Lab on a Chip

Supplementary Information – Experimental Data

Title: Microfluidic system for toxicity testing with integrated electroosmotic pumps, concentration gradient generator and fish cell line (RTgill-W1) – towards water toxicity testing

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Electroosmotic Pump Principles

Here the operating mechanisms of EO pumps are briefly explained. For a more detailed explanation, the reader may consult numerous sources on the subject[1-3]. Consider the simplified schematic of the planar microchannel-based EO pump presented in Fig. 1 of the journal article. The general governing equations for the pressure, P [Pa] and the flow rate, Q [$m^3 \cdot s^{-1}$], generated by planar EO pumps can be derived by applying simplified compact circuit models for the electric and fluid flow fields:[4-6]

$$Q = \frac{IR_e}{R_{eof}}\left(\frac{R_p}{R_p + R_L}\right) \quad (1)$$

$$P = QR_L \quad (2)$$

where I [A] is the applied electric current, R_e [ohms] is the electrical resistance of the EOF channel, R_{eof} [$V \cdot s \cdot m^{-3}$] the electroosmotic resistance, R_p [$Pa \cdot s \cdot m^{-3}$] the hydrodynamic resistance of the pump, and R_L [$Pa \cdot s \cdot m^{-3}$] the hydrodynamic resistance of the pump load:

$$R_e = \frac{L}{\sigma wh} \qquad R_{eof} = \frac{L}{\mu_e wh} \qquad R_p = \frac{12\mu L}{wh^3} \quad (3)$$

where L [m] is the channel length, w [m] the width and h [m] the height, σ [$S \cdot m^{-1}$] is the fluid conductivity, μ_e [$m^2 \cdot s^{-1} \cdot V^{-1}$] the electroosmotic mobility and μ [$Pa \cdot s$] the viscosity. In this study, the pump load for the EO pumps is the concentration gradient generator chip. The more familiar Q-P curve for EO pumps can be derived from the above equations:

$$Q = Q_{\max}\left(1 - \frac{P}{P_{\max}}\right) \quad (4)$$

where Q_{max} is the maximum flow rate (under no load, *i.e.* the gradient generator is absent from the system) and P_{max} is the maximum pressure (under infinite load, *i.e.* the gradient generator has an infinitely resistance) at a specified current.

Direct EO pump Connection Design with Cell Viability

Our previous publication[6] introduced the concept of using EO pumps directly in contact with living cells as shown in Figure S1. The pumps work in a vacuum mode, drawing fluid from the inlet of the cell chambers, over the cells and then through the pump. The voltage is applied in a V- to GND configuration where GND is on the cell chamber side so that the cells do not experience an abnormal electric potential. Pumping culture media is not ideal for EO pumps because the high saline content results in poor performance (low electroosmotic mobility, high current draw, electrolysis). Hence this setup represents an extreme operating condition for the EO pumps.

Cell culture experiments were performed with RTgill-W1 cells to test the EO pump influence. The culture media was a basic solution of L15ex (no FBS). Cells were seeded in fibronectin treated cell chambers for a period of 2.5 hrs in static conditions. The EO pumps were operated at 0.5 µL min^{-1} for a period of 18 hrs. To reach these operating times large reservoirs were used (2 mL) with a strong buffer (1xBufferAll

with 2 µg L^{-1} $NaHCO_3$ titrated to pH 7 with 0.1M NaOH, Sigma Aldrich) to delay electrolysis effects. However, the operation time is greater than the expected buffer depletion of approximately 10 hrs. During the experiments the cells were monitored continuously using a phase contrast microscope.

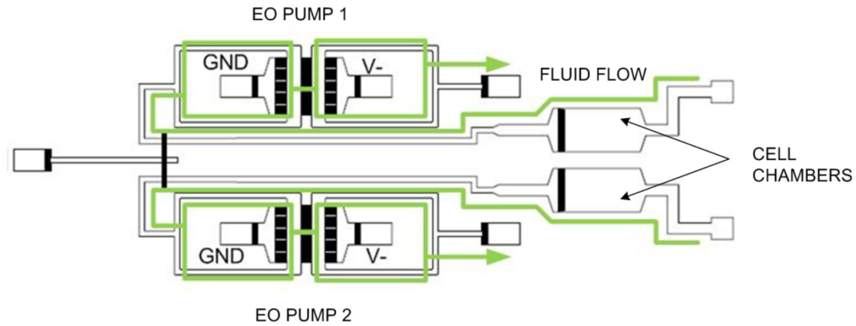

Fig. S1 Schematic illustration of the direct connect EO pump system. Two EO pumps are connected with cell chambers. The EO pumps draw fluid from the inlet of the cell chambers over the cells and to the outlet of the EO pumps. The pumps operate in a V- to GND configuration.

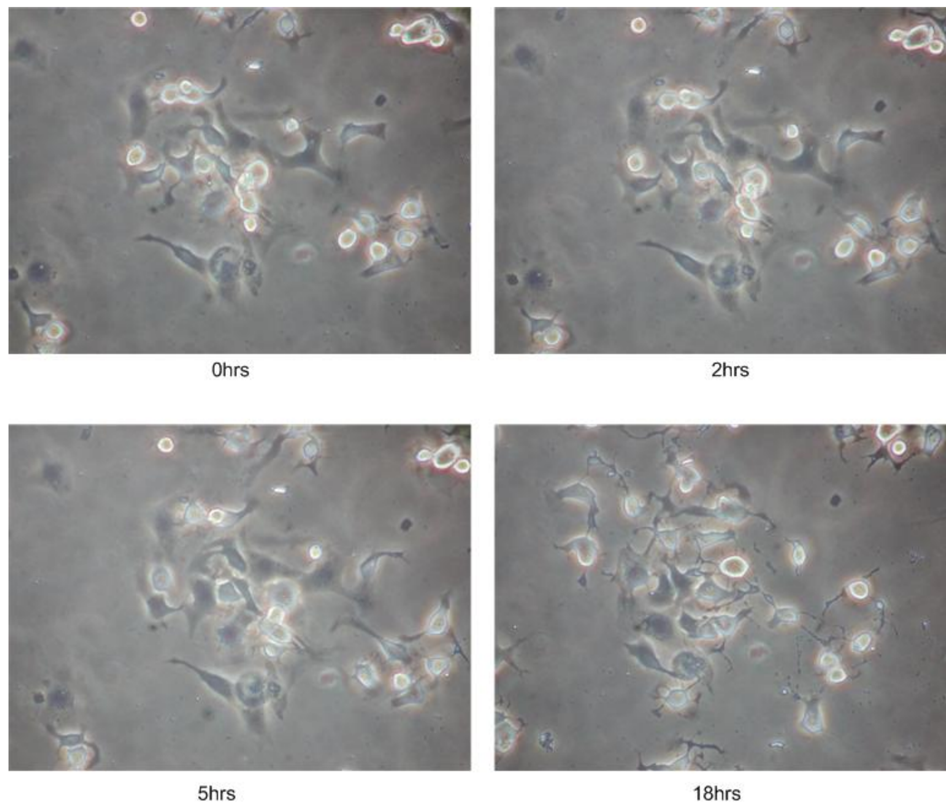

Fig. S2 Phase contrast images of cells while EO pump perfusion was applied with L15ex medium. The same group of cells was photographed at several time intervals after starting perfusion (a) 0hrs (b) 2 hrs (c) 5hrs and (d) 18hrs.

Fig. S2 shows successive images of a single group of cells in the chamber at different times (t=0, 2, 5, and 18 hrs) over the course of the experiment. Considering the first 5hrs, the cells remain viable and continue to attach and spread out on the surface. The rate of spreading is not as dramatic as would be expected if

the cell culture medium contained feeding solution such as FBS. In the image at 18hrs, the cells appear to still remain alive but have begun to recede from the surface preparing for detachment. The reason for this is that the EO pump electrolysis limit had been exceeded and the pump stopped working approximately 8hrs into the experiment (see Figure S3). Therefore, the cells did not receive any newly perfused media for a period of 10hrs. However, during the first 8 hrs the cells showed no ill effects to the EO pumps and it can be gathered that EO pumps can be used with living cells, even if they are in direct contact with the EO pump fluid.

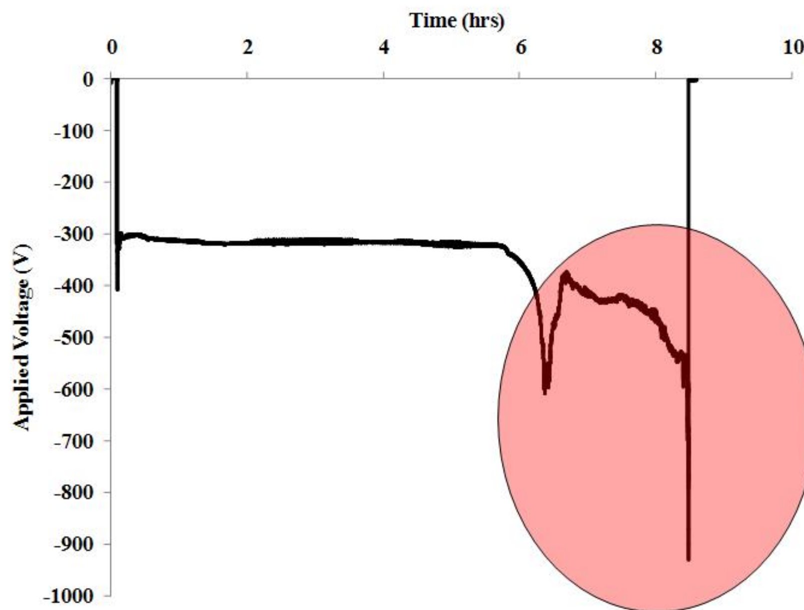

Fig. S3 Voltage plot of EO pump during cell culture test with the EO pumps directly connected. Highlighted area shows the drastic increase in applied voltage caused by electrolysis effects once the buffer in the reservoirs is depleted.

Voltage Current Data from EO Pump

The voltage and current draw from the EO pumps was also recorded during Q-P characterization. The trace data for long term pumping is presented in Figure S4 and corresponds to the flow rate data presented in Figure 4D of the manuscript. Current fluctuations are minimal as the voltage supply is able to effectively regulate the current. The voltage fluctuates approximately 10V around a baseline of 220V over a period of 3 hrs. The voltage steadily increases with time due to the drop in conductivity at the anode side because of electrolysis.

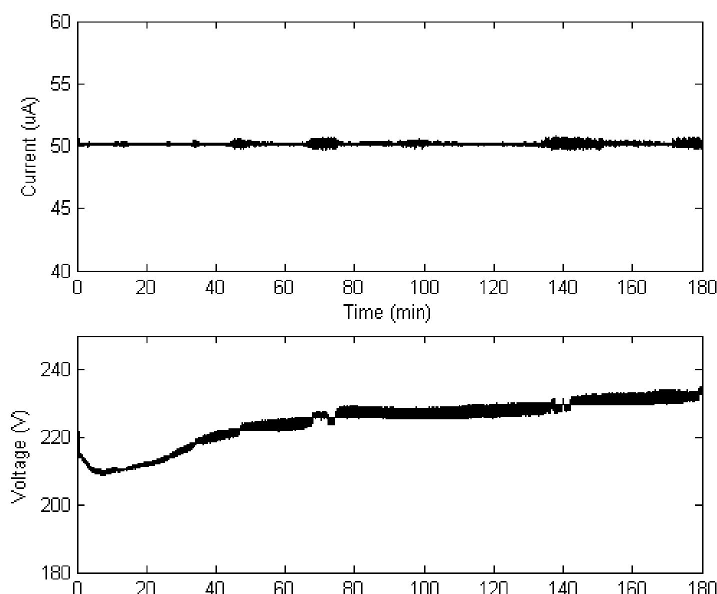

Fig. S4 Current and voltage trace for EO pump under long term pumping corresponding to the data in Figure 4d of the journal article.

Conventional SDS Toxicity Tests

SDS toxicity tests with RTgill-W1 cells were repeated in standard microtitre plates to compare with the chip based assay. Experiments were performed under conditions as close as possible to the chip assay. RTgill-W1 cells were seeded in microtitre plates with L15ex and allowed to attach for only 4hrs. Afterwards, the cells were exposed for 15, 30 and 60 minutes to a range of SDS concentrations. Cell viability was monitored using a fluorometric assay based on Alamar blue (AB) which is a non-fluorescent dye that gets converted by intracellular esterases into a fluorescent product.[7] The experiments were performed in 8 well replicates. Figure S5 summarizes the % of dead cells for different concentrations of SDS at the exposure times.

The EC50 for SDS at 15min was 60.64 µg/ml; at 30min 55.95 µg/ml and at 60min 35.22 µg/mL. In comparison, the EC50 data for the chip based assay was >50 µg/ml, 50 µg/ml and 37.5 µg/ml

respectively. Therefore, the results obtained with the chip based assay are in excellent agreement with the conventional assay.

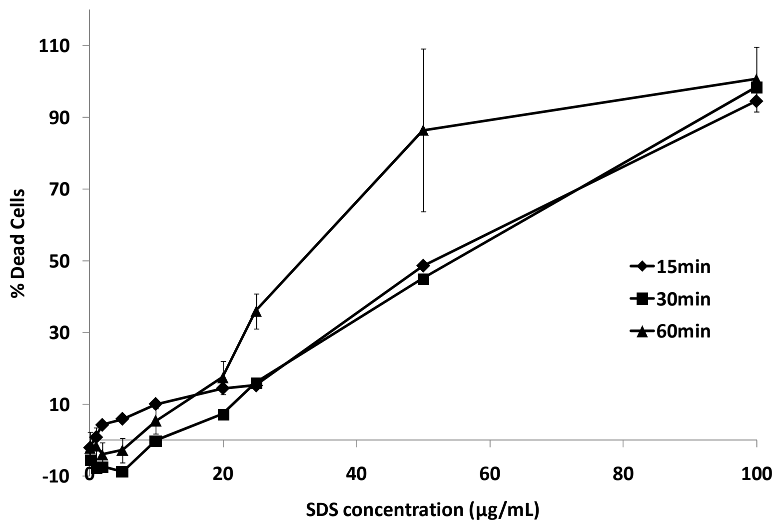

Fig. S5 Results of Almar Blue assay in microwell plates at different SDS concentrations and exposure times.

References

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