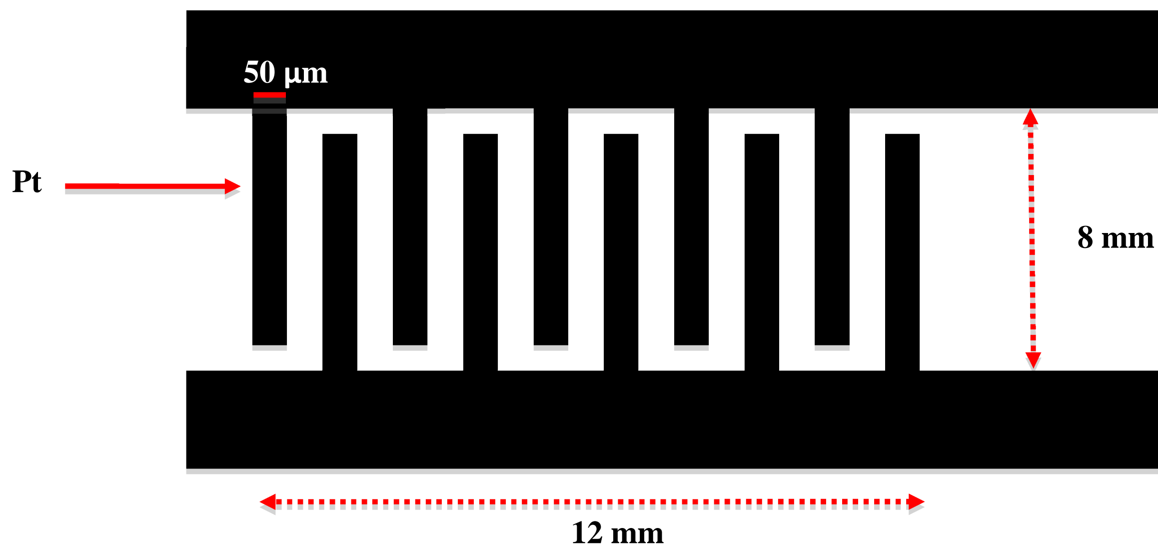
Supplementary data

A



B

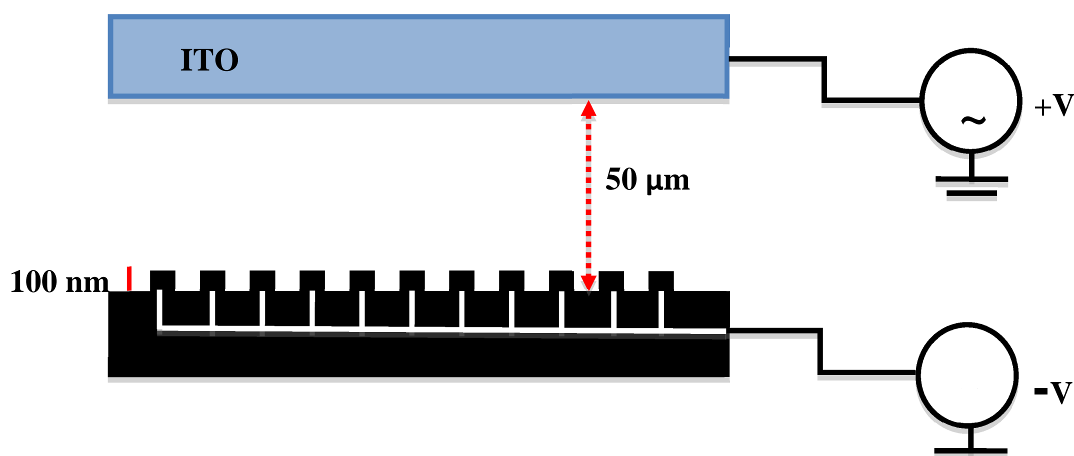


Figure S1. Top view of the Pt-IDA electrode on the glass slide with 50-µm and 50-µm gap distance (A). Side view of the design for the dielectrophoresis (DEP)-based cell patterning experiments in which an indium tin oxide (ITO) electrode was used as the top electrode and a Pt-IDA electrode with a thickness of 100 nm was used as bottom electrode (B).

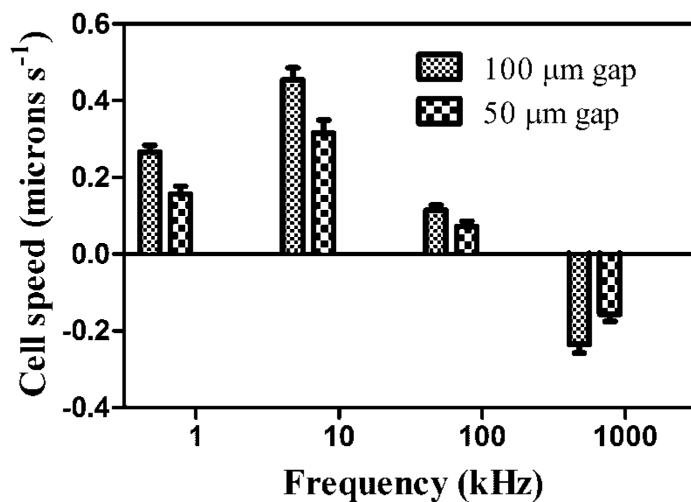


Figure S2. Measured speed of cells in the sucrose buffer and 5% GelMA prepolymer manipulated by DEP forces inside the chamber with a 50-μm electrode–50-μm gap and 50-μm electrode–100-μm gap Pt-IDA design. When cells were stimulated by 1, 10, and 100 kHz, negative DEP behavior was observed, while the cells manipulated by 1 MHz presented positive DEP.

This journal is © The Royal Society of Chemistry 2012

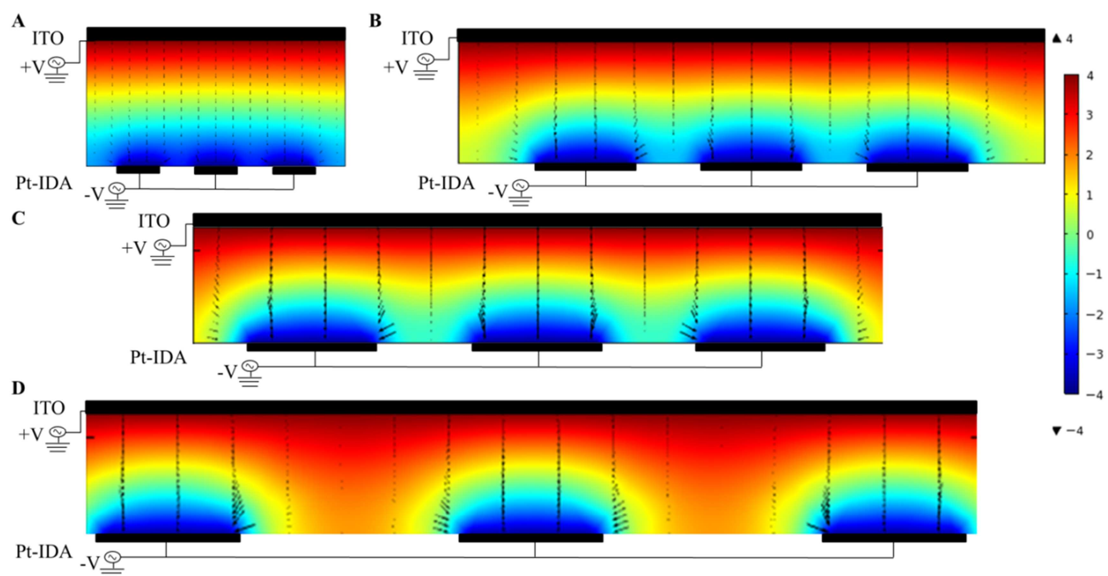


Figure S3. Cross-sectional view of the numerically calculated electric field (V/m) formed in the patterning device obtained by the COMSOL Multiphysics 4.2 software package. Arrows show current density. Upper and lower black bars show the ITO electrode and the Pt-IDA electrode, respectively, with different designs, namely, (A) 15-µm–width electrode–15-µm gap, (B) 50-µm–width electrode–35-µm gap, (C) 50-µm–width electrode–50-µm gap, and (D) 50-µm–width electrode–100-µm gap.

This journal is © The Royal Society of Chemistry 2012

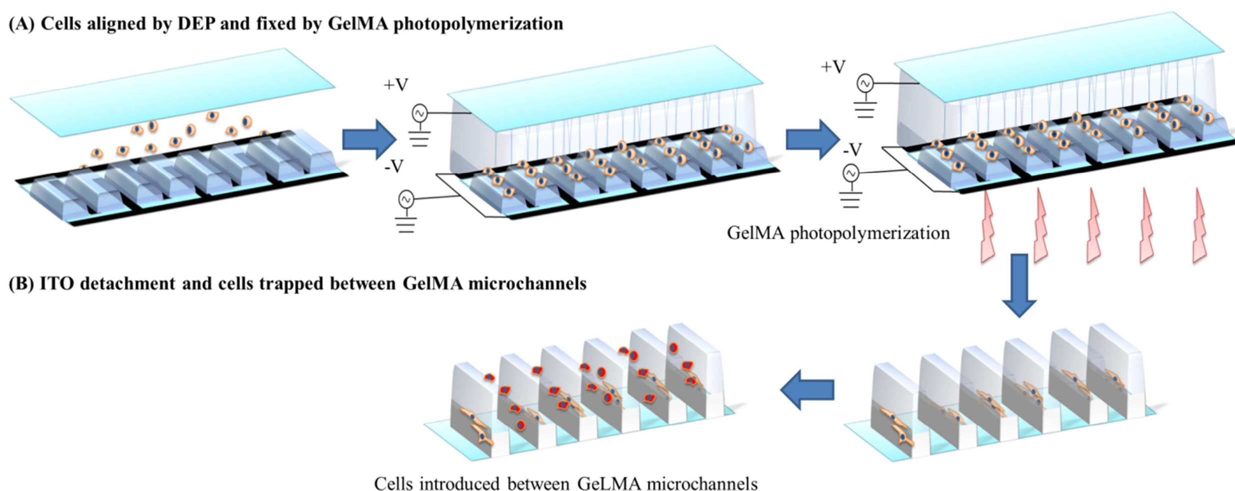


Figure S4. Schematic representation of the process for the co-culture of HUVEC and C2C12 cells. The first cell type, in 5% gelatin methacrylate (GelMA) prepolymer, was injected into the device, aligned by n-DEP, and UV light was irradiated from the underside to cross-link the GelMA prepolymer (A). The ITO electrode was detached, unpolymerized prepolymer was washed out by PBS buffer, and the second cell type suspended in the culture medium was introduced. After 15 min of incubation, the device was gently washed out again with PBS to trap the second cell type in the gap regions between GelMA polymers (B).

This journal is © The Royal Society of Chemistry 2012

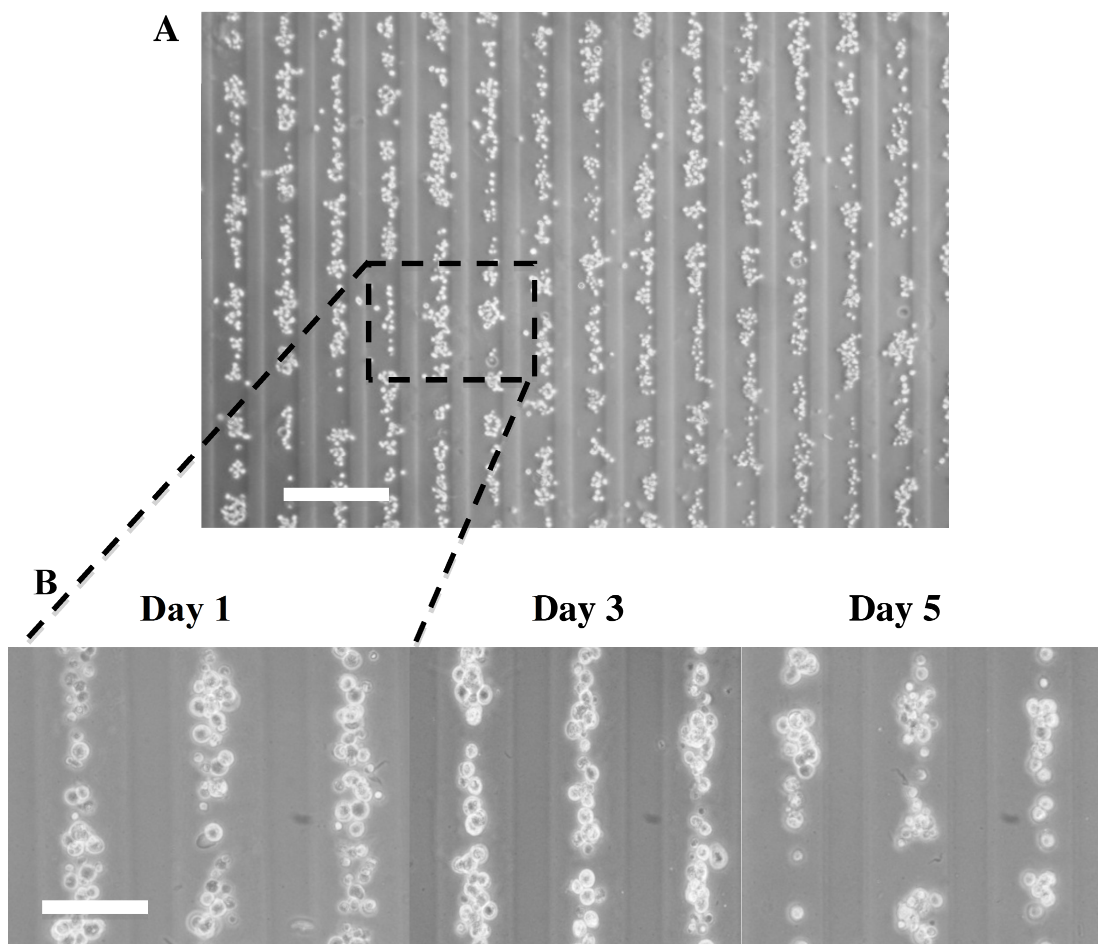
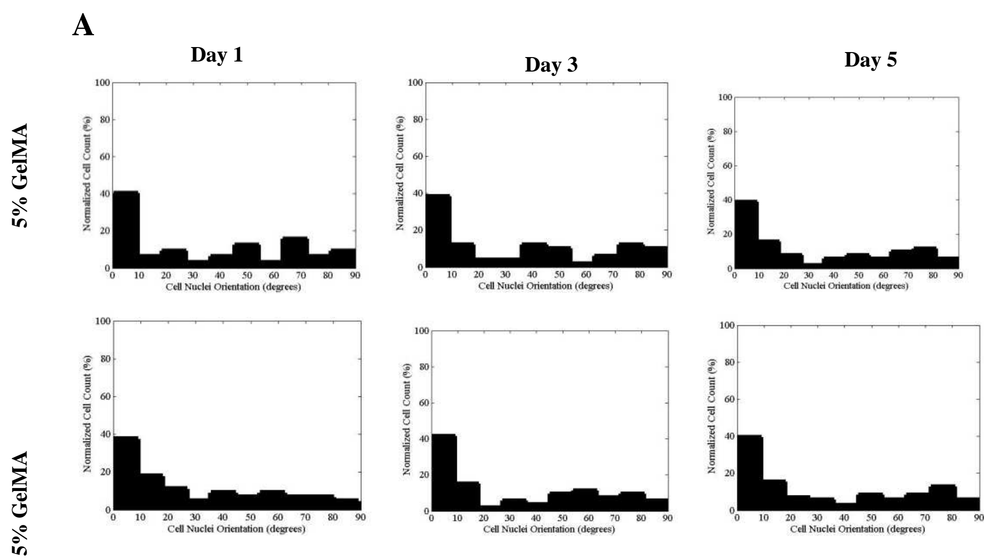
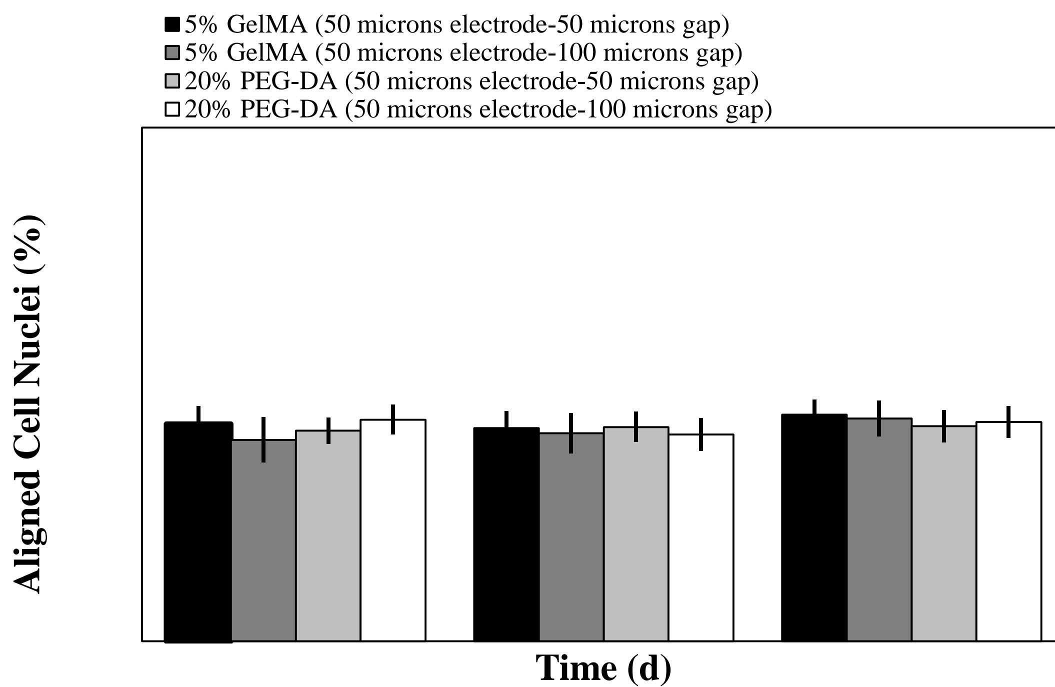


Figure S5. Picture of aligned C2C12 muscle cells within 20% polyethylene glycol (PEG) hydrogel as obtained by the dielectrophoresis (DEP) technique using 50 μm electrode-100 μm gap device (A). Phase contrast images of the aligned C2C12 muscle cells within 20% PEG hydrogel at different culture times (B). Scale bar shows 300 μm and 100 μm in A and B, respectively.


A
Day 1
Day 3
Day 5
5% GelMA
5% GelMA
Normalized Cell Count (%)
Cell Nuclei Orientation (degrees)


B
5% GelMA (50 microns electrode-50 microns gap)
5% GelMA (50 microns electrode-100 microns gap)
20% PEG-DA (50 microns electrode-50 microns gap)
20% PEG-DA (50 microns electrode-100 microns gap)
Aligned Cell Nuclei (%)
Time (d)

C

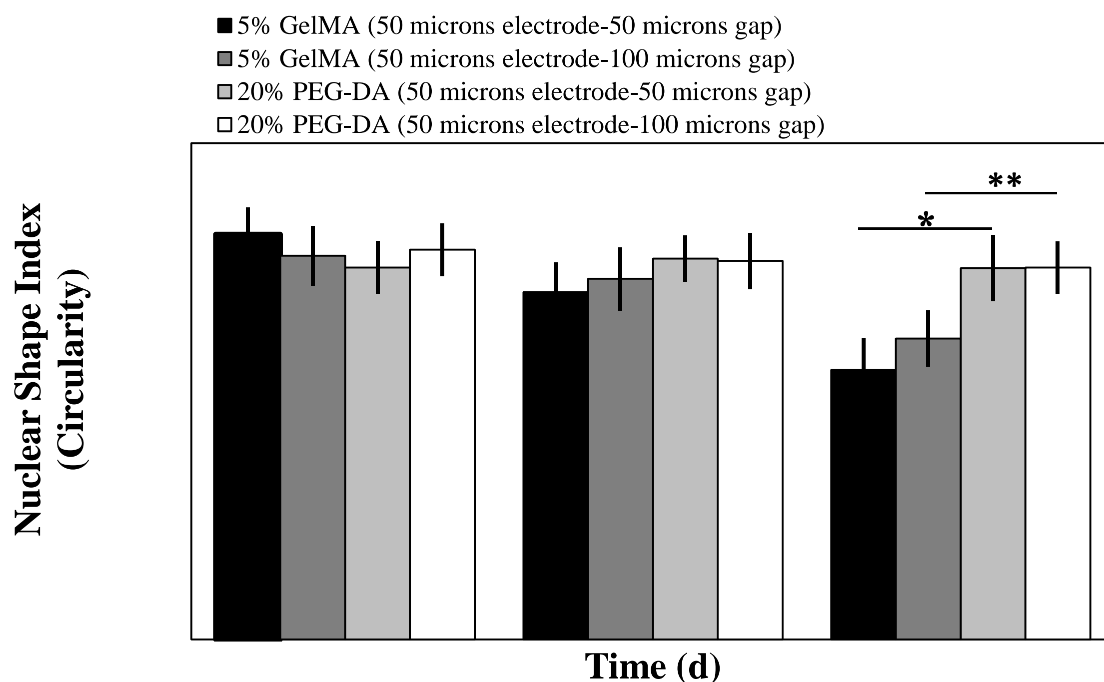
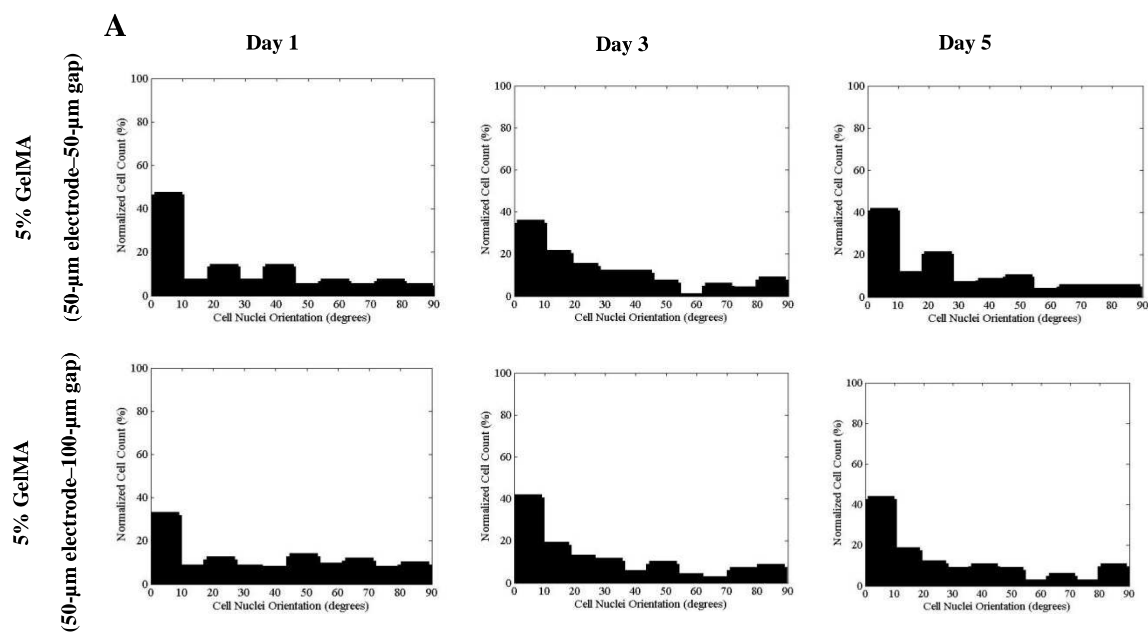
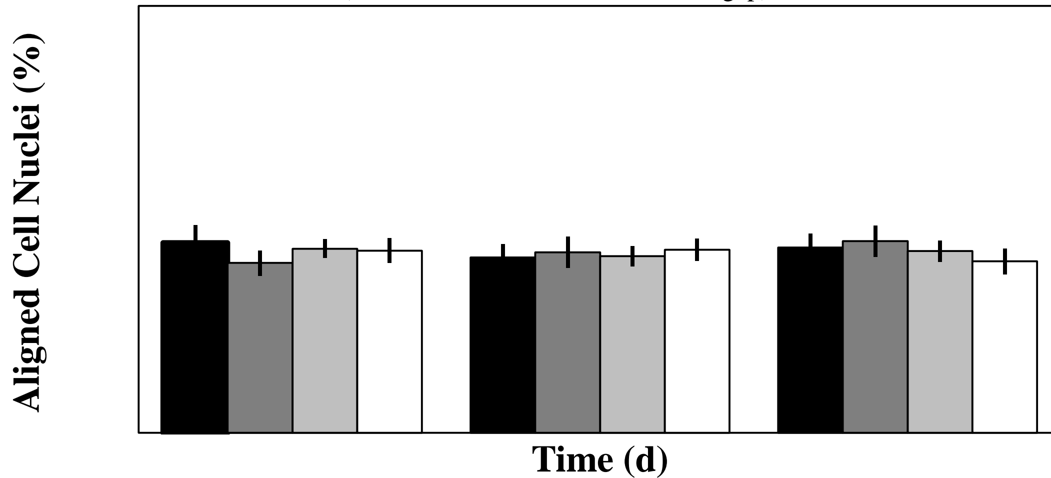


Figure S6. Evaluation of cell alignment and elongation for the patterned HUVEC cells encapsulated in 5% gelatin methacrylate (GelMA) and 20% polyethylene glycol (PEG) using 50-µm electrode–50-µm gap and 50-µm electrode–100-µm gap devices. Representative histograms of the relative alignment in 10 degree increments show highly aligned HUVEC cells in the GelMA hydrogel (A). Both hydrogels demonstrated the ability to keep the aligned cells for 5 days of culture (B). However, the circularity reduction that has a direct relationship with the cell proliferation remained almost constant for PEG and decreased for the GelMA hydrogel (C) (*p < 0.01, **p < 0.05).

This journal is © The Royal Society of Chemistry 2012





C

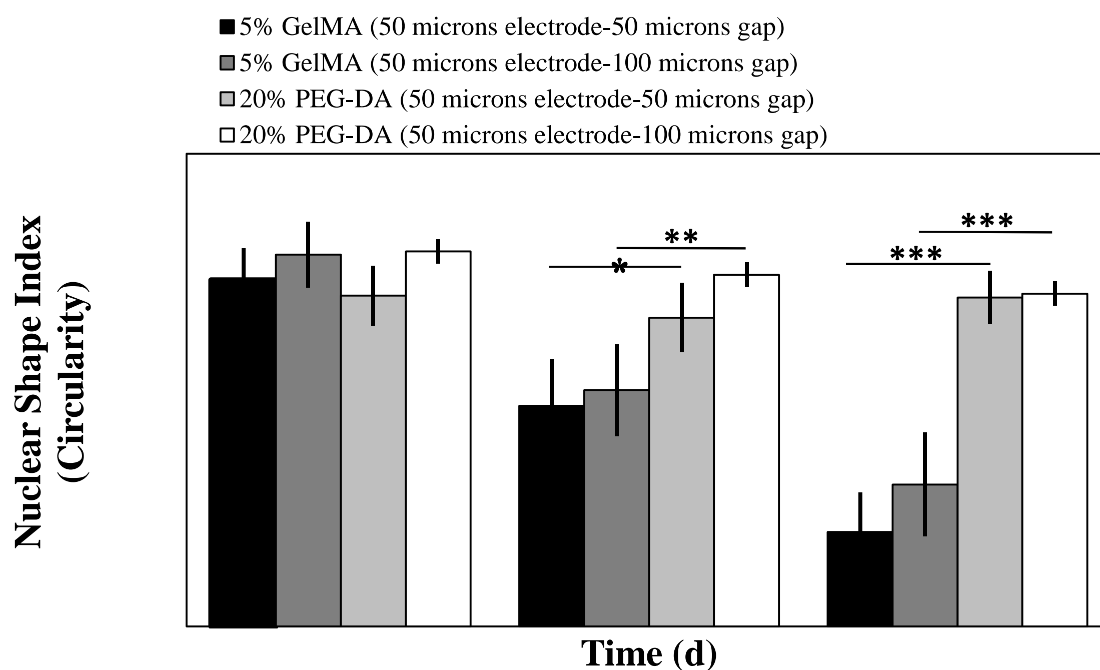


Figure S7. Assessment of cell alignment and elongation for the patterned C2C12 cells encapsulated in 5% gelatin methacrylate (GelMA) and 20% polyethylene glycol (PEG) using 50-µm electrode–50-µm gap and 50-µm electrode–100-µm gap devices. Representative histograms of the relative alignment in 10 degree increments show highly aligned C2C12 cells in the GelMA hydrogel (A). Both hydrogels demonstrated the ability to preserve the aligned cells for 5 days of culture (B). However, the circularity reduction, which has a direct relationship with cell proliferation, remained almost constant for the PEG and decreased for the GelMA hydrogel (C) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Electronic Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2012

Table S1. Characteristics of the media used in the present negative-DEP experiments

Parameters	**Mineral water**	**PBS buffer**	**10 times diluted PBS**	**HEPES[a] buffer**	**DMEM**	**Sucrose buffer**
pH	7	7.5	7.5	5.4	7.2	7.2
Conductivity (mS m^{-1})	-	1500	150	10	1856	8
Cell viability after 1 h (%)	<10	62	44	50	95	88

[a] HEPES buffer is composed of 0.01 M HEPES, 0.25 M sucrose, 0.06 M D-glucose, and 1×10^{-3} M $CaCl_2$.