Electronic Supplementary Material (ESI) for Lab on a Chip

Electrokinetic confinement of axonal growth for dynamically reconfigurable neural networks

Supplementary information

Axon length over time with and without field application

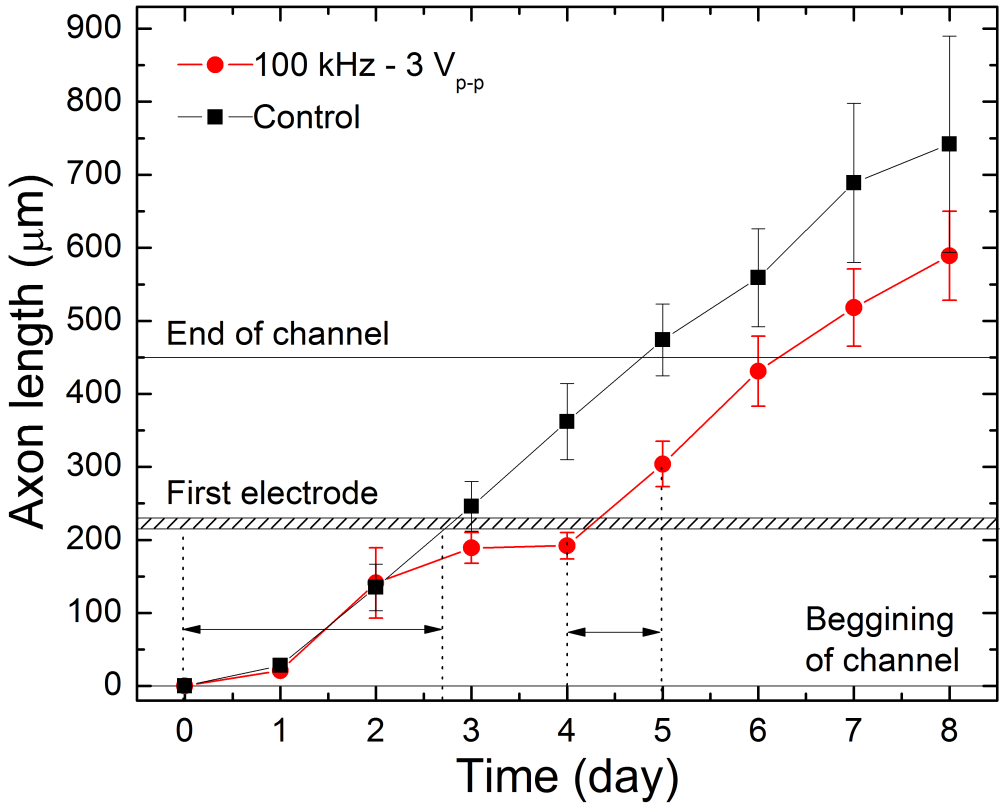

Fig.S1: Length of axons over time in the platform for a control experiment (no field application) and for axons blocked by the AC electrokinetic effect. The distances to the first electrode and to the end of the channel are highlighted. Axons coming from the main body compartment reach the first electrode in ca. 3 days whereas axon elongation after releasing axons from AC blocking takes less than 1 day. The results strongly suggest that observed axon elongation between 4 DIV and 6 DIV results from blocked axon elongation and not new dendrites growing from the cell body compartment.

Joule heating around the electrodes

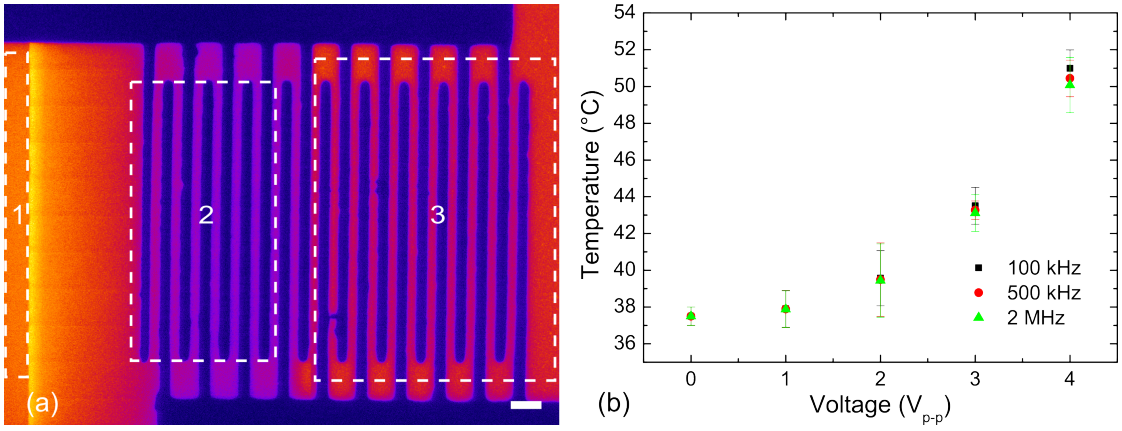

Fig.S2: Measurement of temperature around the electrodes using Rhodamine B, a fluorophore with temperature-dependent fluorescence intensity (1 mM in culture medium).

a) False-color image of Rhodamine B around the electrodes. The color represents the spatial distribution of the temperature. Rhodamine B fluorescence intensity decreases with increasing temperature, so the dark-colored areas indicate a higher temperature compared to the orange ones. Hence, the electrode-free microfluidic chamber (area 1) is orange (cooler) whereas the microchannel with electrodes (area 3) is red (warmer). The highest temperature area can be seen where the electrodes are bonded to the PDMS (area 2), and more specifically in the grooves and closed to the electrodes (left-hand side of area 2), where the axons are found not be growing anymore at voltages > 3.5 V_{p-p}. Scale bar indicates 50 μm.

b) Plot of the extracted temperatures. Each point consisted in applying the voltage for 5 seconds at a given frequency and taking an image (500 ms exposure time, fixed camera gain) with TRITC filter set of three different interdigitated electrodes, each on a temperature-controlled stage set to 37.5 °C. For each image, fluorescent intensities from an area of 240x480 μm (area 2) were averaged spatially and across the three electrodes, and compared to a calibration curve. Calibration was performed using a hotplate to determine the temperature dependence of the Rhodamine B fluorescence intensity, obtaining a measured slope consistent with ones observed in literature (-1.3%/°C). The measured temperature is independent of frequency and increases supralinearly with voltage, which would be expected for Joule heating.

Clausius-Mossotti factor of the growth cone

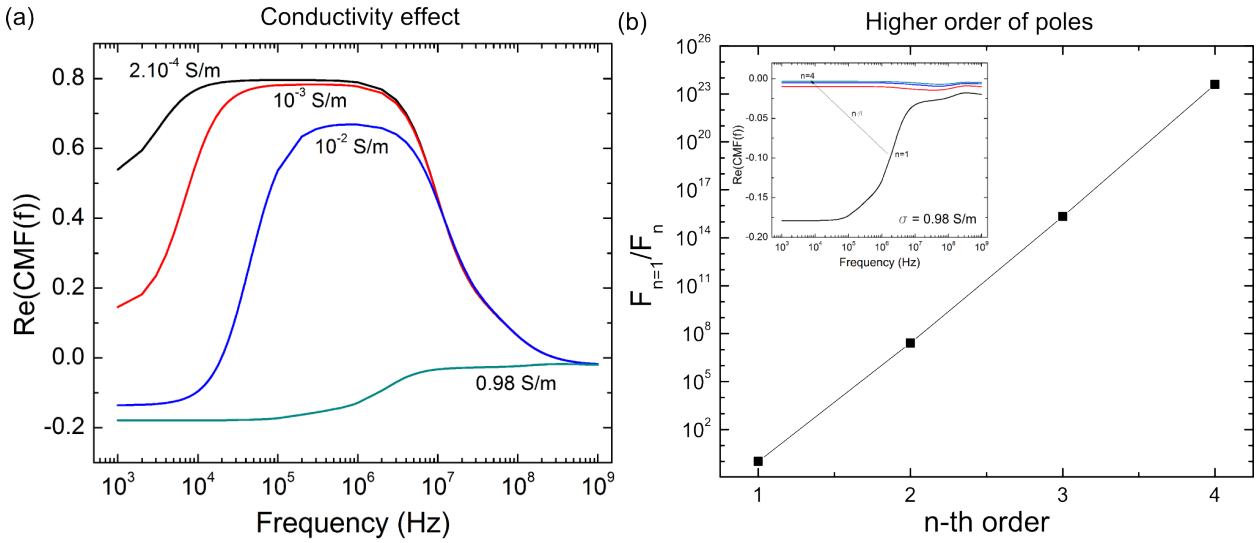

Fig.S3: Simulated Clausius-Mossotti factor of the growth cone model: (a) first-order (dipole) model for several media conductivities and (b) ratio between first-order DEP force and the n-th-order force for increasing numbers of multipoles at neuronal media conductivity. The inset plot represents a detailed view of the n-th order multipole of the CMF of the growth cone. The higher orders of the DEP forces are several orders-of-magnitude smaller than the first-order force.

Forces

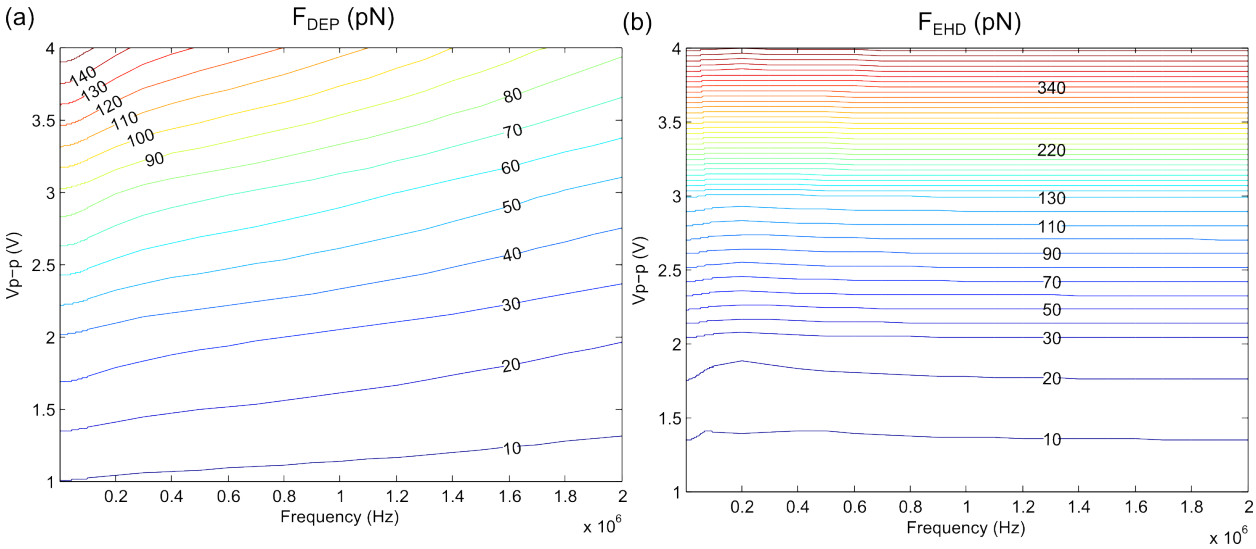

Fig.S4: Simulated values of the dielectrophoretic (F_{DEP}) and electrohydrodynamic (F_{EHD}) forces across the range of frequencies and voltages used in the study. The EHD force has an almost -constant response across frequency, whereas the DEP force magnitude is strongly frequency dependent in the 200 kHz-1 MHz range, which is consistent with the trends observed experimentally.

In vitro platform to apply AC electric signals inside the incubator

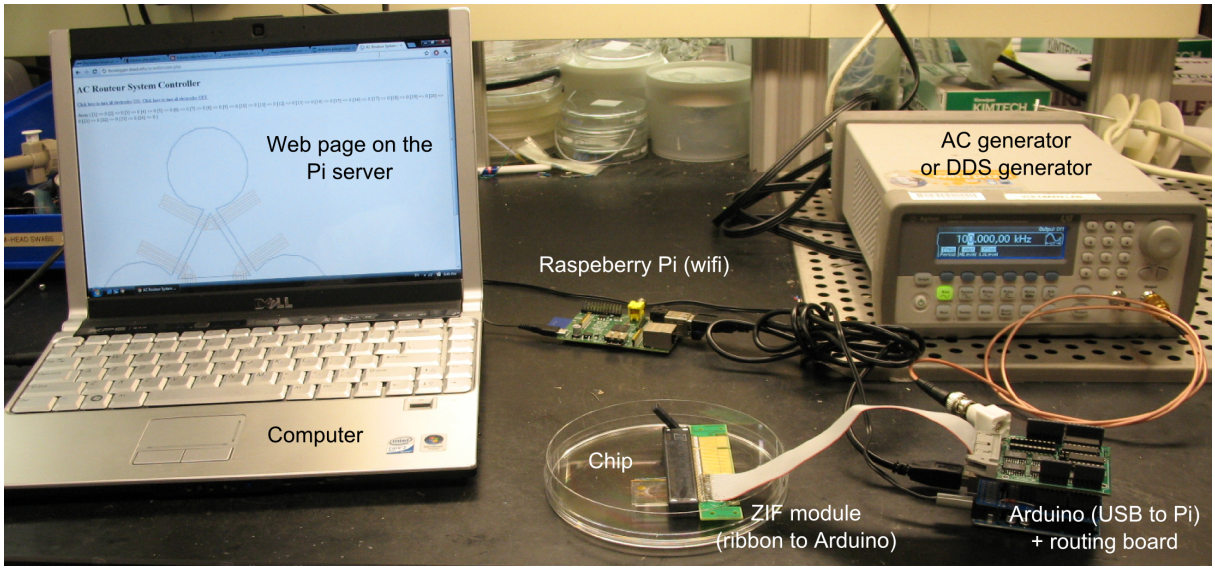

Fig.S5: Image of the electrical stimulation platform. The chip is connected to a Zero Insertion Force connector that acts as a mechanical and electrical holder. The electrical signals are selectively send to each electrode via a home-made arduino board composed of electrical switch and shift registers. The commands to close switches are sent to the arduino by a Raspebbry Pi on which a web-server is constantly running. Both arduino boards and ZIF connectors are finally situated in the incubator.

Flow compartmentalization for local stimulation

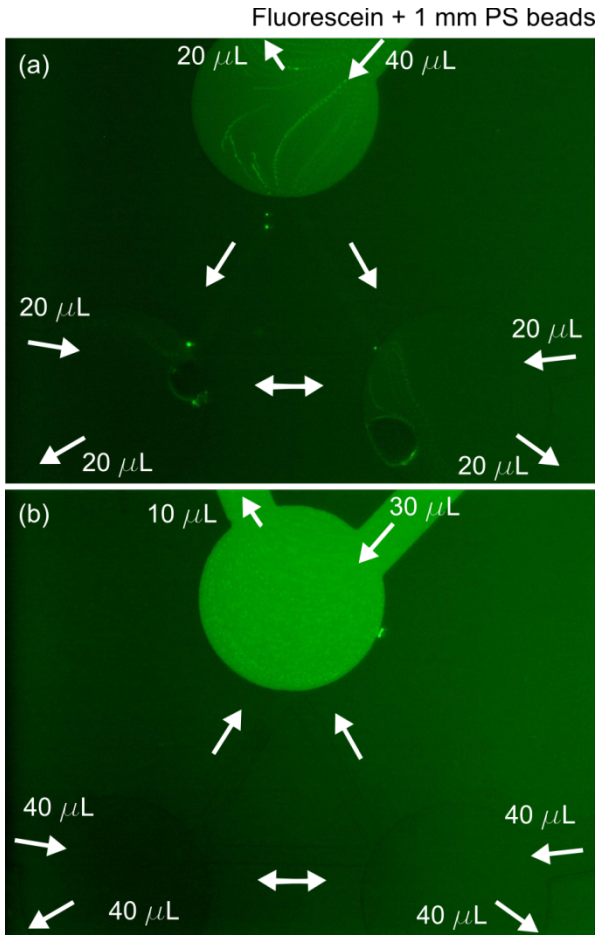

Fig.S6: Compartmentalization of flow by using differential hydrostatic pressure. The pressures are induced by the volume of liquid placed in the inlet and outlet reservoirs connected to each microchamber. Therefore it is possible, by placing the right liquid volume, to direct flow from one microchamber to the other and hence to contain the local application of a chemical to one population of neuron only. Fluorescent images of the three microchambers (a) when the flow is not confined, and (b) when the flow is confined. The arrows indicate the direction of the flux and the volumes the amount of liquid inserted in the in/outlets. The flow was visualized by injecting 10 μM fluorescein and 1 μm fluorescent polystyrene beads in one reservoir.

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Movie of compartmentized excitation of sub-population of the neuron network

The movie presents the ΔF/F0 Oregon-green BAPTA 1 fluorescence intensity of the neuron network. Each sub-population of neurons is excited one after the other by selectively applying 90mM KCl solution.