Single Cell Transfection with Single Molecule Resolution Using a Synthetic Nanopore

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Supplemental Materials

Finite Element Simulations

FIGURE S1

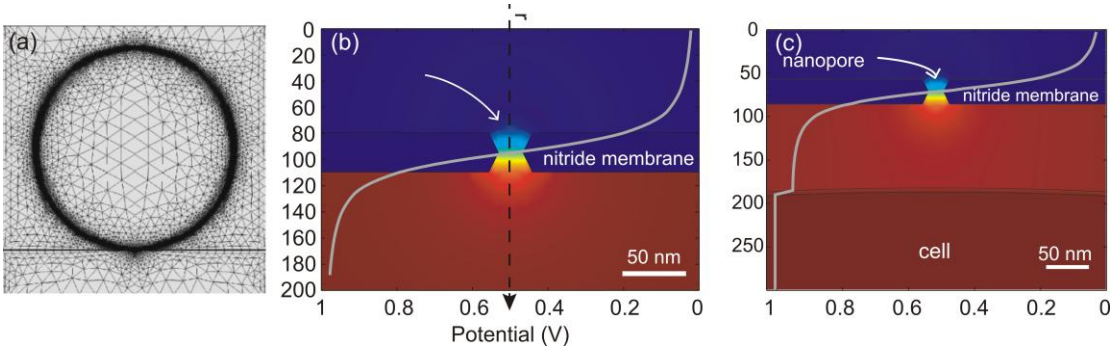

FIGURE S1. Electric fields in the vicinity of a nanopore with and without a cell. (a) A finite element analysis using the grid shown was used to simulate the voltage distribution. The cell is modeled as a sphere encapsulating 150 mM electrolyte in a 8 nm thick lipid layer. **(b)** Electric potential without a cell. **(c)** Electric potential with a cell centered on top of the nanopore with 200nm spacing.

Hydrogel

The poly(ethylene glycol) diacryalate (PEGDA) hydrogel, which serves as a scaffold, only partially encapsulates the cells to enhance viability, motility and morphogenesis. PEG/PEGDA hydrogels are used prevalently in tissue engineering because they are biocompatible and can be easily hybridized with proteolytic oligopetpides or growth factors to promote specific cell interactions, preservation and degradation. The PEGDA used here contains a pendant functional

group, an RGD peptite (arginine–glycine–aspartic acid, N-terminus to C-terminus, American Peptide Company) sequences, that promotes cell adhesion.[1,2] This peptide sequence is covalently bonded to a mono-acrylated PEG of molecular weight 3.4 kDa (Acryl–PEG 3400–NHS, Layson Bio) by reacting equimolar amounts of Acryl–PEG3400–NHS and RGD in 50 mM sodium carbonate buffer at pH 8.2 for 2 h at room temperature. Once the reaction is complete, the product was dialyzed against water overnight using dialysis tubing of molecular weight cutoff 1.0 kDa. The dialyzed product was then lyophilized to obtain the monoacrylated PEG with a RGD sequence (Acryl–PEG 3400–RGD). The hydrogel used is comprised of 5.0 kDa PEG diacrylate (PEGDA) at 10% (w/v), and 3.4 kDa acryl-PEG-RGD with adhesion ligand at 0.5 mM.

Viability Assay after exposure to an Optical Tweezers

The wavelength and power dependence of the lethal dose of IR radiation associated with an optical tweezers were studied in the cell types used in the transfection experiments. Cells were trapped for each 30 s at wavelengths λ = 840, 870, and 900 nm with time-averaged optical power of 50 and 200 mW (after the objective) using a dwell time of 100 μsec distributed over a 3×3 array of nine traps focussed at nine locations separated by 3 μm on each cell. The cells well held in the trap for 30 sec and then partially encapsulated (along with a dark control, 0 mW IR) in 5kDa PEGDA+3.4 kDa acryl-PEG-RGDS hydrogels with a size of 18×18 μm^2 using a 500 ms exposure to 340±20 nm light, and assayed to test membrane integrity and enzymatic activity (Ethidium Bromide and Calcein AM, Molecular Probes). In Figure S2 the scores corresponding to 186 cells cultured for 6 hours after exposure to a time-shared trapping beam and 96 dark controls cultured similarly are reported. There were no significant differences observed between the dark controls and the number of viable cells after exposure to the trap beam. Moreover, the

viability scores were not affected by the wavelength beyond the experimental error. Thus, we infer that cells trapped at λ=868 nm for < 2 min in a time shared trap with a total time-averaged power P < 50 mW per cell to remain viable after transfection.

FIGURE S2

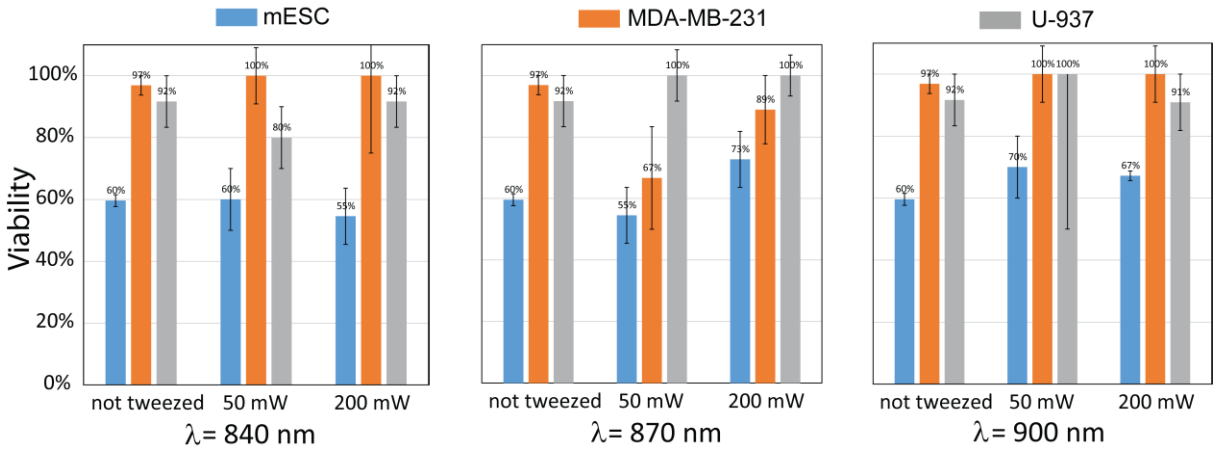

FIGURE S2. Histograms showing viability for various cell types 6 hrs after manipulation with optical tweezers. 116 mESC (blue), 74 U-937(orange), and 92 MDA-MB-231 cells (gray) were assayed after exposre to an optical tweezers in a microfluidic. They were exposed to time-shared optical traps at wavelengths of 840, 870, and 900 nm with 50 and 200 mW per cell. The cells were held in the trap for 30 s prior to gelling in a 5.0 kD PEGDA+PEGA-RGDS hydrogel and then viability was assessed by LIVE/DEAD stains. Both the tweezed cells and the dark controls were subsequently encapsulated in hydrogel.

Transfection controls

OGR1 mESCs transfected with 10 nM siRNA against GAPDH showed uniform EGFP expression in 21 and 39 hrs post transfection (Figure S3). By contrast, OGR1 transfected with 10 nM siRNA against EGFP dramatically reduced EGFP expression in 39 hrs post transfection, such that these OGR1 mESCs exhibited the salt-and-pepper appearance of fluorescence (Figure S2). Next, EGFP gene silencing was monitored in real-time at a single cell level by quantitatively analyzing the fluorescent images. For this analysis, siRNA was labeled with Cy3 using a Cy3-labeling kit according to manufacturer (Life Technologies) to mark transfected

mESCs. OGR1 mESCs transfected with siRNA against EGFP showed either delayed accumulation or a decreasing level of EGFP fluorescence over time (data not shown).

FIGURE S3

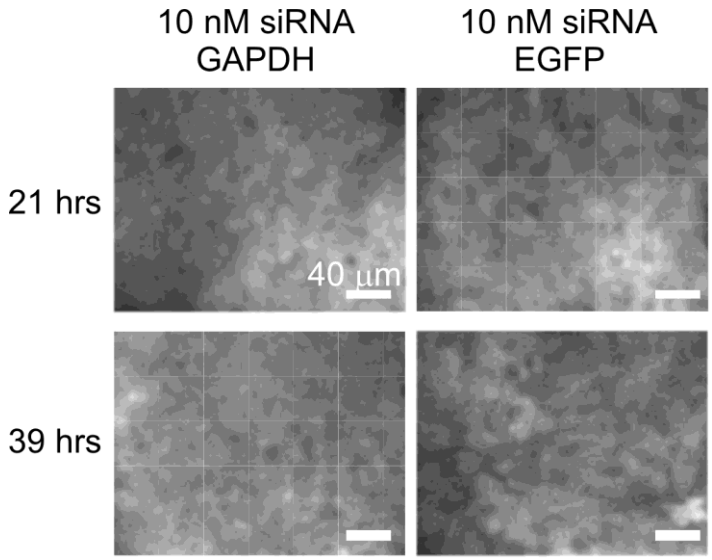

FIGURE S3. **For comparison, efficacy of gene knockdown by a well accepted lipofection method.** Green-fluorescent mouse embryonic stem cells were transfected with 10 nM siRNA against either GAPDH or EGFP. In 21 and 39 hours after the transfection, green fluorescence was observed. Cells transfected with the siRNA against EGFP in 39 hours exhibit prominent salt-and-pepper appearance. Each scale bar represents 40 μm.

Comparison of Molecular Transfer Rates with and without a Cell over the Nanopore.

The top of Figure S4a shows measurements of the fluorescence in a region of interest (ROI 30×20×2 μm; gray bars) at the *trans*-orifice of a 23.8×16.6±0.2 nm^2 pore in a membrane that is nominally 30-nm-thick in relation to an electrolytic current trace associated with an 8.6 kbp circular plasmid interacting at a 1V bias with the pore without a cell positioned over it. The figure illustrates that the electrolytic current through the open pore was approximately 20.5±0.5 nA, but frequently (about every 2-3 s) current blockades were observed occurring in the range

between 19-20 nA. The lower portion of Figure S4a offers a magnified view of the same data in the time frame between 110 and 112 s.

When a fluorescent molecule was found on the *trans*-side of the membrane, a translocation event was tallied. Conditioned on the observation of fluorescence, the coincidence between fluorescence and current blockades observed in the range 19-20 nA was very high—out of 49 fluorescent events tallied, a current blockade was observed every time. In a 142 s window, 367 molecules were observed to translocated across the membrane (2.6 mol/s) at a concentration of 5 pM on the *cis*-side. However, fluorescent events were not tallied every time a current blockade was observed. Short duration current blockades with associated fluorescence on the *cis*-side of the membrane without a corresponding blockade were conjectured to be associated with DNA molecules "bouncing" off the pore in a configuration that does not admit to translocation; the molecules cannot be straddling the pore since no fluorescence was observed there. Finally, other short time, shallow current blockades are observed that are not correlated to fluorescence at all (on either the *cis*- or *trans*-side). These cannot be attributed to smaller fragments of DNA since they would still fluoresce, albeit weakly. Instead, these events must be due to a species without perceptible fluorescence, possibly not even DNA.

This is in stark contrast when a cell was positioned over the pore as in Figure S4b in which the duration of the fluorescent molecules blocking the pore was extended to 10 s.

FIGURE S4

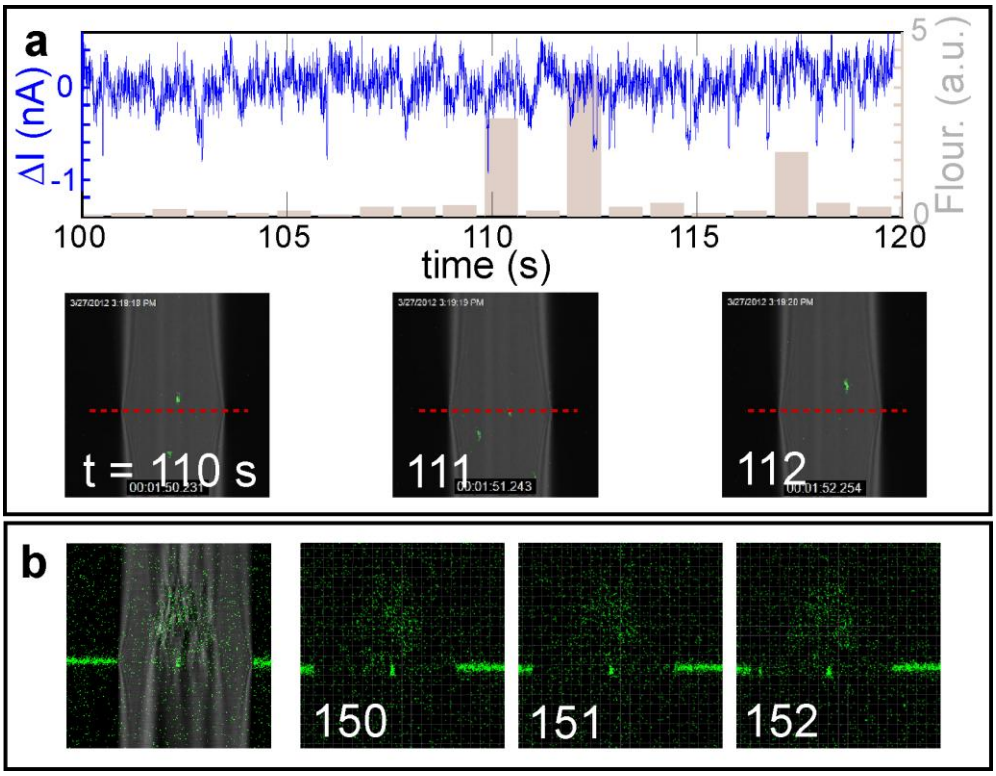

FIGURE S4. Current trace and confocal x-z slices through the nanopore with fluorescent circular plasmids on the *cis*-side and without **(a)** and with **(b)** a cell position over the nanopore on the *trans*-side. The current trace was recorded while circular plasmids were translocating through a 20 nm diameter nanopore with 1 V transmembrane potential to the *trans*-side without a cell. **(a,bottom)** At t=110 s, there is one DNA plasmid on the *trans*-side of the membrane, which correlates to the current blockade event observed at the same time, while at 111 s there is no DNA on the *trans*-side correlating with the absence of blockade events in the current trace, but at 112s there is one DNA molecule observed on the *trans*-side again with a corresponding blockade event. Overall, there is no accumulation of DNA observed at the nanopore during that experiment without a cell. **(b)** In stark contrast, when a MDA-MB-231 breast cancer cell positioned over the pore on the *trans*-side of the membrane as indicated in the composite x-z slice circular plasmids are always found in the neighborhood of the nanopore. However, the fluorescence in the cell body slowly increases over a few minutes indicating an accumulation of transfected DNA.

Nanopore Array Sputtered in a Silicon Nitride Membrane.

Nanopores can be sputtered in silicon nitride membranes with high precision Moreover, pores (with 2 nm diameter) can be configured separately into arrays to reduce the diffusion capacitance and improve throughput, as illustrated in Figure S5.

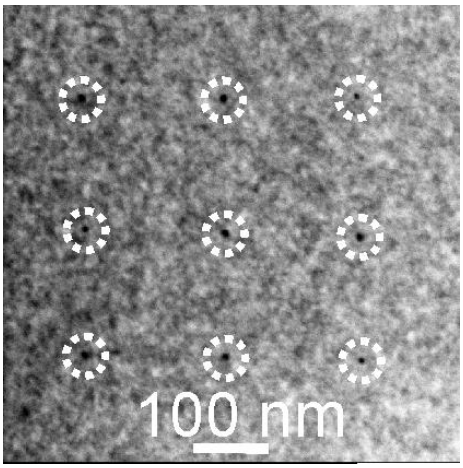

FIGURE S5. Nanopores can be sputtered with sub-nanometer precision in small area membranes. The figures shows a 3×3 array of 2 nm diameter nanopores in a 30 nm thick silicon nitride membrane.

References

(1) Gonzalez, A. L.; Gobin, A. S.; West, J. L.; McIntire, L. V; Smith, C. W. *Tissue Eng.* ***2004****10*, 1775–86.

(2) Yang, F.; Williams, C. G.; Wang, D.-A.; Lee, H.; Manson, P. N.; Elisseeff, J. *Biomaterials* **2005**, *26*, 5991–8.