Integrated Microfluidic Cell Culture and Lysis on a Chip: Supplemental Methods, Figures, and Video Captions

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Supplemental Methods

A 4 ng/µl plasmid DNA solution was prepared. This plasmid contained the HIV gene, *nef*. Two aliquots of this plasmid solution were passed through a device with the power supplied at zero volts and 6 volts. These solutions were collected from the output. The *nef* gene was then PCR amplified using 3 µl (12 ng) of the 0V plasmid DNA and 6 V plasmid DNA as a template. Positive and negative control PCR reactions were also performed using a plasmid DNA solution that did not pass through a device as a template and a solution without a PCR template, respectively. Equal amounts of these PCR products were ran out on a 1.2% SYBR Safe Agarose Gel (Invitrogen E-Gel) and imaged on an Invitrogen Safe Imager.

Supplemental Figures

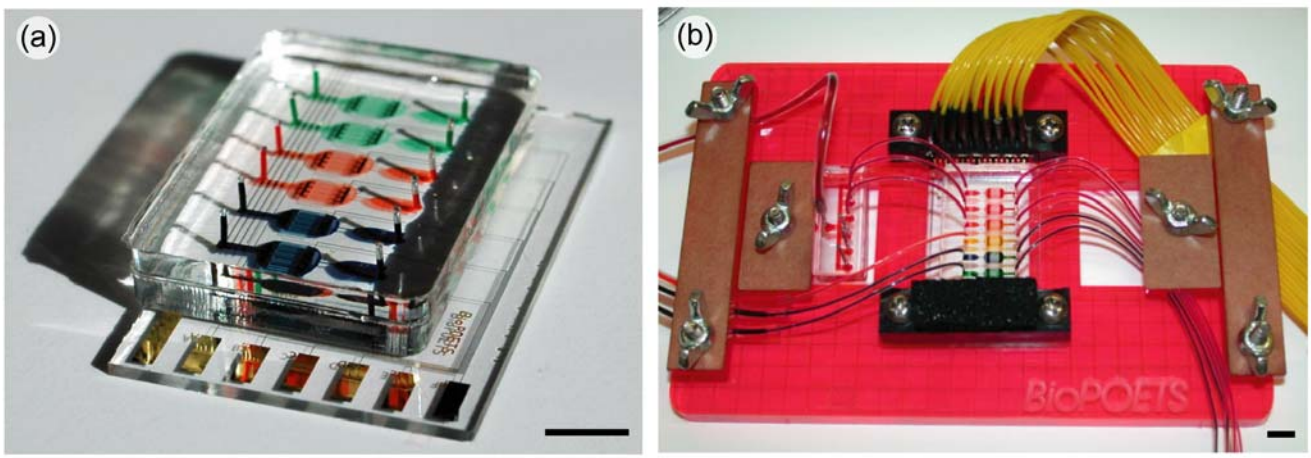

Fig. S1 System integration. (a) Image of a chip with six individual devices aligned to electrodes and filled with food coloring. (b) The platform for holding the chip and making electrical connections is shown here. Scale bars are 6 mm.

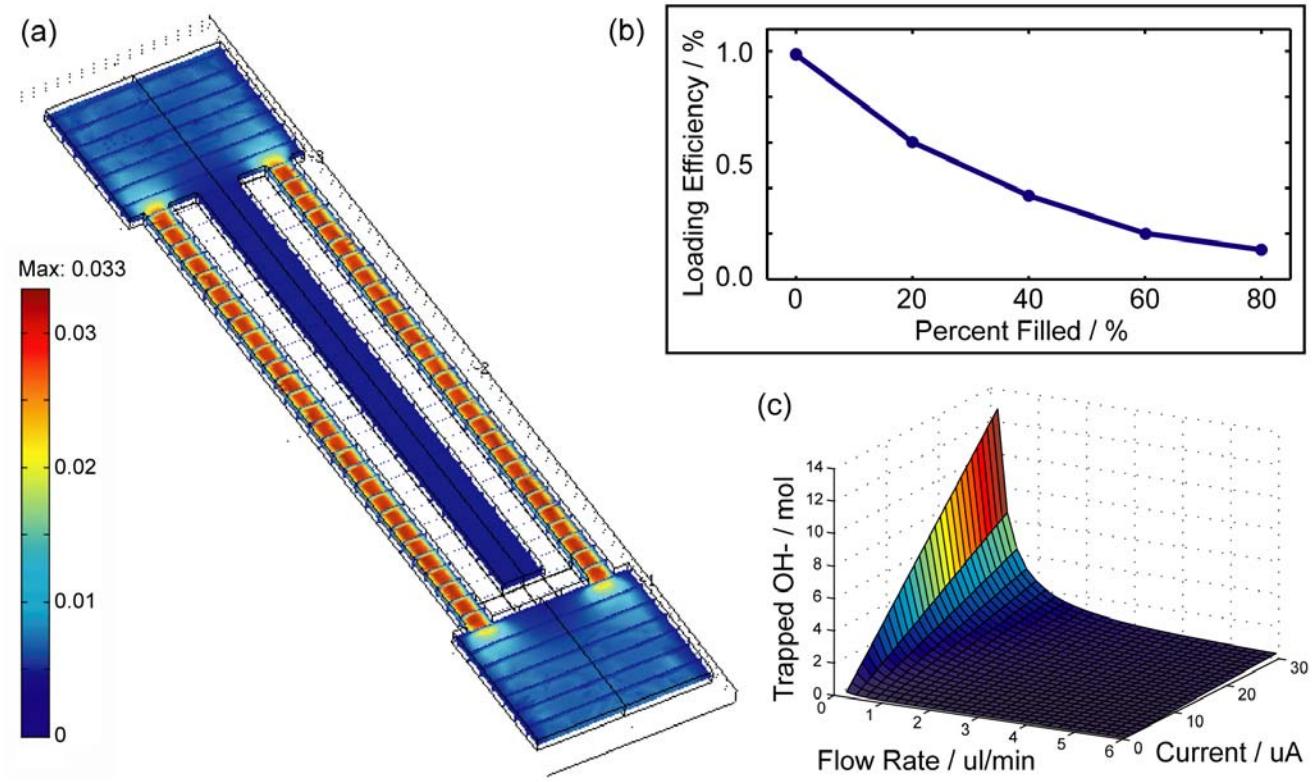

Fig. S2 3D finite element simulation. (a) 3D numerical model of fluid flow in a single trapping device. There is a 2 micron high gap underneath the subtracted cell trapping area. Notice that the flow rate around the trapping region is significantly higher than inside the trapping region, because of the extremely high fluidic resistance of the 2 micron gap. (b) Loading efficiency of the trapping area as a function of how much it is filled. Loading efficiency is calculated as the ratio of the average flow rate inside the trapping region to the average flow rate outside the trapping region. Filling was modeled by adding impermeable blocks inside the trapping area. (c) Number of OH- ions inside in the trapping area (for cell lysis) as a function of the entrance flow rate and the current through the electrodes, at steady state. As the flow rate increases, hydroxide ions are rapidly swept away, leaving few in the trap. Increasing the current increases the number of ions generated, but not dramatically enough to counter the effects of high flow rate. Therefore, lowering the flow rate could increase the rate of lysis much more easily than increasing current. Electrochemical reactions were simulated as in [15].

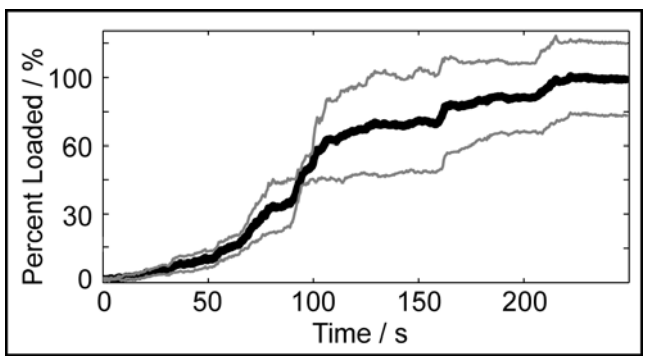

Fig. S3 Cell loading: the percent of a trap loaded with cells as a function of time. The grey lines represent the standard deviation of four traps being loaded simultaneously. .

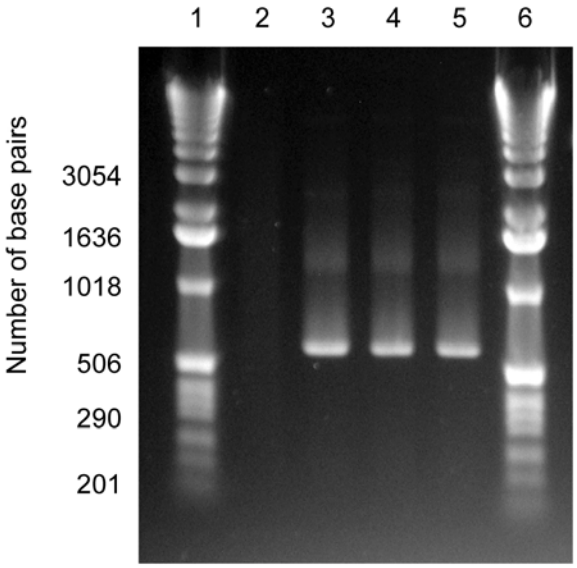

Fig. S4 Results from PCR experiment. DNA Ladders were run in Lanes 1 and 6 (fragment lengths displayed on the left as number of base pairs). Equal amounts of PCR product were run in the following lanes: (2) No template negative control, (3) Positive control plasmid template, (4) Plasmid exposed to OV in device as template, (5) Plasmid exposed to 6V in device as template. These results show that the *nef* gene (620 bp) was readily and equally amplified in all samples except the no template control. The lysing conditions present in this device seem to have no significant effect on the ability to PCR amplify a gene present in plasmid DNA.

Video Captions

Video S1 HeLa cells loading into a single trap. This video is shown in real time, and illustrates that the traps can be completely filled with cells to form a tight three dimensional pack.

Video S2 CHO-K1 cells loading into a single trap. This video is shown in real time. Notice that the cells initially fill in the edges of the trap before filling in completely.

Video S3 Time lapse video of MCF-7 cells cultured over 72 hours. The cells form a spheroid within the cell chamber which is indicative of good cell viability. The device was placed on a heated stage (WIS1, Carel) and the video was recorded using a small inverted microscope (MIC-D, Olympus).

Video S4 A video of HeLa cells being lysed. The lysing electrodes are the large black structures at the left and right edges of the image. A voltage of 2.6V was used for lysis.

Video S5 A video of CHO-K1 cells being lysed under a voltage of 2.6V.

Video S6 Another video of CHO-K1 cells being lysed under a voltage of 2.6V, where these cells are sandwiched underneath the thin section of the cell trap. These cells are confined underneath this 2 μm gap between the glass and PDMS. The blebbing of the cells during lysis is especially apparent because of this confinement.

Video S7 A video of HeLa cells being lysed. These cells were transfected to produce intracellular GFP. The cells that express this protein show up as green spots in these fluorescent images. The GFP can be seen to disappear as the cells are lysed and the membrane is compromised.

Video S8 A video of CHO-K1 cells being lysed. These cells have a membrane protein that is attached to GFP (green), and the nuclear material is stained with Hoescht (blue). This lysis video demonstrates that the cellular material leaks out of the

cell and moves downstream (flow is from left to right) upon lysis. The fluorescently labelled material can be seen as streaks moving downstream, indicating that cellular proteins and genetic material can be found in the lysate.