Electronic Supplementary Material (ESI) for Integrative Biology.
This journal is © The Royal Society of Chemistry 2015

Supplementary Materials

Theoretical calculations for thermal (Joule) heating

The maximum current delivered to a single channel of the ibidi© device is 0.8mA to achieve an EF of 5V/cm. The applied current when 4 channels were stimulated in parallel was 2.4mA. The resistance has been calculated by measuring the voltage across one channel using a standard voltmeter, and at 0.8mA the resistance is 9375 Ω and V is 5 V/cm. The power dissipated over this chamber was calculated using the formula $P=VI$, where P is the power (energy per unit time) converted from electrical energy to thermal energy, I is the applied current, and V is the voltage drop across the channel. As Ohm's law is applicable, the formula can be written in other equivalent forms $P=I^2R=V^2R$, where R is the resistance. Assuming assume the heating of the chamber occurs at a constant rate, and that the rate at which heat is lost to the environment is directly proportional to the temperature in the chamber, the expected temperature of the chamber was calculated using the formula $R= C\,(dT/dt) + hT$, which can be written as $T = \frac{R}{h}(1 - e^{\frac{-h}{c}t})$, where R is the heat applied at constant rate (calculated previously using $P=I^2R$), the proportionality constant C (calories per degree) is the heat capacity of the heater materials, t is the elapsed time in seconds since heat was applied, T is the number of degrees above room temperature expected in the device, and the positive proportionality constant h, is called the heat transfer coefficient. The steady state temperature within the device L was also calculated using the formula $L = \frac{R}{h}(1 - e^{\infty})$. Taking into account the surface area of the channel, the temperature within the device was also calculated using the formula $Q= hA\,(T_{device}-T_{environment})$, where Q is the thermal energy in joules, A is the heat transfer surface area in m^2, T is the temperature is measured in degrees Kelvin (K). Using the values of maximum amount of current applied (0.8mA), and a resistance of 9375 Ω for one ibidi© channel to achieve a 5 V/cm EF, the power converted from electrical energy to thermal energy = the heat dissipation rate (R) = 0.006 *J/s* or 0.0014340344 *cal/s*. T=37˚C within the heated chamber around the time-lapse microscope; t=15 hours/54000 seconds; the heat capacity of water (Cw) at 37˚C is 4.179 *kJ/kgK*= 4.179 *J/g K* ≈ 1 x 20˚C calorie (4.182J). So Cw is approximately 1 *cal/˚C/g*. The volume of media for one channel including connectors ≈ 1 millilitre, C of media (Cm) ≈1 *cal/˚C*. The heat capacity of polystyrene (Cp) is ≈ 0.36 cal/g˚C x 3.2g (weight of device) = 1.152 cal/˚C. Total heat capacity $C = Cm + Cp$ =2.15 cal/˚C. The heat transfer coefficient (h) ≈ $h(water) + h(polystyrene) + h(atmospheric\ air\ at\ 37˚C)$= 500 to 10,000 $W/(m^2K)$ + 0.17 $W/(m^2K)$ + 10 to 100 $W/(m^2K)$ = 10100.17 $W/(m^2K)$ or 0.241243 $cal/s/cm^2/˚C$. Using these values, T = 0.006 ˚C above the heated chamber temperature at 15 hours; L= 37.006˚C; Using $Q= hA\,(T_{device}-T_{environment})$ where $A=0.00014584\ m^2$; T_{device} = 310.15407˚K = 37.00407˚C.

Fluid flow stimulation

Passage 6 human BM-MSCs (obtained from Lonza Pty Ltd), labeled donor D cells, were seeded into a 6 channel ibidi© device as described previously and allowed to attach overnight. Additionally, two lengths of tubing (PE50, 0.58 mm ID, BD Biosciences) were each attached to a sterile blunt needle (22 gauge) on one end, and a sterile needle tips (22 gauge) on the other end. These assemblies were then sterilized using 70% ethanol and dried overnight in a 60°C oven. In a sterile manner, a 1 ml sterile syringe (Terumo) was then filled with the cell culture media and connected to the 22 gauge needle-end of one of the tubing assemblies, which was in turn connected to one end of one of the ibidi© channels (inlet port). The second tubing assembly was connected to the other end of the ibidi© channel and then into a sterile petri dish (outlet port). The media-filled syringe was placed on a syringe pump (NE-1800, New Era, Farmingdale, NY) and continuous fluid flow was delivered at 6 µL/ hour or 20 µL/ hour. The sterile petri dish was placed in the time-lapse microscope incubator, with tubes leading to the syringe pump that was placed outside the incubator at room temperature.

There were no significant differences in directional response for cells under static culture or both fluid flow conditions (figure S1A). There was no statistically significant difference between the mean cell morphology values for 6 µL/hour at 0, 4 or 8 hours (figure S1B-E). There was also no difference for mean cell morphology values for 20µL/hour at 0, 4 or 8 hours (figure S1B-E).

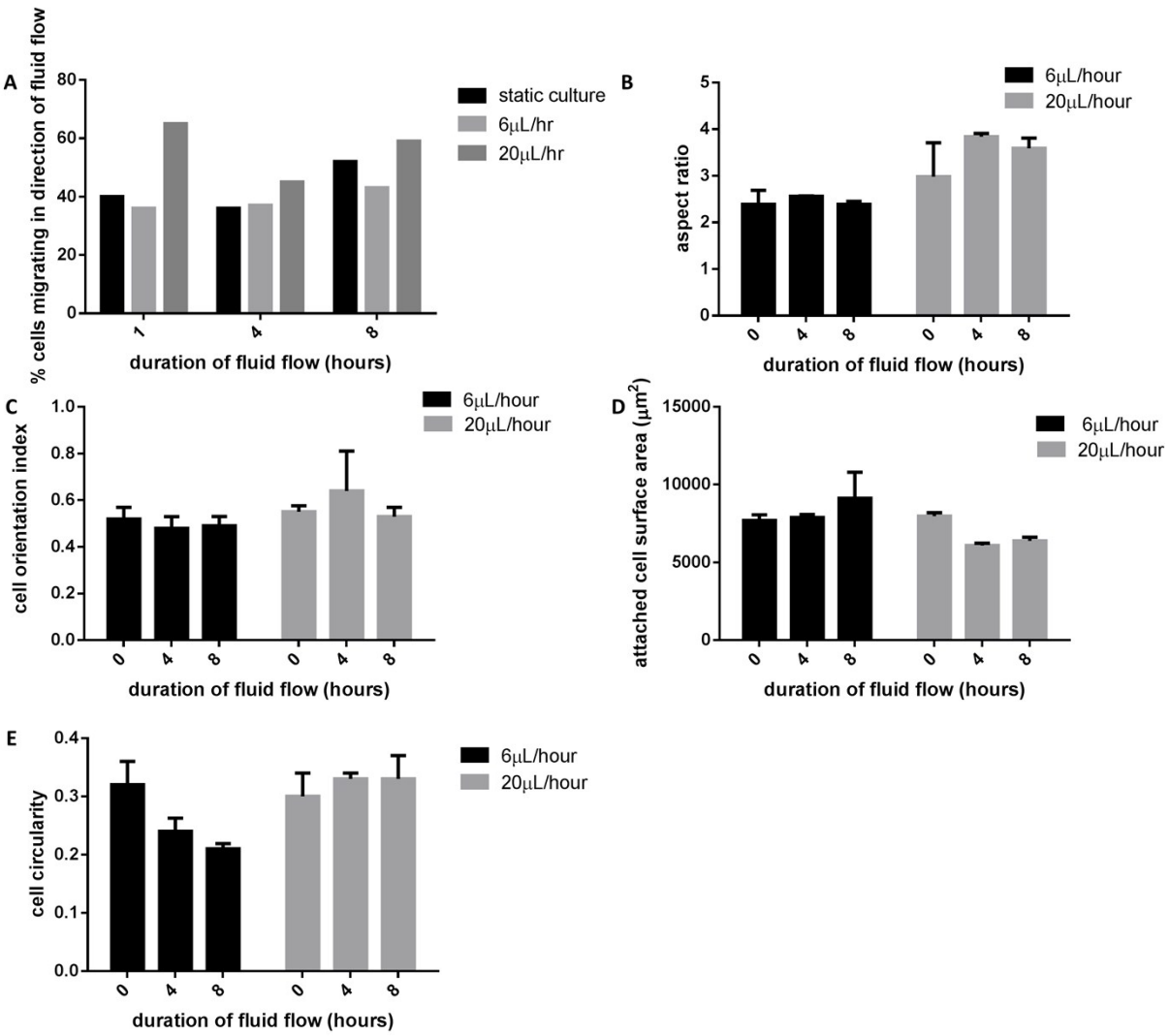


Fig S1. Differences in directional response (A) or the mean cell morphology values (B-E) under static culture or 6 μL/hour or 20 μL/hour fluid flow conditions at 0, 4 or 8 hours.

The speeds travelled by cells under 6 μL/hour fluid flow or static culture conditons were not significantly different. Cells migrated faster at one hour of 20μL/hour fluid flow rate versus static culture but not over longer (4 or 8 hours) time points (figure S2A). There was no difference in euclidean distance travelled at any time point for cells under static conditions versus 6μL/hour fluid flow conditions, and there was no difference in euclidean distance travelled at 1 hour for cells under the 20μL/hour fluid flow condition (figure S2B). Cells did however travel further euclidean distances when stimulated with 20μL/hour fluid flow compared to static conditons or 6μL/hour fluid flow conditons (figure S2B) at longer time points (4 or 8 hours), however the observed delta increases in euclidean distance were significantly smaller than those seen over similar timepoints for an applied EF of 5 V/cm (or lower). There were no significant differences for accumulated distance travelled when comparing cells imaged under static culture versus those imaged under 6 or 20μL/hr fluid flow conditons (figure S2C).

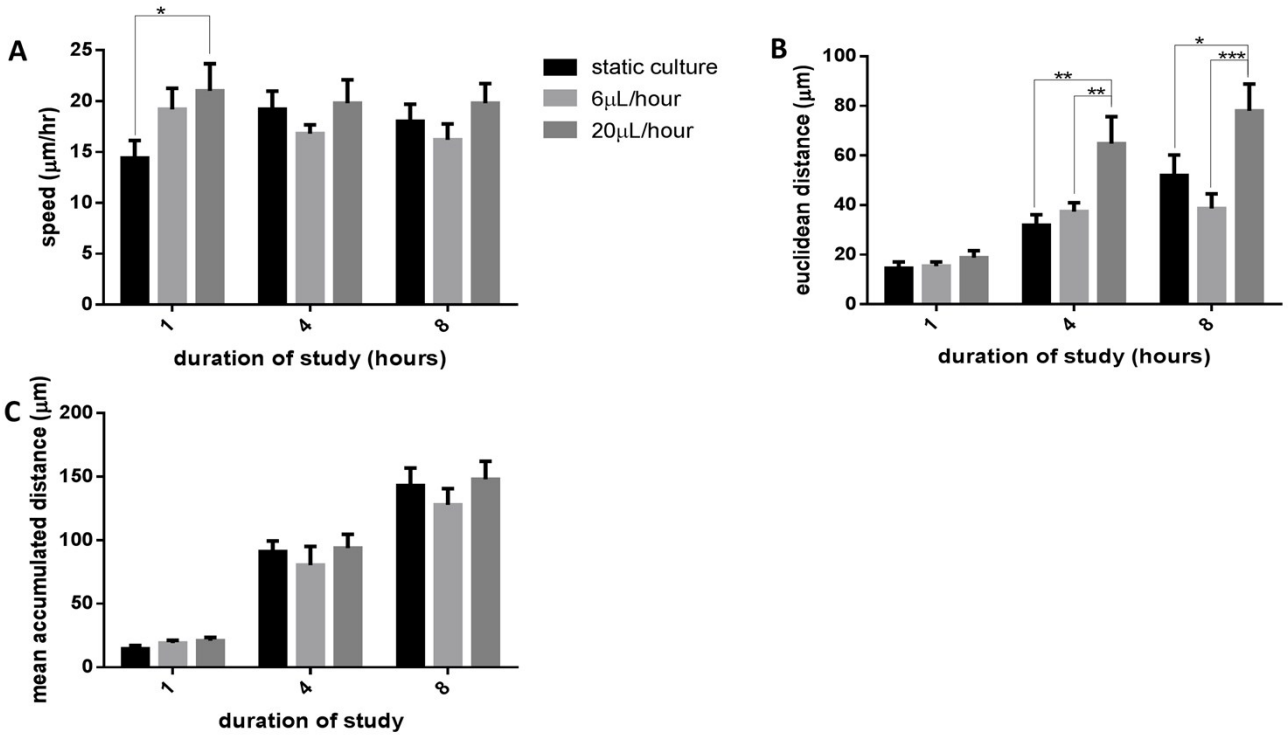
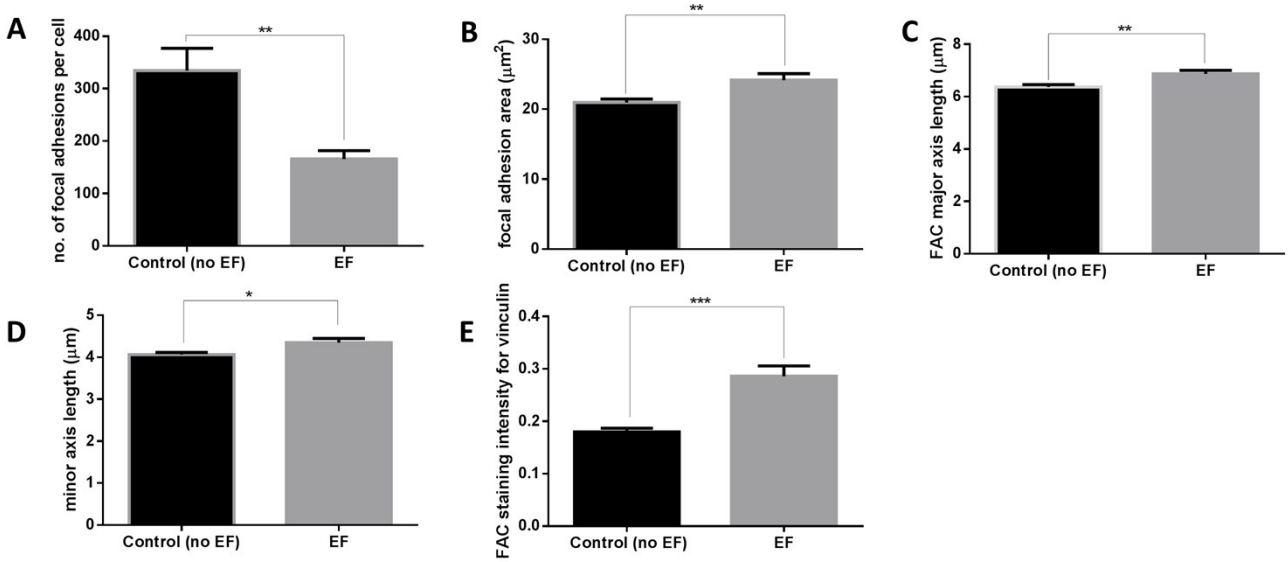


Fig S2. Differences in migration speed (A) or euclidean distance (B) or accumulated distance (C) travelled for static culture, 6μL/hour or 20μL/hour fluid flow rate.



FigS3. For cells stimulated with an EF, FAC were fewer in number per cell, however the FACs that were present were longer and wider and overall larger and stained with greater intensity for vinculin than for control (no EF) cells.

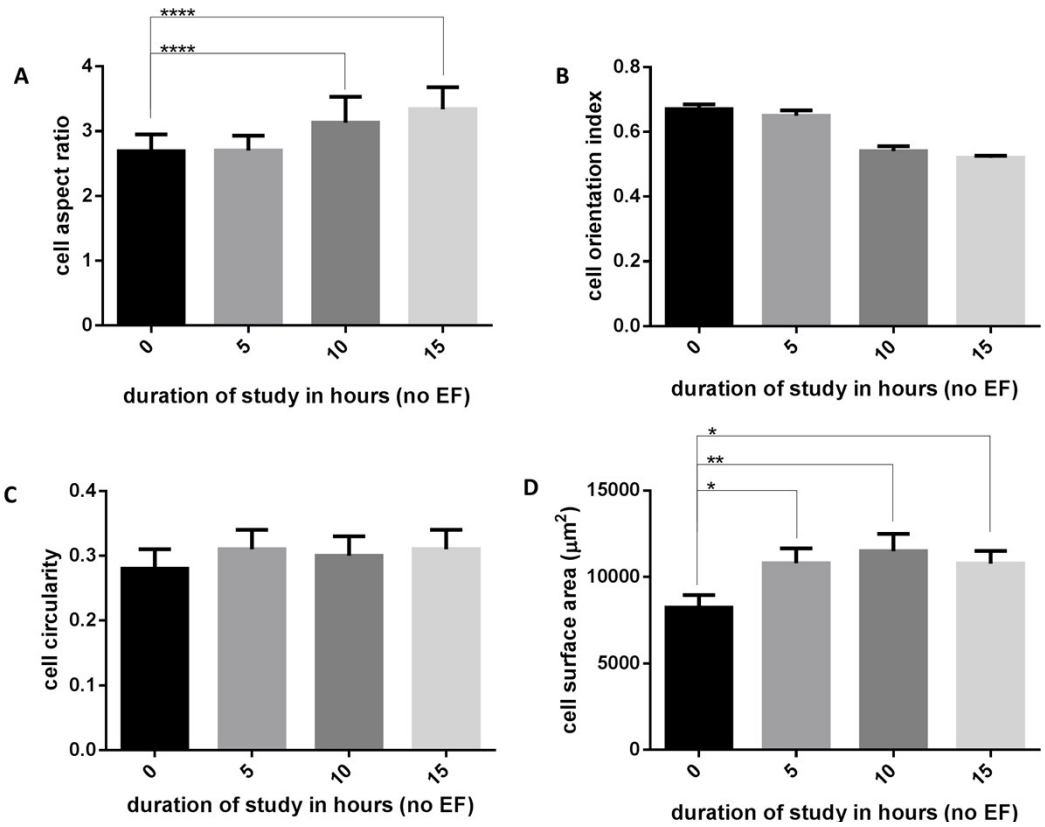
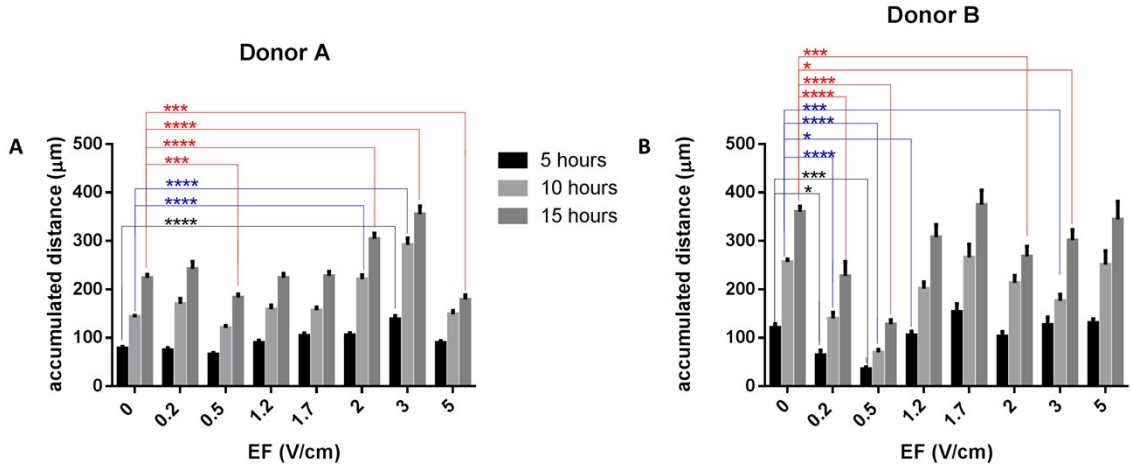


Fig S4. (A) Cell elongation over time was observed with and without exposure to EF, (B) Cells did not align perpendicular to the EF vector without EF exposure, (C) cell circularity did not change in the absence of an EF, (D) attached cell surface area increased over time without EF exposure, in contrast to the decrease in attached surface area found in cells exposed to EFs of ≥1.7 V/cm and ≤3 V/cm.



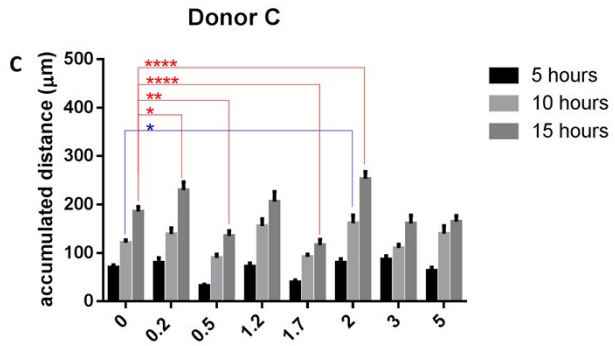


Fig S5. (A) There was a statistically significant increase in the accumulated travel distance for MSC Donor A cells exposed to EFs compared to control cells for 0.5 V/cm at 15 hours, 2 V/cm at 10 and 15 hours, 3 V/cm for all time periods, and 5 V/cm at 15 hours . (B) Donor B cells' control and experimental populations did show differences in accumulated distance (significantly lower in some experimental populations). (C) Donor C cells' control and experimental populations showed significant differences in accumulated distance.

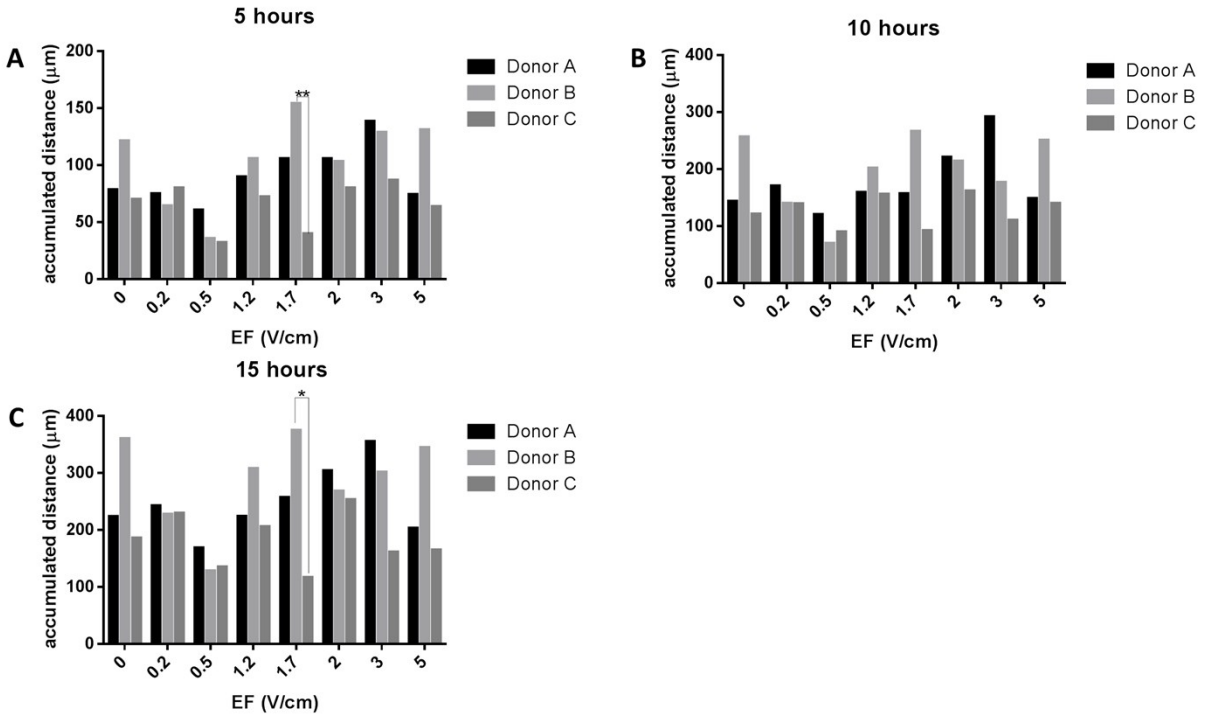


Fig S6. Comparison of various donor MSCs to each other for accumulated distance travelled at (A) 5 hours, (B) 10 hours and (C) 15 hours for various EF applications as shown. Significant differences were found between Donor B and C cells at 5 and 10 hours under a 1.7 V/cm EF.

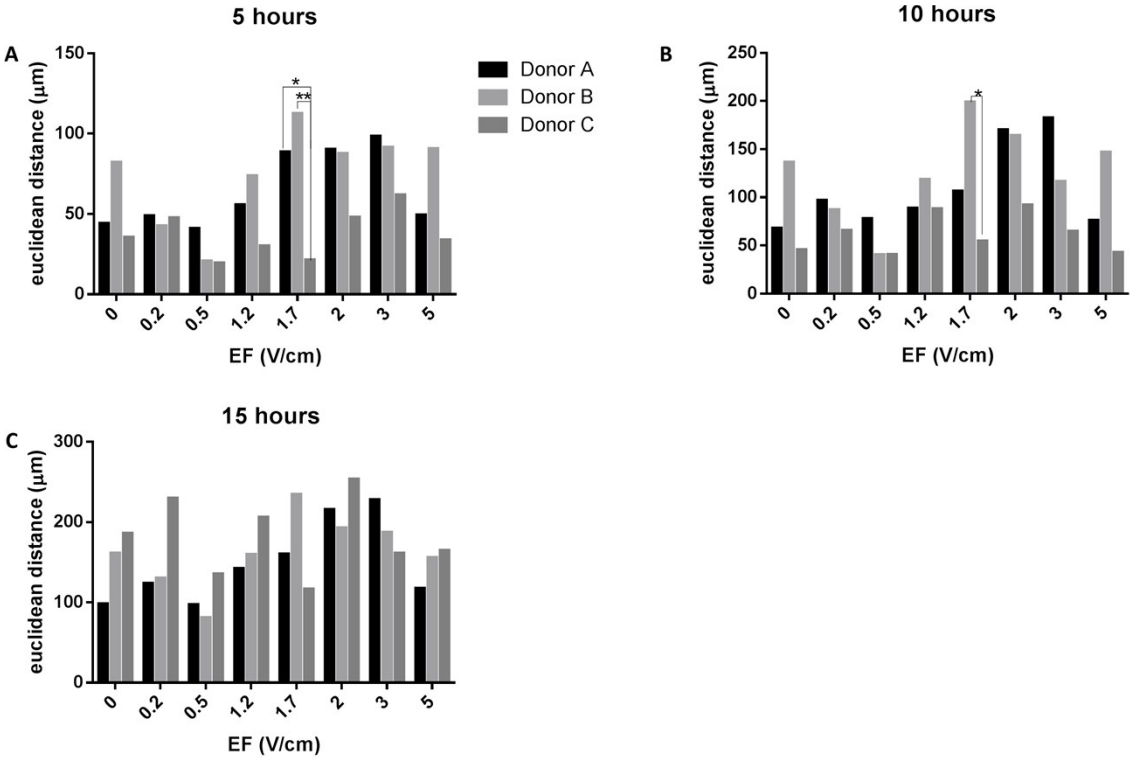


Fig S7. Comparison of various donor MSCs to each other for euclidean distance travelled at (A) 5 hours (B) 10 hours and (C) 15 hours for various EF applications as shown. Significant differences were found between Donor A and C cells at 5 hours, and Donor B and C cells at 10 hours of 1.7 V/cm EF.

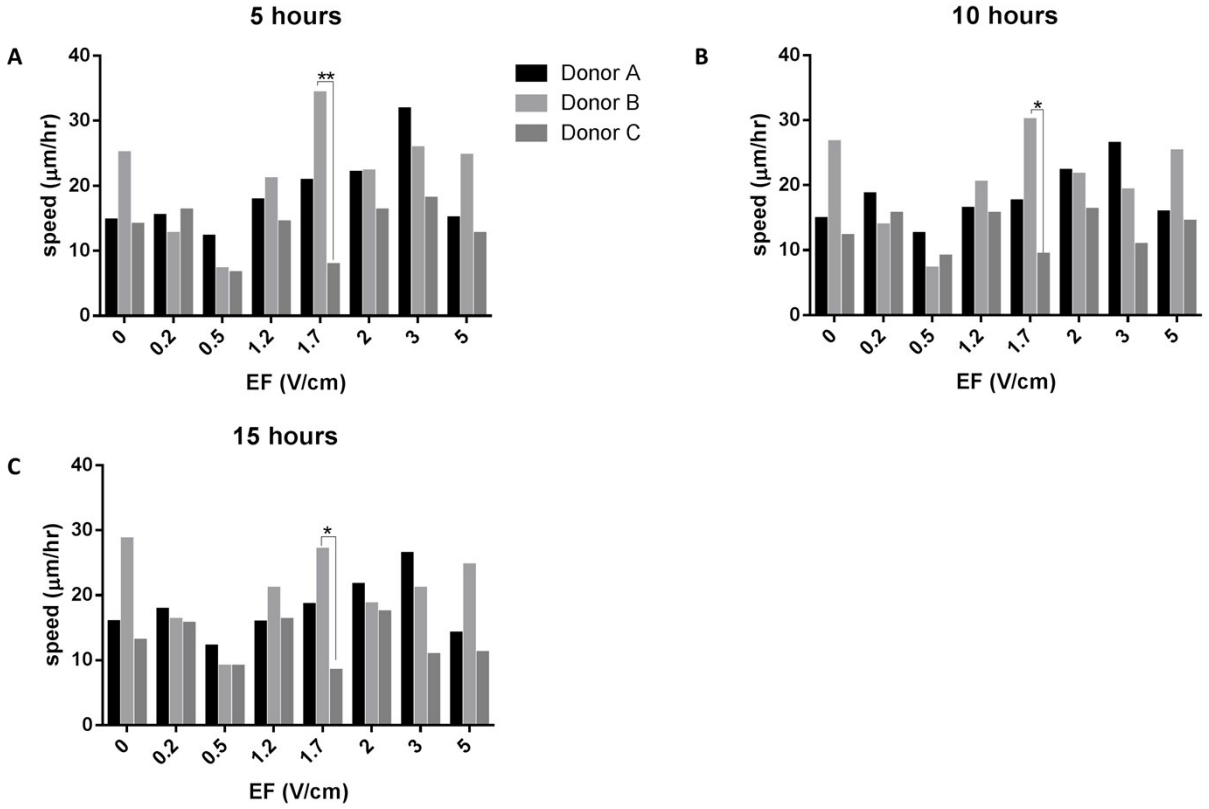


Fig S8. Comparison of various donor MSCs to each other for speed travelled at (A) 5 hours (B) 10 hours and (C) 15 hours for various EF applications as shown. Donor C migrated significantly more slowly than donor B cells under a 1.7 V/cm EF. Note the consistent dip in migration speed at 0.5 V/cm for all donors over all time periods.

Temperature measurements

Cell migration chambers were filled with cell culture media and connected to a customised EF delivery system as before, and kept in a standard 37˚C heated cell incubator. The temperature of the cell migration chamber during 2 or 5 V/cm EF application, or in the absence of EF application, was measured using an infrared laser gun periodically for up to 18.5 hours (device accuracy is quoted as +/- 2 ˚C). There were no significant differences in temperature measured (supplementary figure S9).

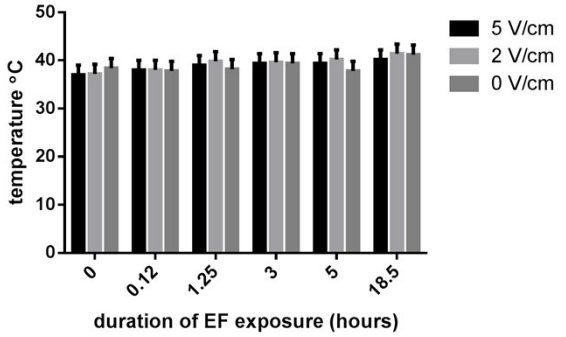


Fig S9. There were no significant differences between time 0 versus 18.5 hours of EF exposure temperature measurements for control devices (no EF), 2 V/cm or 5 V/cm.

Additionally, hBM-MSCs were exposed to the highest EF strength (5 V/cm) with the surrounding temperature heated to 38˚C (1˚C hotter than previous experiments). When comparing accumulated distance, euclidean distance, migration speeds and percentage of cells migrating to the cathode, there were no significant differences between cells treated with a 5 V/cm EF and 37˚C, or the same EF and 38˚C (supplementary figure S10). In a separate experiment, cells were imaged with the surrounding temperature heated to 43˚C for 15 hours in the absence of an EF. These cells migrated further accumulated

distances compared to other conditions, however no other significant differences were seen (supplementary figure S10). Given that cells maintained obvious ability to migrate over a 15 hour period at 43˚C, viability at this temperature was clearly preserved, and as calculated, cell heating does not account for the loss of cellular viability seen at high EF strengths.

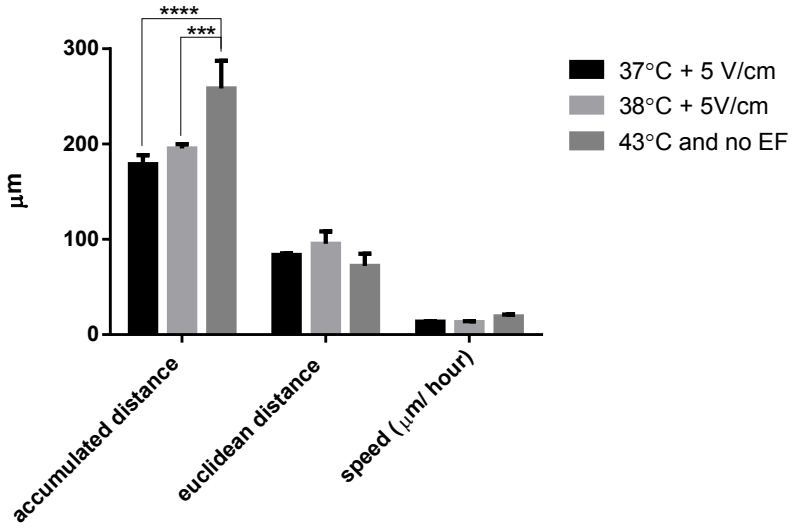


Fig S10. Cell migration distances and speeds over 15 hours at 37or 38˚C under a 5V/cm EF, or at 43˚C and no EF.

Table S2: Control and EF studies of donor A MSC migration

Applied EF	Time (hours)	Mean accumulated distance (µm) ± SEM	Mean euclidean distance (µm) ± SEM	Mean speed ± SEM (µm/hr)	Percentage of cells migrating up/ down	No. of cells counted
0 V/cm (control)	5	78.4 ± 3.3	39.6 ±1	16 ± 0.7	55/45	124
	10	143.6 ± 2.5	67.9 ± 3.5	14.8 ± 0.6	50/50	120
	15	223.5 ± 7.1	98.1 ± 3.8	15.9 ± 0.4	50/50	126
0.2 V/cm	5	74.9 ± 4.3	48.8 ± 3.7	15.4 ± 0.9	58/42	62
	10	170.4 ± 10.8	96.7 ± 7.2	18.6 ± 1.3	67/33	62
	15	242.44 ± 14.9	123.6 ± 11.2	18 ± 1	65/35	68
0.5 V/cm	5	66 ± 3.5	44.8 ± 2.9	13.4 ± 0.7	91/9	87
	10	120 ± 5	77.9 ± 4.9	13.1 ± 0.6	95/5	86
	15	183 ± 7	107.1 ± 6.1	13.1 ± 0.5	96/4	87
1.2 V/cm	5	89.7 ± 5.2	55.8 ± 4.8	17.8 ± 1	95/5	61
	10	159.1 ± 8.2	88.6 ± 6.5	15.4 ± 0.8	90/10	60
	15	223.8 ± 9.1	142.1 ± 7.9	15.8 ± 0.7	100/0	66
1.7 V/cm	5	104. ± 5.3	76.1 ± 5.1	21.4 ± 1.1	100/0	61
	10	156.9 ± 6.3	106.4 ± 5.8	18.4 ± 0.7	95/5	65
	15	227.6 ± 9.1	148.4 ± 8.3	16 ± 0.6	100/0	64
2 V/cm	5	105.7 ± 4.6.	90.1 ± 4.4	22 ± 1	95/5	62
	10	220.9 ± 8.9	170.1 ± 7.8	22 ± 0.8	100/0	67
	15	304.3 ± 11.4	215.5 ± 9.9	21.6 ± 0.7	100/0	61
3 V/cm	5	138.4 ± 7.4	98.3 ± 1.6	31.8 ± 1.5	100/0	63
	10	291.9 ± 13.7	182.4 ± 2.6	32 ± 1.6	100/0	63
	15	355.1 ± 16.9	227.8 ± 2.9	26.4 ± 1.1	100/0	63
5 V/cm	5	89.8 ± 4.3	51.3 ± 4.3	18 ± 0.9	95/5	60
	10	148.4 ± 7.7	75.9 ± 1.5	18.2 ± 0.7	95/5	63
	15	178.9 ± 9.5	83.6 ± 1.8	13.5 ± 0.7	100/0	63

Table S3. Control and EF studies of donor B MSC migration

Applied EF	Time (hours)	Mean accumulated distance (µm) ± SEM	Mean euclidean distance (µm) ± SEM	Mean speed (µm/hr) ± SEM	Percentage of cells migrating up/ down	No. of cells counted
0 V/cm (control)	5	121.2 ± 7.5	82.02 ± 2.9	25 ±0.7	59/41	65
	10	256.6 ± 5.6	136.3 ± 4.3	26.6 ± 0.6	64/36	65
	15	360.07 ± 11.4	161.03 ± 6.3	28.6 ± 10.5	68/32	65
0.2 V/cm	5	64.26 ± 9.9	42.54 ± 6.7	12.6 ± 2	76/24	21
	10	139.83 ± 12.4	86.87 ± 13.1	13.8 ± 1.2	70/30	20
	15	227.52 ± 29.9	130.22 ± 23.3	16.2 ± 2.1	90/10	20
0.5 V/cm	5	35.52 ± 4.2	20.66 ± 3.5	7.2 ± 0.9	54/46	21
	10	69.62 ± 6.4	40.25 ± 7.3	7.2 ± 0.7	86/14	21
	15	128.04 ± 9	80.93 ± 8.6	9 ± 0.7	96/4	20
1.2 V/cm	5	105.77 ± 7.6	73.65 ± 7.7	21 ± 1.6	100/0	21
	10	201.41 ± 14	118.42 ± 13.8	20.4 ± 1.4	91/9	22
	15	307.68 ± 25.8	159.45 ± 18.3	21 ± 1.8	100/0	22
1.7 V/cm	5	154.05 ± 16.1	112.39 ± 13.9	34.2 ± 4.2	100/0	21
	10	266.12 ± 27	198.75 ± 25.4	30 ± 3.5	100/0	20
	15	374.81 ± 30	234.32 ± 24.4	27 ± 2.1	100/0	20
2 V/cm	5	103.28 ± 9.5	87.61 ± 9.5	22.2 ± 2.2	95/5	22
	10	213.7 ± 15	164.13 ± 15.7	21.6 ± 1.4	100/0	25
	15	268.16 ± 20.5	192.59 ± 19.2	18.6 ± 1.3	95/5	20
3 V/cm	5	128.67 ± 16.2	91.44 ± 10	25.8 ± 2.4	100/0	21
	10	176.38 ± 13.4	116.43 ± 12.3	19.2 ± 1.4	100/0	22
	15	301.55 ± 21.4	187.11 ± 18.1	21 ± 1.4	100/0	23
5 V/cm	5	131.08 ± 7.9	90.46 ± 6.8	24.6 ± 1.6	100/0	21
	10	250.95 ± 28.2	146.79 ± 23.9	25.2 ± 3	86/14	21
	15	344.46 ± 36.8	155.66 ± 17.1	24.6 ± 2.2	80/20	20

Table S4: Control and EF studies of donor C MSC migration

Applied EF	Time (hours)	Mean accumulated distance (µm)	Mean euclidean distance (µm)	Mean speed (µm/hr)	Percentage of cells migrating up / down	No. of cells counted
0 V/cm (control)	5	70.08 ± 5	35.44 ± 3.7	14± 1	49/51	60
	10	120.97 ± 6	45.58 ± 4.4	12.2 ± 0.6	43/57	60
	15	185.8 ± 10	59.37 ± 5.3	13 ± 0.6	61/59	60
0.2 V/cm	5	80.11 ± 9.8	47.61 ± 6.6	16.2 ± 1.9	64/36	22
	10	139.07 ± 12.2	65.57 ± 10.7	15.6 ± 2	78/22	23
	15	229.64 ± 16.7	135.67 ± 16.6	15.6 ± 1.2	81/19	26
0.5 V/cm	5	32.09 ± 3.2	19.42 ± 0.7	6.6 ± 0.7	60/40	20
	10	89.67 ± 7.7	40.49 ± 1.5	9 ± 0.7	90/10	20
	15	135.3 ± 10.2	67.06 ± 2.1	9 ± 0.7	100/0	20
1.2 V/cm	5	72.24 ± 6.9	30.04 ± 3.9	14.4 ± 1.3	76/24	21
	10	155.75± 14.2	88.07 ± 14.2	15.6 ± 1.5	90/10	20
	15	205.89 ± 20.7	104.97 ± 15.3	16.2 ± 1.3	76/24	21
1.7 V/cm	5	39.76 ± 4.1	21.28 ± 3.3	7.8 ± 0.8	70/30	20
	10	91.79 ± 5.3	54.54 ± 4.8	9.3 ± 0.6	90/10	21
	15	116.4 ± 11.2	70.04 ± 9.1	8.4 ± 0.8	100/0	20
2 V/cm	5	80.13 ± 7.5	47.87 ± 6.4	16.2 ± 1.5	90/10	20
	10	161.56 ± 16.6	92.02 ± 11.7	16.2 ± 1.7	95/5	22
	15	253.29 ± 14.4	141.26 ± 15.6	17.4 ± 0.9	100/0	22
3 V/cm	5	86.78 ± 7.2	61.82 ± 7	18 ± 1.6	95/5	20
	10	110.15 ± 7.7	64.62 ± 6.2	10.8 ± 0.8	100/0	21
	15	161.04 ± 16.5	76.25 ± 9.6	10.8 ± 1.1	90/10	20
5 V/cm	5	63.47 ± 6.8	33.86 ± 5.5	12.6 ± 1.4	45/55	22
	10	139.86 ± 16.1	42.45 ± 6.1	14.4 ± 1.6	40/60	20
	15	164.73 ± 12	43.09 ± 6	11.1 ± 0.7	24/76	21

Table S5: Reported cellular migration speeds increase with higher EF strengths.

Author	Cell type	EF strength (V/cm)	Mean migration speed (μm/hr)
[4]	hNSCs derived from a hES cells	0	58
		3	76
[5]	CHO cells	0	8
		3	17
[6]	Rat osteoblasts	1	3
		10	5-32
	Rabbit osteoclasts	1	72
		10	138
[7]	Human mammary cancer cells	0	16
		1-4	18-25
	Rat mammary cancer cells	0	25
		1-2	32-45
[8]	Mouse 3T3 fibroblasts	0	10
		4	18
		6	22
[9]	Bovine chondrocytes	0	6
		4	9
		6	14

Supplementary Literature Cited

1. T. Glawdel, C. L. Ren, Electro-osmotic flow control for living cell analysis in microfluidic PDMS chips. *Mechanics Research Communications* 2009, *36*. 75-81, DOI: http://dx.doi.org/10.1016/j.mechrescom.2008.06.015.
2. B. J. Kirby, *Micro-and nanoscale fluid mechanics: transport in microfluidic devices.* . Cambridge University Press: 2010.
3. A. P. Pandhare, S. S. Jadhav, *Fluid Mechanics*. Technical Publications: 2009.
4. J. F. Feng, J. Liu, X. Z. Zhang, L. Zhang, J. Y. Jiang, J. Nolta, M. Zhao, Guided migration of neural stem cells derived from human embryonic stem cells by an electric field. *Stem Cells* 2012, *30*. 349-55, DOI: 10.1002/stem.779.
5. J. Pu, M. Zhao, Golgi polarization in a strong electric field. *J Cell Sci* 2005, *118*. 1117-28, DOI: 10.1242/jcs.01646.
6. J. Ferrier, S. M. Ross, J. Kanehisa, J. E. Aubin, Osteoclasts and osteoblasts migrate in opposite directions in response to a constant electrical field. *J Cell Physiol* 1986, *129*. 283-8, DOI: 10.1002/jcp.1041290303.
7. J. Pu, C. D. McCaig, L. Cao, Z. Zhao, J. E. Segall, M. Zhao, EGF receptor signalling is essential for electric-field-directed migration of breast cancer cells. *Journal of cell science* 2007, *120*. 3395-403, DOI: 10.1242/jcs.002774.

8. E. Finkelstein, W. Chang, P. H. Chao, D. Gruber, A. Minden, C. T. Hung, J. C. Bulinski, Roles of microtubules, cell polarity and adhesion in electric-field-mediated motility of 3T3 fibroblasts. *J Cell Sci* 2004, *117*. 1533-45, DOI: 10.1242/jcs.00986.
9. P. H. Chao, R. Roy, R. L. Mauck, W. Liu, W. B. Valhmu, C. T. Hung, Chondrocyte translocation response to direct current electric fields. *J Biomech Eng* 2000, *122*. 261-7.