer and its curing agent were mixed at the 10:1 ratio and poured onto this template followed by 15 min of vacuum to remove air bubbles. After curing at 70°C for 2 hrs, the PDMS stamp was peeled off from the master mold.

Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) measurements were taken using the CompactStat potentiostat (CompactStat; Ivium Technologies, Netherlands) controlled by a computer equipped with the IviumSoft software package. EIS spectra were acquired for the IDA-Pt electrodes over a frequency range from 10 to 10^5 Hz with perturbation amplitudes of 10 mV and 1 V. The Z-Plot/Z-View software package (ScribnerAssociates, Southern Pines, NC, US) was used for the impedance data analysis and to fit the equivalent electrical circuit. Conductivity measurements were performed in the stimulation medium, which had a conductivity of 18 mS/cm as measured by the SG 3 conductimeter (Mettler Toledo, Zürich, Switzerland).

Assessment of cell viability

The calcein AM/ethidium homodimer live/dead assay (Invitrogen, US) was used according to the manufacturer's instructions to measure the viability of the cells. Calcein AM is a cell-permanent dye that is changed to green fluorescent calcein in live cells through the action of intracellular esterases. Ethidium homodimer is a DNA-binding dye that enters through the damaged membranes of dead cells. Calcein AM and ethidium homodimer fluorescence were observed using the fluorescence microscope. The fluorescence images of live/dead cells were quantified using the NIH ImageJ software package, and at least 5 images from 2 independent experiments were used to quantify cell viability.

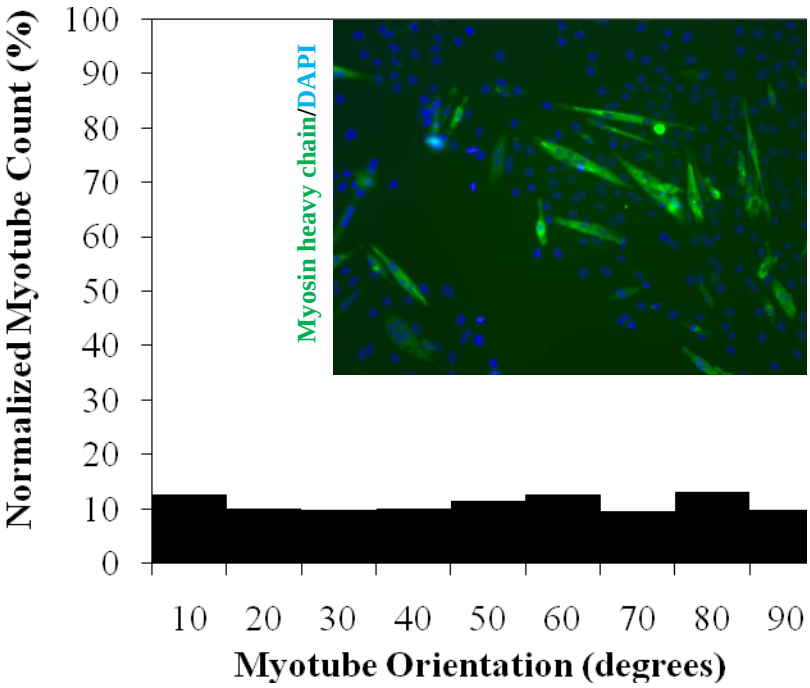

Figure S1. C2C12 myotube alignment within the unpatterned GelMA hydrogel. Histogram of the relative myotube alignment in 10-degree increments showing almost no myotube alignment in the unpatterned control. An immunofluorescence image of myosin heavy chain (green) and DAPI (blue) for the myotubes encapsulated in the unpatterned GelMA hydrogel is presented at the corner of the histogram.

Geometrical parameters of IDA-Pt electrodes

The geometrical parameters of IDA-Pt electrodes define their electric characteristics. These parameters are listed in Table S1 and shown in Figure S2. Key parameters associated with the geometry of IDA-Pt electrodes were the electrode finger width (a), electrode finger spacing (b), electrode substrate thickness (h), the distance between the fingers and the collector electrodes (d), the number of fingers (N), and the distance between the collector electrodes (u). Two additional parameters were digit overlap (W = u-2d) and the equivalent longitude (L = (N-1)W).

Table S1. Geometric parameters of IDA-Pt electrodes.

a (µm)	**b (µm)**	**h (nm)**	**d (µm)**	**N**	**u (mm)**	**W (mm)**	**L (mm)**
50	50	150	50	60	10	9.95	587.05

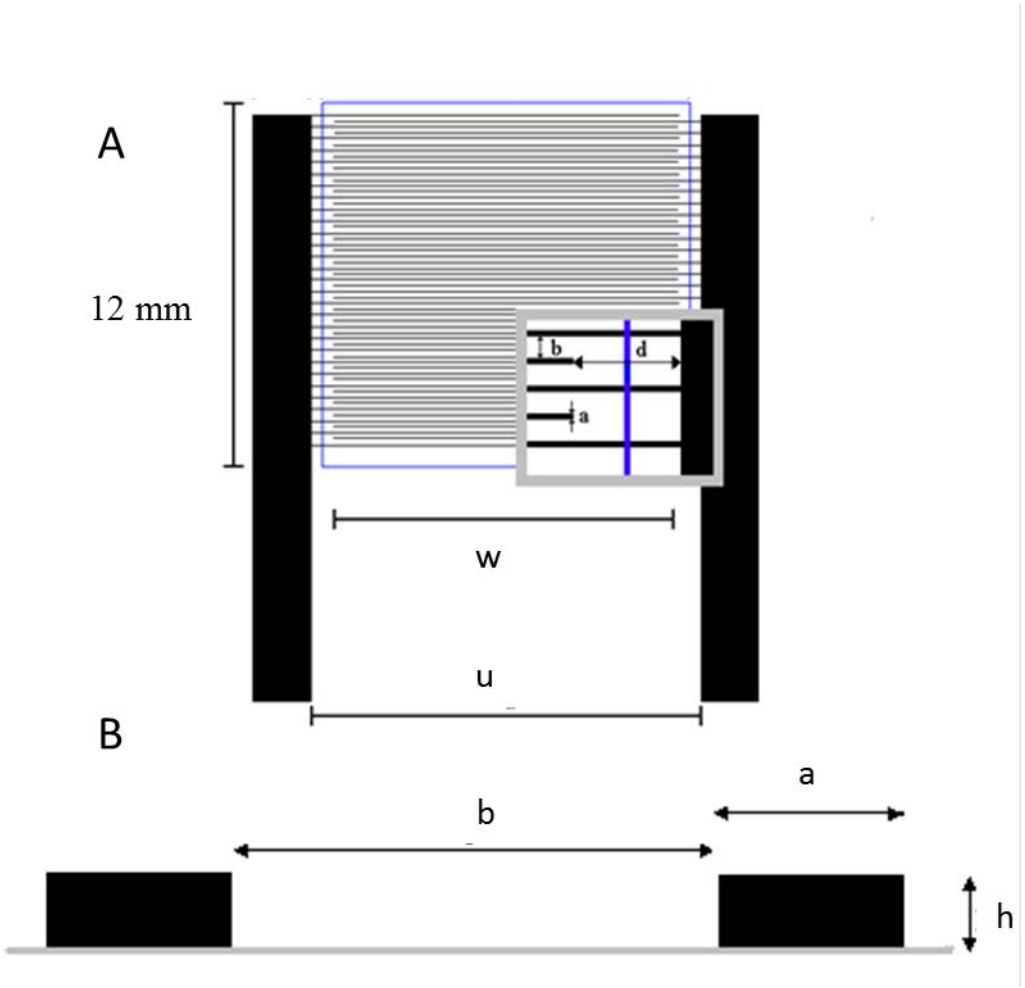

Figure S2. Schematic representation of the IDA-Pt electrodes along with key geometrical parameters. (A) Top view of the device in which the square area with a blue border represents the active area of the device. (B) Side view of the electrode.

Electric parameters of IDA-Pt electrodes

The calculated electric parameters were the electrode capacitance ($C_{IDA\text{-}Pt}$), the cell constant (k), and sensitivity to dielectric changes in the medium (S).

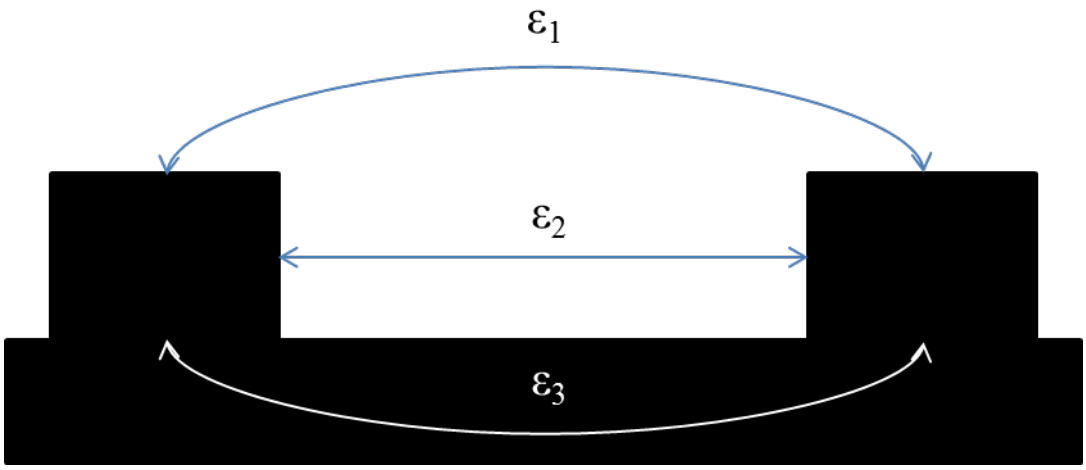

Figure S3. Schematic representation of the electric field lines within the IDA-Pt electrodes.

The capacitance between every two conducting materials can be calculated when the potential distribution for each point between two conductors is known. If we do not have charge storage in the medium surrounding the conductors, the potential should follow the Laplace equation, by which the capacitance between two parallel conductive materials (C) can be calculated according to the following equation,

$$C = \varepsilon \frac{A}{d} \qquad (1)$$

where ε is the electric permeability, A is the surface area of the conductors, and d is the distance between the electrodes. However, in the case of IDA-Pt electrodes it is not this simple because elliptical and longitudinal field lines cross through different materials with different permeabilities (see Figure S3). The capacitance of IDA-Pt electrodes was defined as the sum of capacitance values between all finger electrodes and therefore is given by equation 2,

$$C_{IDA\text{-}Pt} = (C_0 + C_{ED})L \qquad (2)$$

where L is the equivalent longitude and C_0 is the capacitance for the longitude unit and was determined using the Olthuis method (W. Olthuis, W. Streekstra, and P. Bergveld. Theoretical and experimental determination of the cell constants of planar-interdigitated electrolyte conductivity sensors. *Sensors and Actuators B Chemical* **24-25** (1995) 252-256). Olthuis *et al.* defined the capacitance of the longitude unit between two infinite coplanar electrodes (C_0) as,

$$C_0 = \varepsilon_0 \frac{(\varepsilon_1 + \varepsilon_2)}{2} \left(\frac{K\left(\sqrt{1-x^2}\right)}{K(x)} \right) \qquad (3)$$

where ε_0 is the permeability in vacuum, ε_1 is the relative permittivity of the medium, ε_2 is the permeability through the substrate and K(x) is the elliptical integral with the following formula,

$$K(x) = \int_{t=0}^{1} \frac{dt}{\sqrt{(1-t^2)(1-x^2t^2)}} \tag{4}$$

and finally x denotes,

$$x = \cos\left(\frac{\pi}{2}\frac{2\mathrm{a}}{\mathrm{b}+2\mathrm{a}}\right) \tag{5}$$

where a and b are the geometrical parameters of the IDA-Pt electrodes as shown in Figure S2. C_{ED} in equation 2 is the capacity defined by Endres and Drost (H.-E. Endres and S. Drost. Optimization of the geometry of gas-sensitive interdigital capacitors. *Sensors and Actuators B Chemical* **4** (1991) 95-98), which explains the effect of a third medium between the digits of the electrodes (see Figure S3) as follows,

$$\mathrm{C}_{\mathrm{ED}} = \varepsilon_0\varepsilon_3\left(\frac{\mathrm{h}}{\mathrm{b}}\right) \tag{6}$$

where ε_3 is the permeability between the digits, and h and b are the geometrical parameters of the IDA-Pt electrodes as demonstrated in Figure S2. Because $\frac{\mathrm{h}}{\mathrm{b}}$ is very small, C_{ED} is negligible.

The cell constant k (cm^{-1}) of an electrolyte was defined as the proportionality factor between the specific resistance ρ of the electrolyte (kΩ cm) and the measured resistance R (kΩ),

$$R = k\rho \tag{7}$$

and considering an isotropic medium with constant permittivity, the above equation was rewritten as,

$$k = \frac{\mathrm{R}}{\rho} = \mathrm{R}\sigma = \frac{\varepsilon_0 \varepsilon_r}{\mathrm{C}_{\mathrm{IDA\text{-}Pt}}} \tag{8}$$

where ε_0 is the vacuum permeability, and ε_r is the relative permeability. The relative permeability was calculated as the permeability of the medium (Here, KCl) plus the permeability of the substrate (*i.e.*, SiO_2).

Finally, the sensitivity of the device was obtained as,

$$S = \frac{\varepsilon_0}{k} \tag{9}$$

All calculated electric parameters are summarized in Table S2.

Table S2. Electric parameters of IDA-Pt electrodes and permeability values of the underlying media.

$C_{IDA\text{-}Pt}$ (pF)	**k (cm^{-1})**	**S (pF)**
14.11	0.51	0.17
Permeability parameters (unit: pF/m)		
ε (SiO_2)	**ε (KCl)**	**ε_0 (Vacuum)**
3.2	78.54	8.85

Table S3. Primer sequences.

No.	Name	Left primer	Right primer
1	MyoD (1)	5'-GGCTACGACACCGCCTACTA-3	5'-CTGGGTTCCCTGTTCTGTGT-3'
2	Myogenin (1)	5'-TGTCTGTCAGGCTGGGTGTG-3'	5'-TCGCTGGGCTGGGTGTTAG-3'
3	MRF4 (1)	5'-CGAAAGGAGGAGACTAAAG-3'	5'-CTGTAGACGCTCAATGTAG-3'
4	Myf-5 (1)	5'-CTGCTCTGAGCCCACCAG-3'	5'-GACAGGGCTGTTACATTCAGG-3'
5	Mef2c (1)	5'-TCTGCCCTCAGTCAGTTGG-3'	5'-CGTGGTGTGTTGTGGGTATC-3'
6	MLP (1)	5'-TGGGTTTGGAGGGCTTAC-3'	5'-CACTGCTGTTGACTGATAGG-3'
7	MHC-IId/x (1,2)	5'-GCGACAGACACCTCCTTCAAG-3'	5'-TCCAGCCAGCCAGCGATG-3'
8	MHC-IIa (1,2)	5'-GCAGAGACCGAGAAGGAG-3'	5'-CTTTCAAGAGGGACACCATC-3'
9	MHC-IIb (1,2)	5'-GAAGGAGGGCATTGATTGG-3'	5'-TGAAGGAGGTGTCTGTCG-3'
10	MHC-pn (1,2)	5'-ACTGAGGAAGACCGCAAGAA-3'	5'-CAGGTTGGCATTGGATTGTTC-3'
11	Sarcomeric actin (1,2)	5'-ATGGTAGGTATGGGTCAG-3'	5'-GATCTTCTCCATGTCGTC-3'
12	α-Actinin (1,2)	5'-TCATCCTCCGCTTCGCCATTC-3'	5'-CTTCAGCATCCAACATCTTAGG-3'

References

1. M. L. P. Langelaan, K. J. M. Boonen, K. Y. Rosaria-Chak, D. W. J. van der Schaft, M. J. Post and F. P. T. Baaijens, *J. Tissue Eng. Regen. Med.*, 2011, **5**, 529–539.

2. K. J. M. Boonen, M. L. P. Langelaan, R. B. Polak, D. W. J. van der Schaft, F. P. T. Baaijens and M. J. Post, *J. Biomechanics*, 2010, **43**, 1514–1521.