Supplementary materials

Electric Egg-Laying: A New Approach for Regulating *C. elegans* Egg-Laying Behaviour in a Microchannel using Electric Field

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1. Numerical Simulation of Electric Field in the Microfluidic Device

The analysis of the electric field (EF) distribution and uniformity in our microfluidic device was conducted through a two dimensional (2D) numerical simulation. Using the steady-state direct-current (DC) electric module of the COMSOL Multiphysics® software, Ohm's law was solved to determine the EF distribution through the conductive media within the microchannel. The design of the microfluidic features was done in SOLIDWORKS® and consequently analyzed by COMSOL after determination of the boundary conditions and the grid formation on the 2D model. The device comprised of a 3 cm-long and 300 μm-wide channel containing a 100 μm-wide trap in the middle of the design, acting as an on-chip trap for the worm (Figure 1A in the main paper). M9 conductivity was determined experimentally in a similar 300μm-wide channel and measured to be 1.6 siemens/m, a parameter that was added to the numerical model. An electrical insulation boundary condition was applied to all the boundaries of the device. The two end reservoirs were set as an 8V electric potential and the ground, respectively. Grid formation was achieved by using the built-in mesh generation module of COMSOL Multiphysics software, applying a triangular mesh to the features. Figure S1A depicts the EF distribution within the device and the on-chip trap. Figure S1B is showing the EF distribution along line A-A, confirming that the EF is uniform at 2, 4, 6, and 8 V/cm for on-trap exposure. Electric potential of 2.25, 4.5, 6.9 and 9 V were found to be respectively applied at the end-reservoir to achieve the 2, 4, 6, and 8 V/cm EFs required in the trap,

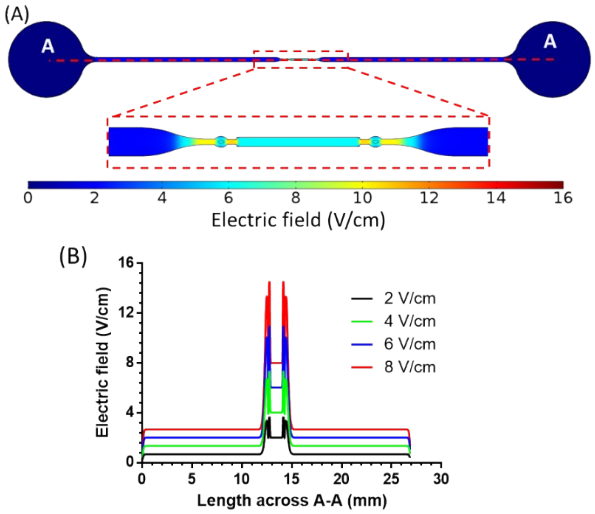

Figure S1: COMSOL simulation of electric field distribution in the microfluidic device when a 7V stimulus was applied along the channel. (A) Electric field distribution in the chip with an inset showing the electric field distribution in the trap region. (B) Electric fields along line A-A in various conditions achieved by applying an electric potential across the reservoirs.

2. Effect of EF exposure on *C. elegans physiology*

Ten worms were randomly collected out of the chip after being exposed to 6V/cm for 10 min (5s on, 25s off pulses) to compare their locomotion behaviours to a group of worms not exposed to EF. At the first glance, the worms were normally swimming in M9 buffer with no apparent morphological defects or abnormal movements. Quantitative analysis in Figure S2 also shows that there is no significant difference in the average speed and body bend frequency of the EF exposed worms compared with the control group. Although these results suggest that the effect of EF in a short time after experiments may be benign on *C. elegans*, but further viability and lifespan experiments are needed if the exposed worms are to be used for extended experiments or off-spring studies.

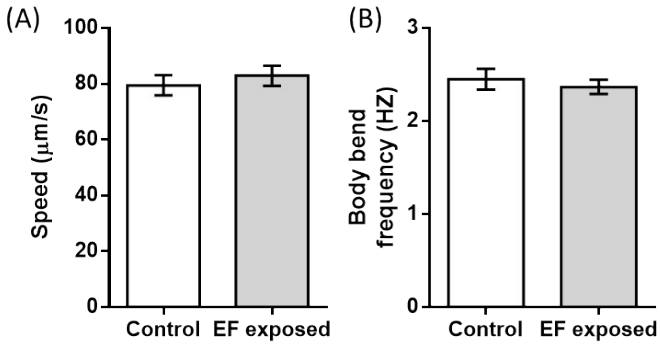

Figure S2: Effect of EF pulses on C. elegans movement behaviour. EF pulses of 6V/cm for 5s on and 25s off were applied for 10 minutes and the (A) speed and (B) body bend frequency of the worms post exposure were compared to the controls with no EF exposure.

3. Comparison of Contraction and Relaxation Rate Constants of Electrical and Optogenetic Methods

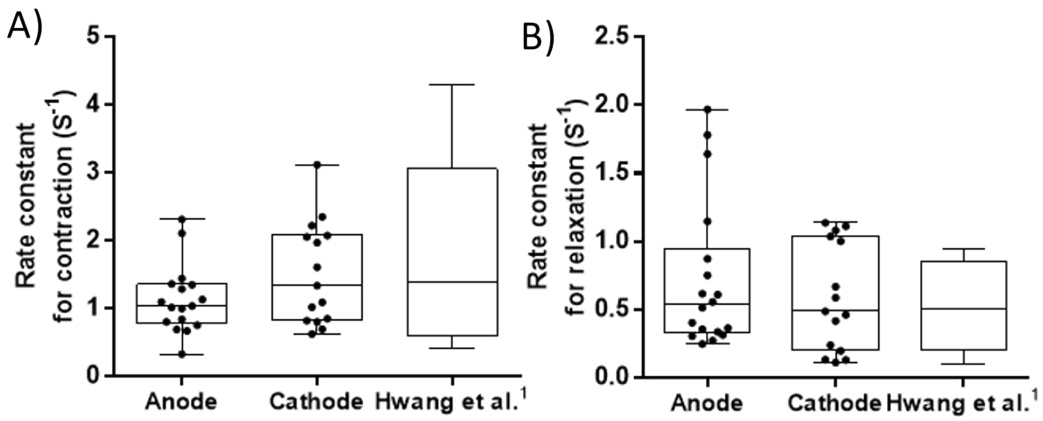

Figure S3: Rate constant for (A) contraction and (B) relaxation of C. elegans muscles using electrical stimulation (our technique) and optogenetics[1]

4. Supplementary Videos

Video S1: Image processing for determination of worm centerline, head and tail movement in pulses of 5s on and 25s off at EF of 6 V/cm

Video S2: EF effect on D1 worm inside a 300µm-wide channel at EF of 6V/cm for 5s pulse duration.

Video S3: The effect of EF direction on *C. elegans* egg-laying behaviour inside our microfludic device at EF of 6V/cm for 5s pulses of anodal and cathodal head stimulation.

Video S4: The effect of EF pulse duration on the egg-laying behaviour at EF of 6V/cm for 40s pulse duration.

Video S5: Vulva muscles Ca^{2+} transients during anodal stimulation at EF of 6V/cm in pulses with 5s on and 25s off cycles.

Video S6: Vulva muscles Ca^{2+} transients during cathodal stimulation at EF of 6V/cm in 5s pulses.

Video S7: HSNs Ca^{2+} transients during anodal stimulation at EF of 6V/cm in 5s pulses.

Video S8: HSNs Ca^{2+} transients during cathodal stimulation at EF of 6V/cm in 5s pulses.

Video S9: VCs Ca^{2+} transients during anodal stimulation at EF of 6V/cm in 5s pulses.

Video S10: VCs Ca^{2+} transients during cathodal stimulation at EF of 6V/cm in 5s pulses.

Video S11: The effect of EF on the worms' egg-laying behaviour for MT1082 strain.

5. References

1 H. Hwang, D. E. Barnes, Y. Matsunaga, G. M. Benian, S. Ono and H. Lu, *Scientific reports*, 2016, **6**, 19900.